Scientific Program

American Association for Clinical Chemistry
40th National Meeting
July 24–28, 1988, New Orleans, Louisiana

Sunday, July 24
9:30 am–4:30 pm  Scientific Workshops (Full Day)
12:30 pm–4:30 pm  Scientific Workshops (Half Day)
5:30 pm–7:00 pm  Opening Plenary Session

Monday, July 25
7:15 am–8:15 am  Breakfast Roundtables
8:30 am–12:05 noon  Symposium
—Drug Abuse
—High Technology Instrumentation for Clinical Chemistry in the 1990's
12:10 pm  AACC Annual Meeting
12:30 pm–1:30 pm  Luncheon Roundtable
2:00 pm–6:00 pm  Scientific Workshops
2:30 pm–4:30 pm  Selected Topics
—Standardization Issues Relating to Ion Selective Electrodes
—Integration of Computers and Instruments Through Interfaces
—Toxicology
5:30 pm  Industry Sponsored Workshops

Tuesday, July 26
7:15 am–8:15 am  Breakfast Roundtables
8:45 am–12:30 pm  Symposium
—Microbiology: A Widening Frontier
—Neurochemistry in Psychiatry and Neurology: Present and Future Developments
12:30 pm–1:30 pm  Luncheon Roundtable
1:00 pm–5:30 pm  Poster Sessions
Immunoassays
—Advances in Immunoassay
Instrument and Product Evaluations—Part A and B
—Evaluations of New Instruments
—Evaluations of New Products
—Evaluations of Other Analytical Techniques
—Evaluation of Random Access Analyzers
Clinical Studies
—Animal Studies
—Clinical Studies
2:00 pm–6:00 pm  Scientific Workshops
2:30 pm–4:30 pm  Selected Topics
—AIDS and Retroviruses
—Advances in Cellular Immunology
5:30 pm  Industry Sponsored Workshops

Wednesday, July 27
7:15 am–8:15 am  Breakfast Roundtables
8:45 am–12:30 pm  Symposium
—Emerging Technology: Visions to Reality via Basic Research
—Quantitative Tissue Analysis in Clinical Chemistry
12:30 pm–1:30 pm  Luncheon Roundtables
1:00 pm–5:30 pm  Poster Sessions
Hormones
—Thyroid Hormones
—Corticosteroids

Thursday, July 28
8:45 am–12:30 pm  Symposium
—Thrombosis and Coagulation in Laboratory Medicine
—Health Care Deliver in the 1990's
12:00 noon–4:30 pm  Poster Session
Drugs Part B
—Miscellaneous Other Drugs
Proteins
—Specific Proteins
Enzymes Part A and B
—Enzymes in Cardiac Disease
—Enzymes in Hepatic Disease
—Miscellaneous Other Enzymes
Tumor Markers
—Tests for Detection of Cancer
Hemoglobin and Coagulation
—Hemoglobin and Glycosylated Hemoglobin
—Coagulant Testing
Nutrition and Trace Metals
—Trace Metals
—Evaluation of Nutritional Status
Test Results and Other
—Factors Affecting Test Results
—Other
Selected Topics
—Fibrinolysis
—Alternatives to Laboratory Testing in Animals
—Geriatric Clinical Pharmacology

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988  1131
Welcome to New Orleans—Opening Mixer  
Sunday, July 24  
7:30 pm–9:00 pm  
New Orleans Hilton  
$15.00 per person  
The Opening Mixer will follow the Plenary Session. There is no better way to begin the week . . . good food, good drink, and a good opportunity to get together with friends and colleagues. Take advantage of this festive gathering . . . we'll ring in the meeting in style and prepare your palate for the week ahead.

Past Presidents’ Reception  
Wednesday, July 27  
7:30 pm–10:00 pm  
Gallier Hall  
$30.00 per person  
Sponsored by Abbott Diagnostics  
Gallier Hall will provide a beautiful setting for an evening filled with dining, dancing and socializing. The old City Hall is an architectural landmark, one of the best examples of Greek revival architecture in the area. The high ceilings and spacious hallways are grand in a typically Southern kind of way. The historic building provides space to dance, indulge in culinary delights and a place to relax. A New Orleans style Past Presidents’ Reception not to be missed! (Shuttle bus service will be provided.)

Plenary Session  
Sunday, July 24  
5:30 pm–7:00 pm  
AACC National Lectureship Award  
Receptor Regulation: An Approach to Treatment of Hypercholesterolemia  
Joseph L. Goldstein, M.D.  
University of Texas  
Southwestern Medical Center at Dallas  
Dallas, TX  
AACC Annual Award Presentations  
(see back cover for listing)

Student Mixer and Poster Contest  
A mixer will be held at the New Orleans Hilton on Monday evening, July 25, from 6:00 pm–8:00 pm. Information about careers in Clinical Chemistry will be provided during informal discussions with the Career Education Committee members and representatives from ComACC. They will be ready to discuss and answer questions on the following topics:  
—Employment  
—Professional Directions  
—Clinical Laboratory Trends  
Chairman: Alan H. Wu, Ph.D.
### PROGRAM AT-A-GLANCE

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Plenary Session
AACC 40th National Meeting

SUNDAY, JULY 24
AACC National Lectureship
Sponsored By: Technicon Instruments Corporation

Joseph L. Goldstein, M.D.

Dr. Goldstein was born in 1940 in Sumter, SC. He received a bachelor's degree from Washington and Lee University in 1962 and an M.D. from the University of Texas Southwestern Medical School in Dallas in 1966. From 1968 to 1970 he was a clinical associate at the National Institutes of Health. He then served two years as a special NIH fellow at the University of Washington School of Medicine in Seattle. In 1972 Dr. Goldstein became assistant professor of medicine at the University of Texas Southwestern Medical School and head of the division of medical genetics. In 1976 he was appointed professor of internal medicine, and in 1977, he was made chairman of molecular genetics at the University of Texas Health Science Center in Dallas.

Dr. Goldstein is a member of the National Academy of Sciences, American Academy of Arts and Sciences, American College of Physicians, American Society of Biological Chemists, American Society for Clinical Investigation, Association of American Physicians, and Phi Beta Kappa. He is also an honorary member of the Harvey Society and a diplomate of the American Board of Internal Medicine.

Together with his colleague Dr. Michael S. Brown, Dr. Goldstein in 1985 received the Nobel Prize in Physiology or Medicine for their discovery of receptors that control cholesterol metabolism. They have received several other awards for this work as well.

Dr. Goldstein has served as a member of the editorial boards of Cell, Annual Review of Genetics, Journal of Biological Chemistry, Arteriosclerosis, and Science. He is currently a member of the Medical Advisory Board of the Howard Hughes Medical Institute, the Scientific Advisory Board of the Welch Foundation, and is a Non-Resident Fellow of the Salk Institute. He is a past president of the American Society for Clinical Investigation (1985-1986).

Receptor Regulation: An Approach to Treatment of Hypercholesterolemia

The low density lipoprotein (LDL) receptor mediates the removal of cholesterol-carrying lipoproteins from blood, and thus controls the concentration of blood cholesterol. When the activity of LDL receptors is reduced, as a result of genetic or acquired abnormalities, LDL builds up in blood and atherosclerosis ensues. Individuals with one mutant LDL receptor gene (familial hypercholesterolemia heterozygotes) have two-fold elevations in plasma LDL levels and develop heart attacks typically in the fourth and fifth decade. Individuals with two defective LDL receptor genes (familial hypercholesterolemia homozygotes) have six to ten-fold elevations in plasma LDL levels and develop heart attacks in childhood. Thirteen different mutant alleles at the LDL receptor locus have been defined in molecular terms through cloning of genomic DNA at the site of mutation. These mutations produce a variety of phenotypes that disrupt receptor behavior, including failure of synthesis; failure of the receptor to move from the endoplasmic reticulum to the Golgi complex; failure of the receptor to bind LDL; and failure of the receptor to cluster in coated pits.

Familial hypercholesterolemia (FH) heterozygotes can be treated with drugs that stimulate their single normal gene to produce twice its normal number of copies of messenger RNA (mRNA), thereby compensating for the mutant gene. The most successful therapies take advantage of the fact that the receptor is normally under negative feedback regulation by intracellular cholesterol. Depletion of intracellular cholesterol in the liver through the administration of bile acid binding resins and cholesterol synthesis inhibitors activates transcription of the LDL receptor gene, and this causes the plasma LDL to fall.

FH homozygotes do not respond to this receptor-stimulating therapy. For them, the most successful approach has been the performance of a liver transplant operation which provides a source of normal LDL receptors. In animals with normal LDL receptor genes the number of receptors can be suppressed by the ingestion of a diet that is rich in cholesterol and saturated fatty acids. This suppression results at least in part from a cholesterol-mediated repression of transcription of the LDL receptor gene in liver. It seems likely that such diet-induced receptor suppression can occur in humans and that it may contribute in part to the widespread occurrence of high cholesterol levels and atherosclerosis among the general population in industrialized societies. Drugs that stimulate transcription of the LDL receptor gene may also be effective in lowering blood cholesterol in individuals who suffer from such receptor suppression.
Drug Abuse Scene

In the early sixties, approximately 2 percent of the population of the United States had tried an illicit drug. By 1985, approximately 37 percent or almost 70 million people from the household population of the United States had tried an illicit drug at least once. Since 1974, the prevalence of cocaine use in the United States had increased fourfold, and medical emergencies associated with cocaine use have increased more than 1000 percent. By the time they graduate from high school, more than half of the seniors have tried an illicit drug. This presentation will draw on data from the National Household Survey on Drug Abuse, the High School Senior Survey, and the Drug Abuse Warning Network (DAWN) in discussing the prevalence and patterns of drug abuse in the United States. Although the paper will focus primarily on marijuana and cocaine use, the prevalence levels for a variety of drugs, including the illicit psychotherapeutics will also be discussed. Data from the High School Senior Survey will be used to review changes in attitudes toward drug use and the perceived harmfulness of various drugs.

Lewis W. Goldfrank, M.D.

Dr. Goldfrank is an Associate Professor of Clinical Medicine at New York University, the Director of Emergency Medical Services at Bellevue Hospital Center and New York University Medical Center as well as the Medical Director of the New York City Poison Control Center.

Dr. Goldfrank's major areas of research interest are in the interface of the fields of Emergency Medicine, Medical Toxicology, and Environmental Medicine. Education in basic medical toxicology is a major goal and his textbook Goldfrank's Toxicologic Emergencies (Goldfrank L, Flomenbaum N, Lewin N, et al, 1987) was developed in conjunction with the full time staff in his department. He received his BA from Clark University. He began his medical training at Johns Hopkins University and received his MD degree at the University of Brussels. He received training in Internal Medicine from...
Montefiore Hospital and Medical Center in New York. He has received Board Certification in Internal Medicine, Emergency Medicine and Medical Toxicology.

**Designer Drugs**

The development of synthetic drugs produced by clandestine drug laboratories in an effort to avoid legal standards has led to serious clinical problems. The three most prominent examples are the development of Pentany derivatives (of extreme potency), of meperidine analogs tragically producing a neurotoxic by-product MPP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and of an amphetamine analog, MDMA (methylenedioxymethamphetamine).

Pentany derivatives have led to marked opioid overdoses with limited toxin (micrograms) responsive to large doses of naloxone.

MPPP led to a drug induced neurotoxicity with a resultant toxic parkinsonism. MDMA was advanced as an amphetamine without toxicity by psychiatrists yet all the clinical manifestations have been typical for amphetamines. The particular clinical aspects of these drugs will be described.

**Wayne R. Snodgrass, M.D., Ph.D.**

Wayne R. Snodgrass, M.D., Ph.D. is Associate Professor of Pediatrics and Pharmacology-Toxicology at the University of Texas Medical Branch, Galveston, Texas. He is the Director of the Clinical Pharmacology-Toxicology Unit and the Texas Poison Center. He is board certified by the American Board of Pediatrics and the American Board of Medical Toxicology. He trained at Indiana University, the National Institutes of Health, and the Rocky Mountain Poison Center. His research interests include metabolic activation of drugs and chemicals to toxic metabolites, and the use of binding agents to accelerate removal of environmental chemicals from deep compartment body storage sites.

**Implications of Drug Levels in Body Fluids**

Direct measurement of drug tissue concentrations rarely is done for clinical purposes. Instead, quantitative serum drug concentrations and qualitative urine drug analysis are done routinely. Drug pharmacodynamic effect occurs in tissue compartments which, at equilibrium, often can be related to drug serum concentration. For many drugs of abuse, true pharmacologic equilibrium/kinetic steady state is not reached under usual conditions of drug use. Thus, quantitative clinical correlation of clinical impairment and serum/urine drug concentration usually is not possible. With random single time-point specimens it is nearly meaningless to attempt to correlate in a precise way for certain drugs of abuse, especially those with a high volume of distribution, a specific serum/urine concentration with a specific degree of clinical impairment.

Marihuana is an example of over-interpretation of quantitative urine drug concentrations. A quantitative urine marihuana carboxy metabolite concentration documents only exposure; it provides almost no information about impairment. The concept of distinguishing exposure versus impairment for drugs of abuse needs further emphasis in clinical toxicology.

**Donald R. Mattison, M.D.**

Dr. Mattison is professor of Obstetrics and Gynecology at the University of Arkansas for Medical Sciences and Director of the Human Risk Assessment Program at the National Center for Toxicological Research.

Dr. Mattison received his B.A. at Augsburg College, M.S. at Massachusetts Institute of Technology and M.D. at the College of Physicians and Surgeons of Columbia University. His research interests include reproductive toxicology, reproductive pharmacology, risk assessment and nuclear magnetic resonance imaging and spectroscopy.

**Drug Use and Reproduction**

**MARIJUANA:** Marihuana use may result in lowered counts and altered sperm morphology and may affect menstrual cycles and ovulation. There is conflicting evidence of fetal wastage. Studies of rodents, and nonhuman primates and women demonstrate that THC is eliminated in breast milk. In animals, intrauterine growth retardation is one of the most common effects of prenatal cannabinoid exposure. Evidence now suggests that cannabinoids do not produce structural malformations in humans although malformations have been produced in mice.

**SEDATIVES, HYPNOTICS AND TRANQUILIZERS:** Maternal use of sedatives and tranquilizers may complicate delivery and leave newborns lethargic, with respiratory difficulties, apneic spells, hypotonia and decreased sucking.

**STIMULANTS AND COCAINE:** An increased incidence of spontaneous abortions has been noted among chronic amphetamine and cocaine
users. Placental abruption has been observed as a complication of cocaine use.

OPIATES AND SYNTHETICS: Opiate use may cause amenorrhea or increase menstrual irregularity. Pregnancy in heroin addicts, however, is not uncommon. Heroin use during pregnancy increases the risk for stillbirth, neonatal death, fetal distress and hypoxia. Withdrawal symptoms are observed in infants born to opiate-addicted mothers.

HALLUCINOGENS: Infants exposed to PCP in utero had the greatest state lability and the worst consolability.

INHALANTS AND SOLVENTS: CNS defects and other anormalies, including abnormal facial features and mental retardations, have been observed in babies whose mothers used inhalants or solvents in combination with alcohol while pregnant.

Session B - High Technology Instrumentation For Clinical Chemistry in the 1980s

John Savory, Ph.D.
Chairman

Richard A. Yost, Ph.D.

Dr. Richard A. Yost is Associate Professor of Chemistry at the Univ. of Florida. He obtained his degree from the University of Arizona in December 1974, after completing research in gas chromatography with Professor Mike Burke. His Ph.D. research was conducted at Michigan State University under the direction of Professor Chris Enke. He was an NSF graduate fellow from 1975-1979, and L.E. Quill Fellow from 1975-1979, and an ACS Analytical Division Fellow from 1977-1978. The concept of tandem quadrupole MS/MS was originated by Yost and Enke. Dr. Yost's research interests are in the development of new analytical chemistry techniques using modern instrumentation and computers, and the applications of these techniques in areas such as clinical, environmental, and forensic chemistry. Special interests include tandem mass spectrometry and integrated separation/identification techniques.

Tandem Mass Spectrometry (MS/MS): Progress and Prospects

Tandem mass spectrometry (MS/MS), although a relatively new analytical technique, has gained widespread acceptance with the analytical community since its introduction in the 1970s. Based upon mass spectrometers capable of providing two (or more) sequential stages of mass separation, MS/MS integrates the two basic separations of chemical analysis, separation and identification, in one instrumental technique. Thus, the acceptance of MS/MS has been due largely to its ability to provide rapid, sensitive, and selective analyses of complex mixtures, often with minimal, if any, sample clean-up. Major emphasis has been placed on rapid screening, confirmation, and quantitation of endogenous and exogenous compounds in physiological fluids and tissues. These techniques have been applied to a wide range of problems, including trace toxicological analysis, drug testing, quantitation of subpicogram levels of neurochemicals and prostaglandins, rapid identification of drug metabolites, and localization of biomolecules in tissues by laser microprobe/MS/MS. This lecture will focus on concepts of tandem mass spectrometry, MS/MS instrumentation, and examples of the use of MS/MS for clinical analysis.

Daniel P. Perl, M.D.

Daniel P. Perl, M.D. is Professor of Pathology and Psychiatry at the Mount Sinai School of Medicine and Director of the Neuropathology Division. He was born and raised in New York City and received his undergraduate education at Columbia College. He earned his M.D. from the State University of New York and trained in Pathology and Neuropathology at Yale University. Prior to joining the staff at Mount Sinai he has held teaching positions at Brown University and the University of Vermont. Based on almost ten years of research in his laboratory, Dr. Perl is one of the leading proponents of the hypothesis that aluminum plays a role in the pathogenesis of Alzheimer's disease and related disorders. Married, with two children, Dr. Perl lives in the suburbs of New York City.

Microprobe X-Ray Spectrometry and Laser Microprobe Mass Analysis (LAMMA) for Trace Element Analysis of Histologic Specimens

Approaches to trace element analysis of biologic tissues have, for the most part, involved the use of techniques which provide only regional concentrations. Methods such as atomic absorption spectrometry and neutron activation analysis provide extremely sensitive quantitative data yet lack the ability to localize elemental constituents within a specimen on a cellular or subcellular level. Through the use of x-ray spectrometry in conjunction with scanning
electron microscopy and/or transmission electron microscopy, methods have been developed to determine the elemental composition of cellular components of tissue sections. In our laboratory, we have identified evidence of aluminum accumulation within the neurofibillary tangle-bearing neurons of cases of Alzheimer's disease (Science 208:297-299, 1980). Similar accumulation have also been identified in the neurofibillary tangle-bearing neurons seen in natives of the island of Guam who suffer from amyotrophic lateral sclerosis and Parkinsonism with severe dementia (Science 217:1053-1055, 1982). We have recently introduced in our laboratory a new technology for trace element analysis of tissue specimens, namely laser microprobe mass analysis (LAMMA). The LAMMA instrument provides mass spectral analysis of sectioned plastic embedded tissues using an optically directed, narrowly focused high energy laser beam pulse. The laser ionizes a minute portion of tissue and the ions produced are drawn into a time-of-flight mass spectrometer for detection. The LAMMA instrument combines a lateral resolution of 1 micron, elemental detection limits in the range of 1 part per million, and the ability to provide elemental concentration data. The operating principles and capabilities of this instrument will be discussed and compared to other forms of microprobe analysis.

Jorge R. Barrio, Ph.D.

Dr. Barrio is Professor of Radiological Sciences (Biophysics and Nuclear Medicine) and Pharmacology at the University of California, Los Angeles. In addition, he holds appointments as Head of the Chemistry/Biochemistry Section in the Positron Emission Tomography Program and as Principal Investigator at the Laboratory of Biomedical and Environmental Sciences at UCLA. He also serves on the Editorial Board of Nuclear Medicine and Biology. Dr. Barrio has made research contributions in the area of bioorganic chemistry, biochemistry and positron-emitting labeled radiotracers, and has participated on various national and international scientific panels on positron emission tomography.

He received a B.S. in Biochemistry and a Ph.D. in Biochemistry from the University of Buenos Aires, Argentina in 1964 and 1969, respectively, and a Ph.D. in Chemistry from the University of Illinois in 1979.

Positron Emission Tomography: An In Vivo Radioassay Technique

Positron emission tomography (PET) is an analytical imaging technique that permits the measurement of local, specific biochemical events in the living human being. Procedures designed to measure specific processes (i.e. glucose metabolic rate, protein synthesis, receptor density and number) should be such that at all times during the tomographic measurements the radioactivity is only contained in the original precursor and one product or, at least, a few defined chemical species. For example, the use of [14C]l-deoxy-2-fluoro-D-glucose to measure cerebral glucose metabolic rates is based upon this principle. The radiolabeled analog competes with glucose for its facilitated transport into the brain and for hexokinase phosphorylation to produce the corresponding hexose-6-phosphates. [14C]l-Deoxy-2-fluoro-D-glucose-6-phosphate is trapped in tissue with minimum metabolic degradation (i.e. dephosphorylation). Accordingly, the rate of accumulated radioactivity is proportional to the tissue glucose metabolic rate under steady state conditions. Protein biosynthesis determination with L-[1-14C]leucine and PET can also be modeled following similar principles to those described for the [14C]l-deoxy-2-fluoro-D-glucose procedure. In vivo pharmacological data can also be obtained from normal human subjects and patients with cerebral disorders. Drugs (i.e. spiperone and derivatives, raclopride) can be labeled with positron-emitters and their pharmacokinetic behavior examined in vivo under tracer conditions or even at concentrations producing pharmacological effects. Following these procedures, the effect of specific pharmacological agents on behavior or symptoms can be observed and correlated with alterations in pharmacokinetics at the sites of action in the brain. These studies are providing for the first time the opportunity to examine the chemical dynamics in the living human brain, as well as the means for assessing the biochemical basis of brain function in health and disease.

Walter Slavin, M.S.

Walter Slavin is involved in the development and characterization of spectroscopic instruments at Perkin-Elmer Corporation, Ridgefield, Conn., 06877. He has written several books and book chapters on different aspects of atomic spectroscopy. He has published widely on analytical instrumentation and instrumental methods development. He is an Editor of SPECTROCHIMICA ACTA, Part B, and a former member of the Advisory Board of ANALYTICAL CHEMISTRY. He led Perkin-Elmer’s development of atomic absorption spectroscopy.

Instrumentation for the 1990s—Atomic Spectroscopy

Atomic spectroscopy is the most widely used technology for the analysis of materials for their elemental composition. There is no apparent limit to what can be expected of the expansion of spectroscopic methods to meet the growing needs of the 1990s. Certainly the needs of the next decade include:

1. Sensitivity: the measurement of ever smaller quantities of the various elements.
2. The routine measurement of more elements.

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3. More efficient handling of more samples and more data.
4. Speciation: the separate measurement of the different bonding states of elements.
5. Reduction of sample handling and preparation efforts.
6. All of this with less skilled laboratorians and at lower cost.

There is no lack of new ideas and new technology on which to base the improvements required in the next decade. Laser spectroscopic systems can already measure femtogram/liter concentrations in microliter volumes of samples. There are a few specialized laboratories detecting single atoms. The computers imbedded within all modern instruments are becoming more powerful, less expensive and much more easily managed. Robotic concepts and hardware are becoming affordable, more easily programmed and capable of a wider range of tasks.

In this talk we will speculate on how these needs and opportunities are likely to come together for analysts in all fields of chemistry, but most especially those chemists whose samples are biochemical.

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**Tuesday Morning, July 26**

**Session A—Microbiology: A Widening Frontier**

**Richard B. Passey, Ph.D.**

Chairman

Richard B. Passey, Ph.D.

Dr. Passey is professor of pathology in the Department of Pathology and in the Graduate School at the University of Oklahoma Health Sciences Center, Oklahoma City. He is also Director of the Clinical Chemistry, Hematology, and Core Laboratories of the Oklahoma Teaching Hospitals. His current national service includes: AACD Liaison to the College of American Pathologists Workload Recording Committee and Chairman of the National Committee for Clinical Laboratory Standards Area Committee for Evaluation Protocols.

He received the B.S. degree, in Medical Technology from Utah State University, and the Ph.D. degree, in Biochemistry, from Colorado State University. He has held academic positions at the University of Texas Medical Branch, the University of Kentucky, and the University of Oklahoma.

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**The Interface of Microbiology and Clinical Chemistry**

Chemistry and Microbiology have developed hand in hand in a logarithmic growth phase over the past 300 years.

Microbes and their impact on man's health both motivated and provided the materials for much of the development of biochemistry and molecular biology. The varied garden world of microbes ranges from a continental peaceful land populated by helpful creatures, to rough frontier foothills and mountains where dwell individuals that have capacity to both live gently or ravage, and still it stretches into the dark and damp jungle of pathogens that care not whether you die as long as they are fed for the moment.

This varied landscape provides more unanswered questions than we have been able to answer. In fact, a truism states, that before one question is finally answered it generates a kaleidoscope of new ones. Such is the world of microbiology.

This symposium explores the boundaries between microbiology and clinical chemistry to better reveal both the opportunities and some of the diagnostic tools that have recently come from this historically fertile field. Koch said "as soon as the right method is found discoveries come as easily as ripe apples fall from the tree."

Prime unsolved mysteries still inhabit the world of the microbes but, by applying the ever improving tools of clinical chemistry, biochemistry, toxicology, physiology, clinical medicine and all their sub-specialties; many will yield their secrets. We have no idea of how many toxic or physiologically active molecules are produced by or from microbes, nor do we understand many of their effects on the human body. Improving, yet inadequate, tools provide ever quicker and more sure diagnosis of infection. The world, plagued with deadly infectious diseases, some new, some old, and some changed from what they were last year, anxiously awaits our answers.

As a model for these problems, we will consider Gram negative septicemia and its attendant endotoxin shock. In the United States, alone, there are approximately 300,000 hospital cases of diagnosed Gram negative septicemia each year. Of these 90,000 to 150,000 die. Effective, yet risky, treatments are available for the fortunate who are accurately diagnosed early. The consequences of these infections are severe and rapid, and current therapies must be applied before irreversible shock has begun, therefore, our limited ability to rapidly diagnose these patients constitutes a severe public health deficiency.

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**Joseph L. Waner, Ph.D.**

Dr. Waner is associate professor of pediatrics in the Division of Infectious Diseases at the University of Oklahoma Health Sciences Center, Oklahoma City. He is also director of the Virology and Serology Laboratories at Oklahoma Children's Memorial Hospital. Dr. Waner is editor of the Newsletter of the Pan American Group for Rapid Viral Diagnosis and serves on the editorial boards of the Journal of Clinical Microbiology and Diagnostic Microbiology and Infectious Disease.

He received his Ph.D. degree from the Stritch School of Medicine, Loyola University, Chicago, Illinois, in 1969. Between 1969 and 1980 he was research fellow, research associate, assistant professor, and associate professor at the Harvard School of Public Health. His research has focused on human cytomegalovirus and the rapid detection of respiratory viruses through the use of defined monoclonal antibodies.
The impetus for development of rapid methods of viral diagnosis has come from the current and future availability of effective antivirals and immune therapies. Rapid techniques employ strategies to identify viruses, antigens, enzymes, or nucleic acids in clinical specimens. Isolation of viruses in cell cultures, however, remains the standard of sensitivity and specificity for rapid procedures.

Identification of viral antigens with antisera depends upon the detection through a signal of an immune reaction; the use of monoclonal antibodies has greatly improved the assays. Fluorescence or a variety of enzymes conjugated to the antisera are commonly used signals. Immunofluorescence (IF), the use of enzyme conjugated antisera, is used primarily to identify antigens directly in cells obtained from the patient; enzyme immunoassay (EIA) can detect soluble antigens. Depending upon the virus, the sensitivity and specificity of both procedures may be greater than 90% of virus isolation. Current EIA constructions, however, may have reached their limit of sensitivity and immunofluorescence is a subjective assay. IF and EIA, however, are being applied to cell culture enhancement procedures wherein, the earliest viral functions are identified for diagnostic purposes. Detection of viral nucleic acids is a specific and sensitive diagnostic procedure. Technical problems associated with availability of probes, specimen preparation and signal, however, have slowed application. A unique application is the detection of viral genome in latent infections.

All methods of viral diagnosis must be effective in aiding the differential diagnosis and determining the role of the virus in pathogenesis.

George H. Parsons, Ph.D.

Dr. Parsons is the Technical Director at GENE-TRAK Systems in Framingham, MA. After two years as an Instructor at Boston University from 1972 to 1974, he joined Clinical Assays in Cambridge, MA from 1974 to 1981, where his last position was Technical Director. After leaving Clinical Assays, George joined Medical and Scientific Designs in Rockland, MA where he worked on automated immunoassays. He joined Integrated Genetics in March of 1986 and became a member of the management team of GENE-TRAK Systems when that joint venture between AMOCO Biotechnologies and Integrated Genetics was formed in July of 1986. George has been involved with research and development of more than 30 different commercial manual and automated immunoassays. He has developed patented technologies for a novel antibody coating methods, for the determination of free T4, for several immunoassay tracers and for a method of making commercial control sera. Publications include an invited chapter on solid phase antibody coating in Methods in Enzymology. Recent research activities at Integrated Genetics and GENE-TRAK Systems focus on the development of nucleic acid probe based tests for food-borne pathogens and for human clinical pathogens.

He received his M.A. and Ph.D. degrees in Physical-Organic Chemistry at Brandeis University in Waltham, MA in 1968 and 1973, respectively, and his B.A. in Chemistry from Boston University in 1967.

Nucleic Acid Probe Assays: The Evolution of a Technology

Probe assays are based on the relatively recent discovery that we can directly access the powerful information transmission medium represented by nucleic acids. As these molecules have evolved over the last billion years, they have become very stable chemically and have the ability to code for extremely specific messages. Given only four nucleotides, there are 64 possible unique three base sequences. These condons in turn code for 21 normally occuring amino acids. This degeneracy in the genetic code means that over 1000 distinct 18 base nucleic acid sequences could code for a single hexapeptide. In theory, at least 10 of these 18 base sequences could be distinguished in an assay by changes in assay temperature or buffer composition. By comparison, the best monoclonal antibody could not distinguish between any of these hexapeptides. Were these peptides to occur in related microorganisms, this inherent unavoidable cross reactivity would prevent the development of specific immunoassays. This potential for increased specificity makes nucleic acid probe assays very attractive alternatives in the search for replacements for or extensions to classical microbiology. For this potential to be realised, however, several problems related to sample preparation, target number, choice of label and assay format must first be solved. The enzymatic self-replicating properties of nucleic acids can be utilized as a means of amplification. Automation may play an earlier role in nucleic acid assays than it has in other chemistries as it emerges from the research laboratory to routine laboratory use.

Myron Sasser, Ph.D.

Dr. Sasser is professor of plant bacteriology at the University of Delaware, Newark. He teaches courses in the diagnosis of plant disease and has research in the areas of bacterial selective media. fatty acid analysis for identification of bacteria and in the biochemistry of disease resistance in plants.

Dr. Sasser received his Ph.D. from the University of Florida in 1968. He has been at the University of Delaware since then with the exception of one year in Hungary as a National Academy of Sciences Exchange Fellow in 1975-1976.

Novel Clinical Applications of a Fatty-Acid-Profiling Microbial Identification System

Fatty acids of bacteria and yeasts are highly reproducible chemotaxonomic markers which lend themselves to automated analysis through use of a computerized gas chromatograph. A commercially available software package allows use of a capillary column gas chromatograph to name more than 200 fatty
The use of a covariance matrix, of principal component analysis and of pattern recognition results in the naming of about 50 unknown bacteria per day.

Future developments (some of which are currently being prepared) will allow automated sample preparation, an increase in the number of analyses to ca. 100 per day, and assays made directly from tissues without culturing of the unknown organisms. A database of yeast fatty acids will allow automated identification of these organisms. The flexibility of the software is such that virtually any group of compounds which can be analyzed by gas chromatography can be compared to a stored database (eg. organic acids from urine to detect defects in metabolism).

**Session B—Neurochemistry in Psychiatry and Neurology: Present and Future Developments**

Charles B. Nemeroff, M.D., Ph.D.

Chairman

Dwight L. Evans, M.D.

Dr. Evans is Associate Professor of Psychiatry and Medicine and Director of the Division of Inpatient Psychiatry at the University of North Carolina School of Medicine at Chapel Hill. He is also Chief of the Inpatient Psychiatry Service of the University of North Carolina Memorial Hospital and Director of the Affective Disorders Unit. He serves as Chief of the Affective Disorders Section of the National Institute of Mental Health Clinical Research Center at the University of North Carolina at Chapel Hill. Dr. Evans is investigating neuroendocrine and immune correlates of depression in psychiatric patients and in oncology patients.

Dr. Evans received his M.S. degree in Physiological and Experimental Psychology at Bucknell University and his M.D. degree from Temple University School of Medicine. He received his Residency training in Psychiatry at the University of North Carolina Memorial Hospital and completed Fellowship training in the Robert Wood Johnson Clinical Scholars Program at the University of North Carolina School of Medicine.

**Current Status of the Dexamethasone Suppression Test In Psychiatry**

The dexamethasone suppression test (DST) was developed and used first in endocrinology as a test for Cushing's Syndrome and recently has been used in psychiatry as a putative biological marker of major depression. As a measure of hypothalamic-pituitary-adrenal (HPA) axis activity, the DST has unprecedented use in clinical psychiatry and has sparked considerable interest in the use of laboratory tests in psychiatry. Evidence suggests the DST is a state marker for depression and approximately 50% of major affective disorder patients will have an abnormal test result. We have found an increasing rate of serum cortisol non-suppression following dexamethasone as well as increasing post-dexamethasone serum cortisol concentrations across the range of diagnoses from depressive symptoms, to major depression without melancholia, to major depression with melancholia, through major depression with psychosis. The specificity of the DST for the diagnosis of major depression appears to be sufficiently high to distinguish (with moderate sensitivity) patients with major depression from normal controls as well as from patients with non-depressive disorders such as schizophrenia. Longitudinal studies suggest that the DST may be a useful marker of treatment response and continued non-suppression may be associated with poor clinical outcome and increased risk of relapse. The DST may help identify and lead to earlier treatment of affective disorders in adult and adolescent patients who present with atypical symptoms and positive DST results. In addition, the DST might be useful for the diagnosis of depression in patients with medical illness. A number of factors must be considered for a technically valid DST and as with all laboratory tests the clinician should use the test as an adjunct in the clinical assessment of the patient. The DST has considerable value as a research tool in probing the pathophysiology of affective illness. Further controlled study and use in the clinical setting will determine the ultimate clinical utility of the DST for the assessment and treatment of major affective disorders.

Charles B. Nemeroff, M.D., Ph.D.

Dr. Nemeroff is an Associate Professor of Psychiatry and Pharmacology at the Duke University Medical Center, Durham, North Carolina. He is also a Senior Fellow in the Center for Aging and Human Development and Director of the Laboratory of Psychoneuroendocrinology at Duke University. He received his B.S. degree from the City College of New York (1970), M.S. degree from Northeastern University in Boston (1973), Ph.D. (1976) and M.D. (1981) degrees from the University of North Carolina at Chapel Hill. He is Board certified in Psychiatry.

Dr. Nemeroff has received a number of research awards in the past several years including, the A.E. Bennett Award from the Society for Biological Psychiatry (1979), the Curt F. Richter Award from the International Society of Psychoneuroendocrinology (1985), the Jordi Folch-Pi Award from the American Society for Neurochemistry (1987), the Anna Monika Prize for Research in Endogenous Depression (1987) and the Daniel H. Efron Award from the American College of Neuropsychopharmacology (1987). He has published more than 200 research reports and has edited four books. In addition, he has been invited to speak on more than 150 occasions. Finally, he has been the
recipient of a number of research grants both from the National Institute of Health and the National Institute of Mental Health including a MERIT award in 1987.

Use of Platelet Receptor Binding Studies in Neuropsychiatry

In the past decade considerable research has focused on neurotransmitter function of the central nervous system (CNS) in psychiatric patients. Unfortunately, this is rendered difficult because of our limited access to the brain, and we therefore must utilize indirect measures of CNS activity. It has long been known that platelets, like CNS tissue, are derived embryologically from neural crest, and are classified as components of the Amine-Pre cursor-Uptake-Decarboxylation (APUD) system. Platelets may, therefore, represent a potential window into the brain. Moreover, abnormalities of neurotransmitter receptor binding sites have been demonstrated in the CNS of psychiatric patients. The reported reduction of 3H-imipramine binding sites in the frontal cortex of suicide victims, taken together with the finding that 3H-imipramine labels a binding site in rat brain located in presynaptic serotonergic nerve terminals, has served as an impetus to study platelet 3H-imipramine binding sites in psychiatric patients. It is also important to note that the kinetics of platelet 3H-imipramine binding sites are identical to those found in the brain. In almost all of the studies conducted, including our own, there is a marked reduction in the number (but not affinity) of platelet 3H-imipramine binding sites in drug-free patients with major depression when compared to controls. This finding may help distinguish patients with depression from those with Alzheimer's disease. Other investigators have focused on platelet α₁-adrenergic receptors which appear to be increased in number in the platelets of depressed patients. Whether these platelet abnormalities can predict response to one or another pharmacologic treatment remains unclear. (Supported by NIMH MH-40159.)

Clinton Kilts, Ph.D.

Dr. Kilts is an assistant professor in the Departments of Psychiatry and Pharmacology, and Director of the Clinical Psychopharmacology Laboratory at the Duke University Medical Center in Durham, North Carolina. In this latter capacity, he supervises the performance and development of quantitative assays for psychiatric drugs and the interpretation of assay results. His research interests involve the determination of the mechanism and sites of action of antipsychotic drugs and the role of drug metabolism as a determinant of the pharmacology of antidepressant and antipsychotic drugs. Other interests include the regulation of the hypothalamic-pituitary-adrenal axis and the role of 5-hydroxytryptamine in brain function and psychiatric disorders.

Dr. Kilts received his Ph.D. degree in neuro- psychopharmacology from Michigan State University in 1979. Prior to joining Duke University, he received postgraduate training in neuropharmacology in the Biological Sciences Research Center at the University of North Carolina at Chapel Hill.

Therapeutic Drug Monitoring in Psychiatry: The Clinical Chemist's Perspective

The use of therapeutic drug monitoring strategies as a means of optimizing and individualizing pharmacotherapy has aided greatly a number of fields of medicine. However, with the exception of lithium, such strategies have as yet unrealized potential in the field of psychiatry. The probable reasons for this are numerous and include the subjective and heterogeneous nature of such disorders, the paucity of clinical studies defining the relationship between the pharmacokinetics and pharmacodynamics of psychiatric drugs, and the rigor involved in applying such strategies. Specific challenges to the clinical chemist relating to this application include the relatively low circulating concentrations of antidepressant and antipsychotic drugs encountered following therapeutic dosing and the complex biotransformation typical of such agents. Such patterns of metabolism generate a variety of drug metabolites possessing both pharmacological activity and quantitative significance and thus merit consideration for quantification.

An increasing number of analytical techniques possessing the requisite sensitivity and specificity have become available to the clinical chemist to address such challenges. Such techniques include gas chromatography with mass or nitrogen/phosphorous selective detectors, high performance liquid chromatography with electrochemical or fluorometric detection, and fluorescence immunoassays.

The problems and promise of therapeutic drug monitoring in psychiatry, the comparative merits of different quantitative assay techniques for psychiatric drugs and their metabolites, and the future concerns and needs of the application of these strategies to the practice of psychiatry will be discussed.

Emel B. De Souza, Ph.D.

Dr. De Souza is Chief of the Neuropeptide Unit in the Neuroscience Branch at the Addiction Research Center of the National Institute on Drug Abuse, Baltimore, Maryland. He also holds a joint appointment as an Assistant Professor in the Department of Pathology at Johns Hopkins University School of Medicine. Dr. De Souza's research has focused on the identification, characterization and localization of various neurotransmitter receptors in brain and in the periphery and in examining changes in receptors and receptor-mediated second messengers in neurodegenerative and neuropsychiatric disorders.

He received his B.A. and Ph.D. degrees from the
University of Toronto, Toronto, Canada, where he carried out neuroendocrine research examining the role of endogenous opioids and brain serotonin in the regulation of the hypothalamic-pituitary-adrenal axis. Subsequently, in 1983, he spent 2 years in the Department of Neuroscience at Johns Hopkins University School of Medicine where he carried out extensive work imaging receptors using autoradiographic techniques.

**Imaging Brain Receptors by Use of Novel Radioligands and Positron Emission Tomography**

The advent of radiolabeled ligands for directly labeling receptor binding sites has revolutionized our understanding of the biochemical aspects and heterogeneity of receptors. In parallel, major advances have been made in understanding the interactions between receptors and their associated second messengers, in characterizing the molecular composition of the receptors, and in the development of anatomical techniques to visualize receptors. The various tools available to image receptors will be described in detail and critically evaluated. The emphasis of the presentation will be on autoradiographic techniques for receptor localization which allow the characterization and high-resolution anatomical mapping of receptors.

The fundamental advantages of microscopic receptor mapping are increased anatomical resolution and greatly increased sensitivity of measurement when compared to traditional "biochemical" approaches for measuring receptor density in homogenates. Receptor maps provide powerful insights into the mechanisms of drug action by identifying those brain regions which have the receptors and, therefore, those areas which are affected by drug administration. Receptor mapping is also a valuable compliment to neurotransmitter mapping. A major use of receptor maps involves identifying changes in receptors in postmortem human tissue in various disease states. Recent developments have utilized positron-emitting ligands to image and quantify brain receptor distributions in normal living humans using emission tomographic techniques and have examined receptors changes in aging and disease. Future directions including the use of cDNA and mRNA probes and antibodies to image receptors will also be discussed. A combination of receptor studies in human autopsy tissues, PET scanning studies in living patients and studies in animal models of disease should help elucidate the role of receptors in both physiological and pathological states.

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**Wednesday Morning, July 27**

**Session A—Emerging Technology: Visions to Reality Via Basic Research**

**Harry L. Pardee, Ph.D.**

**Chairman**

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**John Savory, Ph.D.**

Dr. Savory is Director of Clinical Chemistry and Toxicology Laboratories, and Professor of Pathology and Biochemistry at the University of Virginia, Charlottesville. He received his B.Sc. and Ph.D. degrees from Durham University in England followed by two years of post-doctoral training in clinical chemistry at the University of Washington. Prior to assuming his present position, he held faculty positions and directed the clinical chemistry laboratories at the University of Florida and then at the University of North Carolina at Chapel Hill.

His major research interests are in aluminum neurotoxicity, proton biodemodays by GC/MS, robotics and applications of nucleic acid probes in diagnostic virology.

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**Applications of Emerging Technologies to Clinical Chemistry**

Clinical chemistry plays an important role in taking new analytical techniques from research analytical chemistry and biochemistry laboratories and incorporating them into routine clinical service.

One of the most powerful techniques in analytical chemistry is that of mass spectrometry. For the past decade a few clinical chemistry laboratories have been equipped with this instrumentation mainly for confirming the occasional drug overdose, or evaluating inborn errors of metabolism. However, the recent upsurge in drug testing has made it essential that all of the larger toxicology laboratories possess mass spectrometry capabilities. The analysis of biological specimens, particularly urine, for trace constituents is a considerable analytical challenge. One means of overcoming interferences present in such specimens is to use either high resolution mass spectrometry or tandem mass spectrometry. The initial costs of such equipment are higher than conventional mass spectrometers, but there are distinct advantages in specificity, sensitivity, and throughput. Some applications of these analytical systems are in drug confirmations, anabolic steroids and lipid measurements.

Another area of technology which undoubtedly will play an increasingly important role in clinical chemistry is that of robotics. The most obvious applications are in specimen processing and in performing simple repetitive tests. This latter area is of considerable interest and has led to the development of a fully automated critical care laboratory which can be situated in operating room and intensive care areas. This type of laboratory can perform whole blood analyses for blood gases, electrolytes and hemoglobin without any on-site clinical chemistry personnel. One important component of the system, however, is a clinical chemistry station (satellite central) where monitoring of all of the activities of the automated units is carried out by qualified clinical chemistry personnel.

A review of applications to clinical chemistry of these new techniques will be presented.
Electrochemical Enzyme Immunoassay

The selectivity of an antigen/antibody reaction combined with the chemical amplification feature of an enzyme label enables high specificity and low detection levels to be achieved by enzyme immunoassays. Electrochemical enzyme immunoassays are based on antigen labeled with an enzyme that catalyzes the production of an electroactive product. Hydrodynamic electrochemical techniques such as liquid chromatography and flow injection analysis with electrochemical detection have proved to be very effective for the rapid determination of the enzyme-generated product in enzyme immunoassays. Heterogeneous enzyme immunoassays in which antibody is adsorbed on the walls of polystyrene cuvettes or glass microcapillary hemocrit tubes have been developed. Alkaline phosphatase, which catalyzes the conversion of electroaductive phenolphosphate to electroactive phenol, is the enzyme label. The rate of phenol production is measured by either liquid chromatography/electrochemistry or flow injection analysis/electrochemistry with a thin-layer carbon paste electrode at +0.87V vs. the Ag/AgCl reference electrode. One example is an assay for digoxin, a cardiac glycoside used to treat chronic heart disease. The assay has a detection limit of 50 pg/mL and exhibits good correlation with radioimmunoassay on patient serum samples over the therapeutic range of 0.8-2.0 ng/mL. A second example is a sandwich assay for IgG in which a detection limit of $4 \times 10^{-3}$ attomoles has been achieved.
Novel Approaches to Kinetic and Spectrophotometric Determinations

This talk will focus on two aspects of our work related to new instrumental developments for clinical biochemistry, namely the use of imaging detectors for multiwavelength spectroscopy and nonlinear data-processing methods for kinetic determinations. Nonlinear data-processing methods applied to kinetic data offer some significant advantages including the reduction of errors by as many as two orders of magnitude, extension of the linear range for enzyme substrates, and resolution of mixtures such as the isoenzymes of creatine kinase (CK-MM and CK-MB) based on immunochemical reactions. Specific examples including determinations of glucose, uric acid, immunoglobulins, and the CK isoenzymes will be discussed to illustrate these and other characteristics of the proposed methods.

An imaging detector is a device that permits one to monitor many different wavelengths simultaneously, and to scan optical spectra rapidly if desired. Using multiwavelength data, it is possible to achieve several advantages including compensation for background (blank) absorbances, and resolution of multicomponent mixtures (e.g., hemoglobins, bilirubin, drugs, etc.). Examples involving ordinary absorbance as well as first- and second-derivative spectra will be discussed.

Session B—Quantitative Tissue Analysis in Clinical Chemistry

Morton K. Schwartz, Ph.D.
Chairman

Dr. Schwartz, is Chairman, Department of Clinical Chemistry; Head, Laboratory of Applied and Diagnostic Biochemistry at Memorial Sloan-Kettering Cancer Center; Professor, Sloan Kettering Division, Cornell University Graduate School of Medical Sciences. He received his B.A. degree from Lehigh University and his Ph.D. in Biochemistry from Boston University. He is Past Chairman of the Professional Affairs Commission of AACC and of the IFCC Committee on Education and the Commission on Education of the Clinical Chemistry Division of the IUPAC. Dr. Schwartz has been President of the AACC, President of the NRC, Chairman of the FDA Clinical Chemistry and Hematology panel, Member of the Board of Registry of the ASCP, member of the Evaluation Panel of the Analytical Chemistry Division of the National Bureau of Standards, Chairman of the 1973 and 1983 AACC Annual meetings and Chairman of the New York Metropolitan Section as well as Chairman of the AACC Education Committee. Dr. Schwartz has received the Van Slyke Award, the AACC Award for Efforts in Education and Training, the Certificate of Merit of the New Jersey Section AACC and the Wiley Medal and Citation of the FDA. Dr. Schwartz is author and co-author of more than 250 articles and of a series of books in Clinical Biochemical Analysis. He is an editor of the Journals: Cancer Investigation, Analytical Letters, Tumor Biology, Clinica Chimica Acta and the Journal of International Biological Markers. He has been an editor of Clinical Chemistry, Advances in Clinical Chemistry, Annals of Clinical and Laboratory Science, Clinical Laboratory Automation and Preventive Medicine.

The Role of Tissue Analysis in the Management of the Cancer Patient

For decades the "gold-standard" for tissue identification and the determination of site of origin of metastatic disease has been histopathology. In recent years this technology has been refined by the use of electronmicroscopy and immunohistochemistry. These techniques have all been qualitative. Quantitative assays of analytes in tissue specimens are only now being exploited in the management of cancer patients and are slowly becoming routine techniques in clinical laboratories. In this review there will be considered quantitative procedures which are now in use in clinical laboratories or will be introduced in the near future. Androgen, estrogen and progesterone hormone receptors are essential in the development of a treatment plan for patients with breast cancer. Similar approaches are under consideration for clinical use in...
prostate or endometrial cancer. Cultured tissue cells and uptake of tagged constituents or enzyme assays are now being used to determine the sensitivity of chemotherapeutic agents. It will be an obvious advantage if the clinician can be told which drug will be most effective for first use. Enzyme measurements in tissue specimens have also been used to indicate prognosis and to predict response to therapy. There are indications that tissue enzymes, hormone receptors and tumor marker assays may complement each other in evaluating prognosis and predicting therapeutic response. An area of promise in the management of cancer is measurement of oncogenes. This technology will permit identification of individuals at high risk for cancer. The identification of individuals who are at high risk because of environmental or genetic factors is a high priority requirement if cancer is to be controlled. The clinical laboratory will play an important role in this effort.

Gregory A. Grabowski, M.D.

Dr. Grabowski received his B.A. degree in mathematics and his M.D. from the University of Minnesota (Minneapolis, MN). After his internship and residency in Pediatrics, he did a post-doctoral fellowship in Medical Genetics at the University of Minnesota Hospitals. He then joined the faculty at the Mount Sinai School of Medicine in 1979 as an Assistant Professor of Pediatrics and Genetics. Dr. Grabowski is an Associate Professor of Pediatrics and Genetics in the Department of Pediatrics at Mount Sinai School of Medicine. His current positions include: Director of the Comprehensive Gaucher Disease Clinic, Co-Director of the Mount Sinai Center for Jewish Genetic Diseases, Co-Director of the Tay Sachs Disease Screening Program and Director of the Biochemical Genetics Diagnostic Laboratory. His research has focused at the biochemical and molecular basis of Gaucher disease and at the development of recombinant DNA methods for improved diagnosis of genetic disease.

Molecular Analysis of Tissues

Recent advances in recombinant DNA technology and "reverse genetics" portend the development of specific markers for genetic traits and tissues. Already, for diseases in which the genetic defects are known recombinant DNA methods have facilitated improved diagnostic testing in at-risk populations (e.g. sickle cell anemia, hemophilia A and B). For such disorders, which are single gene traits, application of differential oligonucleotide hybridization to normal and abnormal DNA sequences permits the specific identification of affected individuals as well as carriers of the disease gene. In those diseases in which the specific defect has not been defined at the protein level, the principals of "reverse genetics" are defining the nature of the DNA mutations as well as their defective proteins. For example, in cystic fibrosis and Duchenne Muscular Dystrophy, the protein defect is not known. However, the genes responsible for the diseases have been mapped to specific chromosomal segments and the tissue specific proteins will soon be identified from the DNA sequences. These proteins as well as other tissue specific proteins can serve for disease specific markers or potentially for monitoring tissue damage. An additional advance in molecular methods has been the use of a thermostable DNA polymerase to amplify specific genomic DNA sequences for direct sequencing, i.e. the polymerase chain reaction (PCR). This technique can and will permit the direct identification of mutations or polymorphisms for diagnostic purposes without the need for laborious cloning procedures. The application of these techniques will be discussed in the framework of direct clinical use of diagnostic tests and, potentially, for the analysis of tissue specific damage.

Alan Waggoner, Ph.D.

Dr. Waggoner is Associate Professor of Biological Sciences and a member of the Center for Fluorescence Research in the Biomedical Sciences, Carnegie-Mellon University, in Pittsburgh. His research is directed at the development, integration, and application of fluorescence detection systems for basic biological research and medicine. Major effort is devoted to the design, synthesis, characterization and application of fluorescent probes. Early work centered on the development of membrane potential sensing probes and on the determination of the mechanism by which these dye molecules respond to changing electric fields within membranes. More recently the laboratory has concentrated on the design of dyes that fluoresce brightly at long wavelength regions of the spectrum and can be used to label proteins and nucleic acids.

Dr. Waggoner received his PhD from the University of Oregon and was an NIH Postdoctoral Fellow at Yale University. After 11 years in the Chemistry Department at Amherst College, he joined Carnegie Mellon in 1982.

Fluorescent Probes for Flow and Image Cytometry-Multiprobe Analysis

There is a revolution occurring in the capability to detect and quantify molecular and physiological properties of living cells and tissues. There are two essential and integrated parts of the revolution: fluorescent probe

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Reagents and fluorescence detection instruments. Reagents include fluorescent antibodies, RNA and DNA hybridization probes, functional biomolecular analogs, and physiological indicators, such as pH, calcium, membrane potential, and enzyme activity probes. These probes, when combined with flow cytometers and modern microscope imaging workstations, provide powerful tools for dissecting the composition, 3-dimensional structure, and function of biological specimens.

The classes of existing fluorescent probes and their applications will be briefly reviewed. Recent advances in fluorescent labeling reagents will be discussed. As a result of the development of new fluorescent tags and other probes that fluoresce in different regions of the spectrum, the simultaneous and continuous measurement of more than 4 structural and functional properties of single living cells is now a reality. The signal from each probe can be selectively detected by using appropriate combinations of optical filters.

**David Cowburn, Ph.D., D.Sc.**

Dr. Cowburn is associate professor at The Rockefeller University, New York. He is also on the adjunct faculty of New York Hospital/Cornell Medical College, and consultant at Memorial Sloan-Kettering Cancer Center. He is actively involved in developing new applications of magnetic resonance biological research. He is especially experienced in stable isotope labeling for MR uses, and the application of indirect detection methods.

He received a B. Sc. degree from the University of Manchester Institute of Science and Technology, and Ph. D. and D. Sc. degrees from the University of London (England). He carried out postdoctoral studies at the College of Physicians and Surgeons of Columbia University.

**Non-Invasive Tissue Studies Using Magnetic Resonance**

Magnetic Resonance techniques can be used for studies of chemical composition, by magnetic resonance spectroscopy (MRS), or for determination of spatial organization by magnetic resonance imaging (MRI), or, to a limited extent, for the combination of both spectroscopy and imaging in localized spectroscopy. For clinical chemistry purposes, the positive features of MR techniques, specific chemical information, and quantitation, must be weighed against the relative insensitivity of MR detection, and the problem of 'hidden' signals, broadened by association with higher molecular weight materials. The nondestructive nature of MR methods is particularly important to time-dependent studies. Phosphorous MRS can be used to assess intracellular pH, concentrations of phosphates associated with energy metabolism, and regulation of glycolysis. Fluorine MR can be used to identify and measure levels of fluorinated compounds, e.g. 5-fluorouracil, and metabolic products. 13C NMR can be employed to study the fate of 13C labeled precursors. In tissues, proton NMR is limited by technical considerations arising from the intense signal from water, reducing the spectral range which may be usefully examined. Various tests have been proposed involving markers in plasma, possibly for neoplasms, and the basis of these tests and their range of applicability are controversial.

The installation and routine operation of MR equipment is costly, and special attention to type and intensity of uses is desirable.

**Thursday, Morning, July 28**

**Session A—Thrombosis and Coagulation in Laboratory Medicine**

**Philip C. Comp, M.D., Ph.D.**

Chairman

**Robert B. Francis, Jr., M.D.**

Dr. Francis is Assistant Professor of Medicine in the Division of Hematology at the University of Southern California School of Medicine in Los Angeles, and is Director of the Coagulation Laboratory at the Los Angeles County-University of Southern California Medical Center. He received his B.S. degree from Harvard University, and his M.D. degree from Temple University. He completed his internship and residency in Internal Medicine at St. Luke’s-Roosevelt Medical Center in New York City, and his fellowship training in Hematology and Oncology at U.S.C. His research interests are in abnormalities of the protein C anticoagulant pathway and of the fibrinolytic system relevant to the risk of hemorrhage and thrombosis.

**Laboratory Management of Fibrinolytic Therapy**

Safe, effective use of fibrinolytic therapy requires proper patient selection, adequate documentation of thrombolysis, and laboratory monitoring of the thrombolytic state. Of these, proper patient selection is the most important. Subjects should have objective documentation of thrombotic disease for which throm-
bolytic therapy is likely to be beneficial, and no
contraindications to thrombolytic therapy. Invasive
procedures which create potential bleeding sites
should be minimized. Objective documentation of the
extent of thrombolysis achieved is essential to
ascertain effective and safe treatment. Laboratory monitoring of fibrinolytic ther-
apy has two goals: to document that increased fibrin-
olytic activity has been achieved, and to detect per-
turbations of normal hemostatic mechanisms which
would increase the risk of bleeding. The first goal
is most important in subjects receiving streptokinase,
in whom inhibitory antibodies may be present, and is
most easily accomplished using the dilute whole blood
clot lysis time assay. To monitor the effect of fibrinolytic therapy on normal hemostasis, the
activated partial thromboplastin time and clotting
fibrinogen level are most useful. Replacement therapy
with cryoprecipitate should considered in subjects
with marked depletion of fibrinogen. Possible
potentiation of the risk of bleeding by concomitant
heparin therapy should be kept in mind. It is
important to remember that thrombolytic agents cannot
distinguish between "good" and "bad" fibrin, and that
a certain irreducible minimum risk of catastrophic
bleeding is probably attendant on therapy with any
of the currently available fibrinolytic agents.

Jeffrey I. Weitz, M.D.

Dr. Weitz is associate pro-
fessor of medicine in the
Hematology Division at
McMaster University Medical
Centre, Ontario, Canada. He
is a member of the McMaster
University Thromboembolisim
Program and directs a special
research coagulation labora-
tory at the Henderson General
Hospital. Dr. Weitz contrib-
utes to many committees
working in the area of
thrombosis and hemostasis and has a special interest
in fibrinolytic and anticoagulant

He received the M.D. degree from the University
out internship and residency programs at the Toronto
General Hospital. After completing his research
training at Columbia University, New York, in 1983,
he joined the Faculty of Columbia University College
of Physicians and Surgeons. In 1986, Dr. Weitz
assumed his position at McMaster University.

William Keith Hoots, M.D.

Dr. Hoots is associate
pediatrician and associate
professor in the Division of
Pediatrics at the University
of Texas System Cancer Center M. D. Ande-
son Hospital and Tumor In-
stitute, Houston, Texas. He
also holds the following
positions: associate professor of pediatrics
Department of Pediatrics,
associate professor of pathology, Department of Internal
Medicine, The University of Texas Medical School at
Houston; medical director and principal investiga-
gator, Gulf States Hemophilia Diagnostic and Treat-
ment Center, The University of Texas Health Science
Center at Houston. Dr. Hoots contributes to many
committees working in the areas of pediatric hema-
tology and AIDS. He is also a Charter Member of the
Special AIDS Review Study Section NIAID of the NIH,

He received the A.B. and M.D. degrees, respec-
tively from the University of North Carolina,
Chapel Hill, North Carolina in 1975. Between 1975
and 1980 he carried out internship and residency
programs at the Parkland Memorial Hospital, Dallas,
Texas and fellowship program at the University of
North Carolina, School of Medicine Chapel Hill,
North Carolina.

Present research interests include pathogenesis of
disseminated intravascular coagulation following
head trauma, viral risk factors and natural history
among hemophila patients exposed long term to
clotting factor concentrates (particularly hepatitis and HIV infection) and inter-relationship
between endothelial injury and the vasculitis of
Kawasaki's disease. Recent work has also included
clinical investigations of bone marrow growth fac-
tors such as colony stimulating factor granulocytes
and macrophages (CSF-GM).

The Clinical Utility of Monitoring Intravascular Clotting and
Fibrinolytic Activities

The balance between thrombin and plasmin action on
fibrinogen has been postulated to be an important
determinant of thrombosis. This balance can be asses-
sed by measuring the products of proteolysis. Throm-
bin acts on fibrinogen in a two-step fashion. The
first step releases fibrinopeptide A (FPA) from the
NH2-terminal region of the Aα-chain producing an
intermediate known as fibrin I, while the second step
involves cleavage at the NH-terminal of the Bα-chain,
releasing fibrinopeptide B (B81-14 or FPB) and pro-
ducing fibrin II. In the initial digestion of fibrin-
ogen by plasmin, large segments of the COOH-terminal
Aα-chain and B81-42 from the NH -terminal of the Bα-
chain, are cleaved and fragment2X is formed. In con-
trast, plasmin attack on fibrin II, from which FPB
has already been released, results in cleavage of
B81-42 during fragment X formation.

Specific radioimmunoassays for the products of
these reactions allow quantitative analysis of in
vivo thrombin and plasmin action on fibrinogen. Thus,
plasma FPA and FPB levels reflect thrombin-mediated
fibrin I and fibrin II formation, respectively, where-
as B81-42 reflects plasmin action on fibrinogen or
fibrin I, and B81-42 reflects plasmin action on fib-
rin II. Tests of in vivo enzyme activity are useful
in the diagnosis and management of thrombotic diseases
and disseminated intravascular coagulation. Further,
B81-42 and B81-42 levels provide a sensitive index
of the relative fibrinolytic and fibrinogenolytic
effects of thrombolytic therapy thereby facilitating
the development of optimal dosage regimens for the
newer clot selective lytic agents. Finally, plasma
FPA levels can be used to determine the optimal inten-
sity of anticoagulation in patients with recurrent
thrombotic disease.

In summary, quantification of the products of
thrombin and plasmin action on fibrinogen provides
a powerful tool for testing the hypothesis that the
balance between these two enzymes is an important
determinant of thrombosis. More widespread clinical
use of these tests however, will depend on the
development of faster assay techniques.

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Hemophilia for Clinical Pathologists—Laboratory and Clinical Implications

Laboratory diagnosis of either hemophilia A or B traditionally has relied on the functional clotting assay for either (F.VIII) or F.IX that relies on plasma deficient in the specific factor for substrate analysis. Despite sequencing of the F.VIII and F.IX genes (thereby making possibly large quantities of pure F.VIII or F.IX protein), the clotting assay system remains the gold standard at the present time. However, differentiation between functional and antigenic F.VIII can be made by comparing results between F.VIII coagulant antigenic assay and the F.VIII level by clotting assay. The former utilizes either a purified anti-F.VIII antibody derived from a patient with a high titer F.VIII inhibitor or more recently monoclonal antibody against known epitopes on a recombinant purified molecule. In addition, cloning of the F.VIII gene has significantly improved the availability and decreased the fetal morbidity of antenatal diagnosis of hemophilia A. This is now routinely available along with carrier testing and utilizes genetic pedigree comparisons of restriction enzyme fragment length polymorphisms in fetal cells of male infants at risk obtained from chorionic villi. Similar applications for hemophilia B are in the developmental stage. The DNA recombinant technology also has allowed for production of purified, gene derived F.VIII for replacement therapy of hemophilic patients. Clinical trials are underway with this product.

Prior to the development of this gene produced F.VIII and presently available monoclonal therapy of purified F.VIII preparations, hemophilia A and B was significantly complicated by the presence of viral contaminants in plasma-derived products. HIV and the hepatitis group of viruses have been particularly problematic. The management of HIV infection, AIDS complications and chronic hepatitis now, more than ever, require a collaboration between the clinician and the broad expertise of the clinician pathologist. Management of the approximately 70% of hemophilia patients unfortunate enough to have become virally infected with HIV, hepatitis or both requires expertise in clinical chemistry, microbiology/virology, serology, immunology, and hematology, since the coagulopathy now represents only a portion of the morbidity disease in such patients.

As implied above, recombinant and other technology has essentially removed the morbidity infectious risk for the newest generation of hemophiliacs. In the short term this is creating a therapeutic dilemma for hemophilia treaters because of significant cost increase and decreased availability of F.VIII concentrate preparations as manufacturers switch to the new technology. This has given impetus to efforts at reducing over-treatment of individual patients. Careful laboratory assessment of each hemophilia patient utilizing Factor infusion survival (half-life) studies thereby have become more essential in day to day management of hemophilia.

Phillip C. Comp, M.D., Ph.D.

Dr. Comp is an Associate Professor of Medicine at the University of Oklahoma at Oklahoma and he directs the Thrombosis and Coagulation Laboratory at Oklahoma Memorial Hospital and the Adult Division of the Oklahoma Hemophilia Treatment Program. Dr. Comp's research interests center on the natural anticoagulant proteins and the influence of inflammation on the regulation of coagulation.

He received his B.A. from Reed College, Portland, Oregon and his M.D. from the University of Washington, Seattle. Dr. Comp holds a Ph.D. in Biochemistry from the University of Oklahoma.

The Natural Anticoagulants: Protein C and Protein S

Protein C and protein S are vitamin K-dependent plasma proteins which regulate clotting. Unlike the vitamin K-dependent clotting factors, these proteins stop clots from forming and help dissolve clots which are already formed. Patients who are heterozygous deficient in either of these proteins are at risk of recurrent venous thrombosis. Patients who are partially deficient in protein C are at risk of tissue necrosis when the oral anticoagulant warfarin is started. Homozygous protein C deficient newborns are subject to purpura fulminans neonatalis, which is characterized by necrosis and thrombosis in the skin.

Acquired deficiencies of protein S occur during pregnancy and during oral contraceptive use. This decrease in protein S may predispose to the thromboembolic complications which may occur in these conditions.

Laboratory screening of patients with a personal or family history of recurrent thrombosis should be carried out. Protein C, protein S, antithrombin III, plasminogen, fibrinogen and lupus anticoagulant should be measured in these patients. At present, one of these tests will be abnormal in approximately 20% of patients with a family history of thrombosis or a personal history of repeated episodes of venous thromboembolic disease.

Session B—Health Care Delivery in the 1990s

David E. Bruns, M.D.
Chairman

David E. Bruns, M.D.

Dr. Bruns serves as Associate Professor of Pathology and Associate Director of Clinical Chemistry and Toxicology at the University of Virginia, Charlottesville, VA. Dr. Bruns received the B.S. in Chemical Engineering and A.B. at Washington University, St. Louis, and his M.D. at St. Louis Univers-

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sity. His residency and fellowship training were taken at Washington University. For the past 15 years, Dr. Bruns' major research interests have been in the areas of clinical enzymology and cellular calcium metabolism. His work has been supported by NIH, the American Cancer Society and the National Dairy Council. Dr. Bruns has been active in a variety of AACC committees. He was Workshops Chairman for the 1984 National Meeting and serves on the ENDO Committee. He has been a member of the Editorial Board of Clinical Chemistry since 1983 and a member of its Executive Committee since 1985. In 1987, he received the AACC's Award for Outstanding Contributions to Research in a Selected Area (Roche Award) and the Sunderman Award of the Association of Clinical Scientists. Dr. Bruns helped to organize the symposium on Health Care Delivery in the 1990's in his capacity as chairman of the AACC's new Laboratory Utilization Committee.

The Impact of Technological Changes on Health Care Delivery

The development of new technologies has shaped the practice of medicine in the United States. In clinical chemistry, the introduction of biochemical testing and automated methods has influenced patient care in unforeseen ways and in each branch of medicine. We are now entering an era in which resources may increase or decrease. A key and central issue for medicine, and for clinical chemistry, is the trade-off between cost and quality. Clinical chemists have faced this issue repeatedly in the past. In the new era, clinical chemists will be placed, more than ever before, at the nexus of technological advances and costs. Physicians will demand more sophisticated testing, while multiple groups demand decreasing medical costs. Laboratorians will be buffeted by pressures from both sides. In addition, the striking, rapid advances from the field of molecular biology will continue to offer startling opportunities in the clinical laboratory. In only some cases will new technology decrease costs. The nature of the new technologies will require innovative approaches to the organization of medical laboratories. The implications of these forces for Clinical Chemistry and for Clinical Chemistry will be discussed by three panelists who bring expertise in the areas of Laboratory Medicine, Hospital Administration and Health Policy.

Donald S. Young, M.B., Ph.D.

Dr. Donald Young is Professor of Pathology and Laboratory Medicine at the University of Pennsylvania and Head of the Division of Laboratory Medicine of the Department of Pathology and Laboratory Medicine. He is also Director of the William Pepper Laboratory of the Hospital of the University of Pennsylvania. Previously, he was Head of the Section of Clinical Chemistry at the Mayo Clinic and before that Chief, Clinical Chemistry Service, National Institutes of Health.

He has been President of the American Association for Clinical Chemistry and Chairman of the Board of Editors of Clinical Chemistry. He is the current President of the International Federation of Clinical Chemistry.

Transformation of the Clinical Laboratory: The Nineties and Beyond

Although more clinical laboratory tests are performed each year, there is little evidence of an improvement in patient care, reduced hospital stay or better quality of life because of the added testing. This suggests that much of the testing may be inappropriate, possibly because the tests used are not adequate for their application. Thus, few tests to diagnose malignant disease have the capability of detecting all advanced tumors and none perform well in the early stages of the disease when it is most treatable. Liver function tests, as used now, are tests of organ damage rather than tests of the liver's metabolic capabilities. We do not currently have tests to predict the likelihood of an individual disease responding to treatment, nor to select the optimal treatment. We also lack adequate tests to identify the likelihood of a particular individual developing specific diseases. The next decade will provide many opportunities to develop better tests.

Some of our present failings probably result from preoccupation with the analysis of body fluids rather than the study of the cells from diseased organs. Currently, image analyzing systems have the potential to biochemically evaluate biopsy specimens but this application has still to be exploited. The development of such techniques will give the clinical chemist an important interpretive and consultative role, which will be reinforced by the use of various in vivo analyses for which the clinical chemist should assume responsibility. The expanded decentralized testing will require a greater involvement of the clinical chemist in hospital practice.

As newer diagnostic modalities such as those involving DNA probes gain acceptance, it will be necessary to develop a technological orientation to laboratory testing, as is now being done by several companies as they incorporate tests from different laboratories, e.g. chemistry, coagulation and toxicology, onto a single analyzer. The future clinical laboratory would have groups of technical staff expert in a specific technology reporting at the same time to the clinical chemist, hematologist and toxicologist. Yet the increasing shortage of skilled technologists may mean that individuals with different skill levels will have to be employed in the laboratory. Thus the training of the future technologist and clinical chemist will have to be very different from now.
Medical Technology in Hospitals: Is the Cost Greater Than the Revenue Under Prospective Payment Systems?

Investment in new technology has been the hallmark of successful hospitals and medical centers over the past four decades. Under cost reimbursement, any investment in new technology was assured of reimbursement from various third party payors who recognized those costs as part of the reimbursememt system. A hospital making an investment in technology was assured of the compensation for the cost incurred, as well as the benefit from having introduced that new technology in serving patients and conducting educational and research programs.

The fundamental shift in philosophy of compensation for healthcare services of the past five years has modified the incentives for technology investment. Institutions now must balance the benefit and cost of investing in new technology and the ability of their institution to attract their existing and new patient referrals because of the availability of technology. The ability to attract and retain a high quality medical staff and other clinical staff is highly dependent on the quality of technology available in the hospital setting. Loyalty of staff and loyalty of patients using hospitals is crucial in maintaining a stream of revenue to support the cost of technology, as well as all other costs within a hospital setting.

The change in reimbursement no longer assures compensation for the introduction and application of technology in a hospital setting. In those instances where the new technology improves the attractiveness of the hospital for patients, and reduces the cost of providing a service, the decision to make an investment is quite easy. When the new technology helps attract patients, but increases the cost of providing services, perhaps even to the extent of making costs greater than the revenue, the decision is more difficult. When the application of new technology has no direct influence on attracting the patients, but assures the continued participation by faculty and results in higher cost of care, the decision is most difficult.

Every hospital must address the issue of introducing and applying new technology in the context of its mission. Those hospitals that are focused on primary care and dependent on a revenue stream only from patient care activities, may have little choice but to be highly selective in the choice of new technology to be utilized. Those institutions in a highly competitive market where their patient volumes are dependent on their identity as a center of excellence may have difficult decisions, but may be obliged to invest in the latest proven technology that is developed. Those hospitals which have a mission involving more than patient care and includes teaching, research and the development of new technology, may have no choice but to continue to make their investment in applications of new technology. Those institutions must become far more effective in assessing the potential cost and benefit of each new technology and develop systems involving scientists, technologists, as well as marketing staff and other administrative and financial staff, to make crucial decisions about which technology investment should be made.

This paper describes how such decisions can be made and the type of analysis which can be conducted which will permit institutions to make these decisions with relative security.
a national precedent. His research and writing address health care systems, strategic planning and health policy.

Health Care Delivery in the 1990s

Sovereign Profession
During the '50s and '60s the essentials of the practice of medicine were free choice of physician, fee-for-service, and solo practice. Physicians served on voluntary staffs of community hospitals funded by cost-based reimbursement. Medicare poured hundreds of billions of dollars into this system, undisciplined by the market or the public sector. Costs escalation challenged governmental and third-party financing.

Rise of a Vast Industry
The health care industry is challenged by
(1) Competition
(2) Corporate Restructuring
(3) Metamorphosis of a Social Service to a Market Good
(4) Search for Productivity in a Labor Intensive Industry
(5) Metamorphosis of a Social Service to a Market Good
(6) Search for Productivity in a Labor Intensive Industry

Retrospective reimbursement of costs are shifting to prospective pricing with Diagnostic Related Groups (DRGs). This combined with HMOs, PPOs, PROs, multi-institutional systems, and joint venture are precipitating profound, provocative, and pervasive change in our way of thinking about health care.

Health Care Megatrends Towards the 21st Century
Strategic planning and environmental forecasting is critical to all institutions. The following "Megatrends" seem inevitable.
(1) The Corporate Practice of Medicine
(2) From DRGs to Weighted Capitation
(3) The Emergence of Closed Budgets
(4) Challenge of Side Effects of Scientific Discovery
(5) The Transition of Patients to Constituents
The implications of these potential developments are of high priority for my students since they will spend the vast majority of their professional careers in the 21st century, until a median year for retirement of 2030.
Abstracts of Meeting Papers

Ed. Note: These abstracts have been reproduced directly, without editorial alteration, from the material supplied by the authors. Infelicities of preparation, grammar, spelling, style, syntax, and usage are the authors’.

Organizing Committee Note: All papers will be posted for 2 hours. The presenting author will be in attendance during the second hour.

Tuesday Afternoon—July 26

Poster Sessions 1:00pm—3:00pm

IMMUNOASSAYS

Advances in Immunoassay

001 EVALUATION OF FLUOROMETRIC ENZYME IMMUNOASSAY FOR HBsAg

FERRITIN: David C. Jirinzu, Capt., USAF, BSC, SSgt Rhonda Scarbrough, Sg t John Williams. (Clinical Chemistry Section, Epidemiology Division, USAF School of Aerospace Medicine, Human Systems Division (AFSC), Brooks Air Force Base, Texas 78235-5301.)

Ferritin, one of the analytes monitored for the diagnosis of anemic states, has routinely been measured by radioimmunoassay (RIA). A new method, fluorometric enzyme immunoassay (FEI) using double antibody sandwich technique, rabbit anti-ferritin preimmobilized on glass fiber paper, clinical sample, and conjugate containing enzyme labeled rabbit-anti-ferritin IgG (American Dade Stratus), was evaluated. Pooled sera and tri-level control sera were used for the initial evaluation after the calibration of the Stratus Fluorometric Analyzer that was used in the studies. Serum samples were collected and their ferritin levels measured and compared to a commercial RIA reference method.

The standard curves were linear up to 300 ng/mL and dilution studies showed good linearity. The within-run CV for tri-level control sera were 7.05%, sensitivity limit of 0.5 ng/mL was established, and the assay over between high and low control sera was 0.238; recoveries for ferritin averaged 113%.

Ferritin levels from 60 patient specimens (sera) were determined by FEI and compared to RIA, the reference method. The correlation coefficient was 0.999, slope 1.11. Triglyceride levels up to 2100 mg/dL did not interfere.

In summary, FEI is sensitive and specific, with excellent performance and an easy-to-follow protocol. The automation of the Stratus offers speed and accuracy as opposed to the labor intensive RIA procedures.

002 DETECTION OF HEPATITIS B VIRUS SURFACE ANTIGEN USING MEMBRANE TECHNOLOGY. Rrysman Loor.

S.T. Siebert, J. Roberts and James P. Lee (Lecco Diagnostic, Southfield, MI 48075) (Spon.: R. Loor)

We have developed a diagnostic test for the detection of Hepatitis B Virus Surface Antigen (HBsAg) in human serum or plasma using membrane technology, which can be performed in hospital and physician's office to promote the disease treatment and monitoring. The HBsAg test employs the principle of enzyme immunosay. The monoclonal antibody immobilized on membrane binds HBsAg from specimen and the antigen subsequently reacts with polyclonal antibody conjugated to enzyme. The enzyme reaction product is proportional to antigen concentration. The assay procedure only consists of an incubation of 30 minutes at RT. The assay end result reads in a simple format, i.e. + + + for the positive and - - - for the negative. In the assay, a scavenger antibody was specifically selected and built in the system to eliminate any cross-reactivity or false positive result.

As demonstrated by the inter and intra assay, and the recovery study, the Lecco HBsAg test is precise and accurate. For the assessment of assay sensitivity, a FDA reference panel (#01 to 513) including negative, weak positive and strong positive for both ad and ay

serotypes were tested. The result indicates that this assay sensitivity sufficiently meets the FDA requirements for the "third generation" HBsAg test. A total of 1045 specimens containing antigen or positive HBsAg was also evaluated to determine the diagnostic sensitivity and specificity. The data gave a correct identification as compared to those assayed by another FDA licensed test kit, and a diagnostic sensitivity of 99% and a specificity of 99.7%.

In summary, a simple and fast assay for the detection of HBsAg in human serum or plasma has been developed using membrane technology. The assay is accurate and precise. It shows sufficient sensitivity on the FDA reference panel and provides correct identification of HBsAg in a total of 1045 specimens.

003 Development of an Enzyme Immunoassay (EIA) for SQ 27,519, the Active Phosphonic-Carboxylic Acid Diacid of the Prodrug Fosinopril, in Human Serum: A Comparison with the SQ 27,519 RIA. E. Siebert, R. M. Ray, DiPietro, D. T. Siebert, D. T. Si... (The Squibb Institute for Medical Research, New Brunswick, NJ 08903) (Spon.: R. M. Ray) (The Squibb Institute for Medical Research, New Brunswick, NJ 08903) (Spon.: R. M. Ray)

An enzyme immunoassay (EIA) for the determination of SQ 27,519, the active phosphonic-carboxylic acid diacid of the prodrug Fosinopril, is presented. Fosinopril represents a new chemical class of Angiotensin-Transforming Enzyme (ACE) inhibitors currently under development by The Squibb Institute for Medical Research. A radioimmunoassay (RIA) for SQ 27,519 has been previously reported and is currently in use as an analytical support for Fosinopril clinical studies. The SQ 27,519 EIA uses the same specific SQ 27,519 antibody developed for the RIA. The enzyme-antigen conjugate was prepared by a carbodiimide-mediated coupling of SQ 27,519 to beta-galactosidase. The assay utilizes cysteine as an activator and o-nitrophenyl-D-galactoside as the substrate with detection at 405m. The use of cysteine represents a significant improvement over the generally used i-mercaptoethanol in terms of toxicity and convenience. A set of SQ 27,519 human clinical specimens were analyzed using both RIA and EIA. The mean values for the RIA and EIA were 105ng/mL and 92ng/mL (n=49), respectively, with a correlation coefficient of 0.948. The close-reactivities for Fosinopril and the major metabolite of SQ 27,519 (phenolic-SQ 27,519) were within acceptable limits (8.162 and 1.3L, respectively). From the data presented, the SQ 27,519 EIA represents a suitable alternative to the RIA.

004 ENHANCED LUMINESCENCE IMMUNOASSAYS FOR FREE THYROID (T4) AND FERRITIN. D. A. Arbmsbury, D. A. C. Jirinzu, and J. V. Williams (Clinical Chemistry Section, Epidemiology Division, USAF School of Aerospace Medicine, Human Systems Division (AFSC), Brooks Air Force Base, Texas 78235-5301) (Spon.: D. A. Arbmsbury)

Enhanced luminescence is the basis for an integrated immunosay system, the Amerlite analyte and reagents (Amerahm Corp, Arlington Heights, IL). Noteworthy features of this system are: antibody coated plastic microtitr well; a horseradish peroxidase (HRP)/H2O2/luminol chemiluminescent reaction stabilized by an enhancing compound; an automated lumimeter with stored standard curve ability.

The FTA assay uses competitive binding between patient T4 and HRP-labelled T4 for sheep anti-T4 which in turn binds to anti-sheep antibody coated on the wells. Within-run CVs ranged from 2.7-6.5%; day-to-day ranged from 6-15.5%. Sensitivity is 0.03 ng/mL. T3 concentrations up to 8 ng/mL produced no cross-reactivity. Recovery studies ranged from 97-105% (ave = 99%). Dilutional linearity was exhibited. Lipids (210 mgL added...
triglycerides) and lcterus (2 mg% added bilirubin) produced positive interference. The Amerlite ferritin assay binds patient ferritin in a "sandwich" between monoclonal anti-ferritin coated on the well and HRP-labeled anti-ferritin. Within run CVs ranged from 4.0–10.5%; day-to-day CVs from 5.8–12.5%. Sensitivity is less than 1 ng/ml. Recovery studies ranged from 99–103%. Dilutional linearity was exhibited. Lipemia (420 mg% added triglycerides) caused a positive interference; hemoglobin (155 mg%) caused a negative interference. Linearity regression analysis of Amerlite and RIA ferritin values yielded: Amerlite=1.0 RIA + 11.1, r = .96, n = 55.

The Amerlite system offers simple, relatively rapid, semi-automated immunoassays using a methodology that has comparable sensitivity to RIA.

005 ASSESSMENT OF BIOLOGICAL REAGENTS FOR PRESENCE OF HUMAN IMMUNODEFICIENCY VIRAL ANTIGENS, Peter J. Howmanitz, James H. Werneck, Robin H. Pennington, Dept. of Pathology, UCLA Sch. Med., Los Angeles, CA 90024-1732. (Spon.: Peter J. Howmanitz)

Recent work has indicated that the human immunodeficiency virus antigen(s) (HIV Ag) test is more sensitive, rapid, and reliable than measurements of reverse transcriptase for identification of the virus associated with AIDS. Thus, we tested 122 biological reagents including laboratory quality control, proficiency, and therapeutic human immunoglobulin products using an immunoenzymatic assay (IEMA) for HIV Ag supplied by Abbott Laboratories, IL. Biological reagents from 21 manufacturers were classified as 64 HIV antibody (Ab) nonreactive, and 44 HIV Ab reactive quality control samples, and 14 HIV Ab reactive human immunoglobulins. HIV Ab was identified using the Abbott HIV Ab IEMA kit. Thirteen immunoglobulins and nine HIV positive quality control samples studied were previously reported as Western blot positive.

In the Abbott HIV Ag procedure, polystyrene beads coated with human Ab to HIV were incubated with sample or control and after washing, rabbit IgG to HIV was added. Goat Ab to rabbit IgG conjugated with horseradish peroxidase was added resulting in a complex sandwich. Bound enzyme was detected on addition of substrate (OPD) containing H2O2 with color development in proportion to the quantity of Ag present.

All 122 samples tested were nonreactive for HIV Ag. Lack of Ag reactivity may reflect that sera perhaps for these biological reagents was not obtained from donors early in the course of AIDS when only HIV Ag was present. Alternatively the incubation of sera in sera late in the disease when they were symptomatic and HIV Ag reactive. Also Ag nonreactivity may reflect assay insensitivity or destruction of viral Ags reactivity. Our results support other data that these samples do not contain viable HIV.


Standardization of assays for autoantibodies is a problem. First, positive control sera are not available in sufficient amounts. Second, the titer of patient sera varies from patient to patient and even in a single patient the titer will change with time; therefore results from different laboratories are often not comparable.

Through the development of the assay for insulin autoantibodies (IAA), we solved this problem by preparing a synthetic standard. For this purpose a mouse monoclonal antibody against insulin was covalently bound to human IgG. This conjugate mimics human IAA: the binding of the standard to an insulin coated microtiter plate is mediated by the affinity of the monoclonal antibody to insulin; analogous to the real human IAA the standard is detectable by an antihuman Fc-region peroxidase conjugate.

For the sera investigated their absorbance values in the IAA assay are calculated as percent value of the standard. Thus, comparison of the results obtained on different days or at different time intervals after starting the substrate reaction is possible, though the absolute signals may vary.

450 serum samples from healthy blood donors were analysed for IAA and 97.5% of them were located in a range of 0–2 % of the standard. Therefore, unknown serum samples can easily be classified for the presence of IAA by comparison with a 1:50 dilution of the standard. Specificity for IAA is verified by competition of the wallfused with free insulin. The undiluted standard can be used as control reagent for all the solutions used in the particular assay.


A fluorescent immunoassay for the detection of antibodies to human Immunodeficiency Virus (HIV) has been developed for use with the Abbott IMX Instrument. This assay utilizes microparticles (less than 1.0 micron in diameter) to decrease the total assay time and increase the sensitivity. After the addition of the samples and controls to the instrument, the assay is fully automated in 20 minutes. All controls can be run at one time with results for all of the samples ready within 40 minutes.

The test is a sandwich assay utilizing recombinant antigens from the env and gag regions. HIV antibodies directed towards gp120, p41 and gp120 are detectable by this assay.

The sensitivity is two fold dilutions more sensitive than current viral based blood screening assays. Of the 20 anti-HIV positive samples classified as Western blot positive.

Use of this automated assay for anti-HIV on the Abbott IMX Instrument allows minimal hands on time, rapid turnaround and optimal sensitivity.


A rapid, fluorescence enzyme immunoassay for the detection of HBS Ag has been developed for use with the Abbott IMX automated EIA processing system based upon microparticle technology. The heterogeneous assay utilizes a sandwich assay format with anti-HBs coated latex particles for antigen capture and an anti-HBs probe. A fluorescence signal proportional to the amount of HBS Ag present is generated by the enzyme reaction of alkaline phosphatase and methylumbelliferophosphate. Total time required to process 24 samples is 40 minutes.

The assay sensitivity for all nine major HBS ag subtypes is 0.84 ± 0.36 ng/ml. The dynamic range for the assay is linear to 50 ng/ml (r = 0.996) and extends to greater than 1 mg/ml with a slighthook effect such that a 1.2 mg/ml specimen produces a signal 6% less than 50% saturation (maximum assay signal). Assay reproducibility is typically 6-10% CV for the negative standard, 5-9% CV for a cutoff standard (0.9 ng/ml) and 2-5% for moderate signal samples (10 ng/ml).

An HBS Ag negative population of 257 random donors, 158 serum and 99 plasma, tested with overall initial and repeat reactive rates of 0.84 and 0.05% respectively. 95 anti-HB vaccine positive, HBS Ag negative samples were tested in parallel with the Abbott AUSZYME Monoclonal EIA with a resultant specificity agreement of 95.0%.

009 TOX LINEAR DOSE RESPONSE CURVES USING MONOCLONAL ANTIBODIES, F. Lucas, M. Ferrer, J. Ibanez (Coulter Diagnostics Div., Hialeah, FL 33014) (Spon.: J. Ibanez)

Pauling & Pressman (J. Am. Chem. Soc. 64:781–792, 1944; Meth. Med. Res. 10:124–127, 1964) described curvilinear characteristics are typical of polyclonal antibody dose-response curves. Cambio (Biol. Fluids Proc. Coll. 21:505–531, 1973) demonstrated that curvilinear response of polyclonal nephelometric haptan immunoassays were the result of antibody affinity hierarchy. This work deals with attempts to linearize a dose response curve using high affinity monoclonal antibodies.

DART™ Theophylline reagents optimized for 5-parameter logit calibration were used in the study. Linear calibration points are 5.0, 10.0, 20.0, 30.0 ppm/ml while curvilinear points are 0, 2.5, 5.0, 10.0, 20.0 & 40.0. Results on DACTM are as follows:
homogeneously by a ligand-specific binder, and whose enzymatic activity can be monitored potentiometrically. Specifically, an immunoassay for biotin based on the enzyme adenosine deaminase (ADA) and the strong and specific biotin/avidin interaction will be described. The enzymatic activity is monitored by using well-characterized ammonia detection schemes. These involve either the use of an ammonium-selective polymeric membrane electrode in an automated gas-sensing arrangement or a ready-made ammonia gas-sensing electrode.

The basis of this assay is the competition between an ADA-biotin conjugate and free biotin for a fixed number of binding sites of avidin. Several conjugates of ADA were prepared which exhibited as high as 99% inhibition of their enzymatic activity in the absence of avidin. The addition of free biotin reverses this inhibition in an amount proportional to the concentration of biotin. Relatively steep dose-response curves were obtained which reflect the concentration of biotin in standards to the inhibition of the enzyme conjugate. Changing the concentration of the biotin conjugate and the avidin, as well as the incubation time alters the detection limits in a predictable fashion. Dose-response curves with ED_{50} values as low as 4 \times 10^{-9} M were obtained.

The proposed method provides sufficient detection limits for a fast and simple determination of biotin in real samples. In addition, potentiometric detection circumvents the problem of background absorbance, avoiding the need for sample dilution inherent in photometric detection.

\[ 013 \] PROTEIN LABELING WITH THE FLUORESCENT PROBE, 4,7-BIS(CHLOROSULFOPHENYL)-1,10-PHENANTHROLINE-2,9-DICARBOXYLIC ACID (BCPDA), PROCEDURES AND APPLICATIONS. T. E. Diamandis, B. G. Morgan (CyberFlour Inc., 179 John Street, Toronto, Ontario, Canada M5T4X4) (Spon.: Karen Conway).

We describe methods for protein labeling with a new fluorescent probe, BCPDA, which forms stable and highly fluorescent complexes with Eu^{3+}. This fluorescent lanthanide chelate has a large Stokes shift (290 nm) and a long fluorescent lifetime, making it suitable for time-resolved fluorometric immunoassay applications with sensitivities in the picomolar range.

The protein labeling procedures were optimized with respect to the reaction pH, protein concentration, BCPDA excess and incubation time. The labeling proteins were isolated by gel filtration and concentrated and quantitated by absorbance at 325 nm. Maximal labeling was obtained at protein concentrations of 2 - 5 mg/ml after 30 minute reaction in NaClO_{3}, pH 9.1 by using a 5-fold excess of BCPDA over the free amino group concentration of the protein. However, in order to maintain the biological activity of proteins such as streptavidin, avidin and monoclonal and polyclonal antibodies, the protein concentration was reduced to 0.15 - 0.20 mg/ml. The excess BCPDA required was found to be 2-fold for streptavidin and 0.5-fold for avidin and the antibodies. The biological (binding) activity of the labeled proteins, as assessed by their ability for solid-phase binding in suitably coated microtiter wells, was preserved when a maximum of 14, 5, 17 and 12 hours were incorporated per molecule of streptavidin, avidin, monoclonal antibody to streptavidin and polyclonal goat anti-mouse IgG antibody, respectively.

The directly labeled antibodies, both monoclonal and polyclonal, were used successfully to generate standard curves for a competition type assay of cortisol, using the immobilized antigen approach. BCPDA labeled streptavidin, in conjunction with biotinylated antibodies, was also applied successfully for the development of other immunoassays of the competitive or non-competitive type.

\[ 014 \] TIME-RESOLVED FLUOROMETRIC IMMUNOASSAYS WITH EUROPIL CHELATES AS LABELS. A NEW GENERATION OF ALTERNATIVE IMMUNOASSAYS. F. P. Diamandis, R.A. Evangelista, A. Pollak, E. F. Templeton and J.A. Lowden (CyberFlour Inc., 179 John St., Toronto, Canada M5T4X4 and HSC Research Development Corp., 88 Elm Street, Toronto, Canada M5G 1X8) (Spon.: Alfred Pollak).

Time-Resolved Fluorescence Immunoassays (TR-FIA) are rapidly becoming established alternatives to isotopic immunoassays. The main advantages of this technique is that background fluorescence can be effectively excluded, thus, achieving very high detection sensitivity, which is equivalent or better than that obtained with radiolabeled agents. Europium ion fluorescence is long-lived and at 590 nm, and is very well suited for time-resolved measurements. A new europium chelator, 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid (BCPDA) has been synthesized recently. BCPDA can be covalently linked to streptavidin, other proteins and monoclonal or polyclonal antibodies. Competitive immunoassays (immobilized antigen approach) and 'sandwich' type immunoassays can be developed with the new fluor using either directly labeled antibodies, directly labeled second antibodies or
biotinylated antibodies and labeled streptavidin. An instrument has also been developed which is suitable for solid-phase time-resolved fluorometric measurements after pulse excitation with a nitrogen laser. This complete system (instrument plus chemistry) can be used for the routine assay of small and large molecules (drugs, peptide, thyroid, steroid hormones, cancer markers, etc.) in serum. The main advantages of the new system in comparison to other alternative immunoassays are as follows (a) high sensitivity (b) invulnerability to exogenous Eu³⁺ contamination which is the major limitation of currently used time-resolved immunoassays (c) stability of the fluorescent complex for months (d) handling of dry microtitration plates is very convenient. The instrument has automatic data reduction capabilities with a reading time of about 2 min per 96-well plate.

015 USE OF SYNTHETIC PEPTIDES FOR THE DETECTION OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) PROTEINS, B. Parekh, M. Altin, V. Huebler, P. Balaubramanian, and R. Walker (Bio-Rad Laboratories, Clinical Division, 100 Alfred Nobel Drive, Hercules, CA 94547) (Sponsor: Sandy Sweet)

Peptides were selected from HIV protein sequences, synthesized on a peptide synthesizer and assayed by dot-blot and by ELISA using a panel of positive and negative sera. Immunoreactive peptides were identified from each of the gag, pol and env gene products. A highly sensitive and specific ELISA was developed using a mixture of reactive peptides and was used for the detection of antibodies to HIV in more than 500 serum samples. Results demonstrated a complete correlation with Western blot analysis, with 100% of biot reactive peptides giving positive ELISA results, while all negative sera were ELISA negative. A group of problem sera, 30% of which yielded false positive results on another screening ELISA, were all negative on this peptide-based ELISA, demonstrating the high specificity of this assay. Thus, short synthetic peptides, representing antigenic sites of protein, can be usefull in a very sensitive and specific assay for the detection of antibodies to HIV.

016 DETERMINATION OF REACTIVITY OF HIV-1 SYNTHETIC PEPTIDES WITH AIDS/ARC PATIENTS' SERA, Musaffer Altin, Bharat Parekh, Vreul Huebler, P. Balaubramanian, and Roger Walker (Bio-Rad Laboratories, Clinical Division, 100 Alfred Nobel Drive, Hercules, CA 94547) (Sponsor: Michael Watkins)

Two hundred thirty three peptides (15 a.a. long with 5 a.a. overlap) covering most of the HIV-1 genome were synthesized on polystyrene beads using the method of Geyser, et al. (Proc. Nat. Acad. Sci. (USA) 81:3998 (1984)). Individual beads were arranged according to a 96-well microtiter plate format. Immunoreactivity of these synthetic peptides with AIDS/ARC patients' sera was determined by ELISA. The reactive peptides corresponding to all structural gene products: gag (p15, p18 and p24), env (gp41 and gp120), pol (p32) and regulatory proteins (tat, orn, trn, 3'orf and 5'orf) were identified using a panel of HIV-1 positive sera. These synthetic peptides can be used for the detection of antibodies to HIV-1 proteins.

017 EVALUATION OF A RADIOIMMUNOASSAY FOR THE DETERMINATION OF SERUM NEOPTERIN, J. Thames, J. Lukefahr and Thomas E. Worthy (MetPath Inc., Teterboro, N. J. 07608) (Sponsor: Diane Tyna)

We evaluated the performance of the Henning (Berlin) serum Neopterin RIA c assay kit in terms of precision, recovery, parallelism, standard curve stability, sensitivity, selectivity and established a reference range. Precision studies on replicate analysis of control material yielded coefficients of variation (CV) ranging from 4.9-14.8% for the normal control and 8.8-14.3% for the abnormal control. Inter-assay control precision was 10.0 and 13.2% respectively. Recovery studies demonstrated a X of 96% with a range of 96.1-97.4%. A patient sample when diluted in zero standard gave results which were parallel to the standard curve. Standard curve stability was assessed by measuring the variability of the standard curve over seven days. The CV's for the 80/80 and backfit doses ranged from 3.19-15.8% and 4.89-13.1% respectively. The assay sensitivity was determined by measuring the minimum detectable dose 2 SDs above the X zero standard. The assay demonstrated a sensitivity of 1.0 nMol/L which corresponded to a 92% 8/B. An evaluation of specimen type showed a frozen serum protected from light is the specimen of choice over plasma EDTA or heparin. A reference range determined on 50 healthy donors gave a range of less than 8.54 nMol/L. Eighty seven 82 microglobulin specimens assayed for Neopterin demonstrated a direct correlation and gave the following linear regression data: Y=7.01x - 8.10; r=0.82. Conversely, specimens with low T subset ratios (less than 1.04) had elevated Neopterin levels. We conclude that this assay is useful as a non-specific indicator of various diseases.

018 PERFORMANCE OF A RADIO-RECEPTOR ASSAY FOR THE RECOGNITION OF ACETYLCHOLINE RECEPTOR ANTIBODY, A. Judy Famulare, Isabel Reyes and Thomas E. Worthy (MetPath Inc., Teterboro, N. J. 07608) (Sponsor: S. Raymond Gambino)

We evaluated the performance of the Krons I-125 Acetylcholine Receptor Antibody Assay based on control and patient precision, tracer variability, established a reference range and comparison of methods. Precision was measured by monitoring the coefficient of variation (CV's) for each control over a period of 5 assays. The intra-assay CV's of the positive control counts per minute (CPM) ranged from 1.0-2.5% and for MoI/L ranged from 1.0-3.0%. The inter-assay CV's of the positive control counts per minute ranged from 12.3-18.7% and for the inter-assay precision (N=4) 28.5%. A patient sample assayed over three assays (N=30) gave a mean (X) of 11.0 MoI/L and a CV of 6.2%. Tracer variability, was demonstrated between lots of tracer by assaying the total counts over two lots of reagents. The X CPM 16521 (N=20) with a CV of 13.08 between lots. Fifty normal MetPath employees when assayed demonstrated a reference range of 0.00-0.4344 MoI/L. Comparison of methods with Duke University (N=59) demonstrated excellent qualitative agreement when analyzed by coned. We conclude that the Krons Acetylcholine Receptor Antibody Assay is simple to use and accurately determines the presence of antibodies to acetylcholine receptor.


The use of chromium dioxide magnetic particles as the solid support for enzyme immunoassays has been pioneered by Du Pont. Here we report on the use of this solid support in an assay for alpha-fetoprotein (AFP).

The competitive two-site enzyme immunoassay format uses a 20 μL serum sample, which is incubated with the monoclonal antibody-coated chromium dioxide reagent and the monoclonal antibody-alkaline phosphatase conjugate. This analyte capture incubation step requires only 30 minutes because of the large surface area of the solid support. Three washes follow the capture step. The fluorescent response is developed by incubation of the magnetic particle pellet with the substrate 4-methylumbelliferyl phosphate for 5 minutes.

Correlation studies versus the Amersham RIA method yielded the following results: y=0.915x + 3.1; Sxy=6.55; r=0.98 (n=41). The assay employed range from 2 to 1000 ng/mL. The within-run precision (CV) of the assay, with serum samples, is 2.3% at 20.1 ng/mL (n=10), 2.5% at 94.3 ng/mL (n=10) and 2.2% at 290.9 ng/mL (n=10). We have challenged the assay with elevated levels of AFP of up to 1000 ng/mL, while observing no evidence of a "hook-effect". Recovery of AFP spiked into serum samples is 100 ±10%. The sensitivity of the assay has been established at 3.5 ng/mL.

This assay is sensitive, can provide fast access to results and shows excellent performance.

020 METHODS OF ANTIBODY IMMUNOFLUORESCENCE FOR USE IN FIBER OPTIC FLUORESCENT IMMUNOASSAY, Stephen L. Lin, P. J. Maguire and J. M. Baltas (Biocleanse Laboratories, Allied-Signal, Inc., P. O. Box NO132, Morristown, NJ 07960-1021) (Sponsor: S. E. Diamond)

1196 CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988
Optical fiber waveguides have recently shown great promise as a simple, rapid, and sensitive means of monitoring the results of fluorescent immunosassays. When antibodies are immobilized on the fiber surface, sandwich immunosassays may be performed by successively exposing the derivatized fiber to antigen and to labeled antibody. An advantage of this procedure is that the fluorescent labelled antibodies which are bound near the fiber interface are subject to evanescent wave excitation by the light propagating within the fiber. In contrast, when non-derivatized antibodies remaining in solution are not stimulated by the light propagating within the optical fiber. A disadvantage of this procedure is that the glutaraldehyde method typically employed to couple antibodies to the fiber surface, as background fluorescence and relatively low antibody binding activities.

In an attempt to overcome these limitations, we investigated a number of other chemistries for the attachment of antibodies to optical fibers, and evaluated both the optical and the immunoenzymological characteristics of the resultant antibodies and antibody-derivatized fibers. Antibodies were coupled to suitably derivatized fibers using glutaraldehyde, carbodimide (CDI), succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), and succinimidyl-4-(N-maleimido)stilbene-4,4'-disulfate (SMLD). In addition, dial-derivatized fibers were activated with either trifluoroacetylethanesulfonic or with sodium periodate. That anti-rabbit IgG was the antibody used in this study. The amount of bound antibody was measured by blocking fluorescent labeled goat anti-1gG to the fibers and monitoring the fluorescence. To determine binding capacity, fibers coated with unlabelled goat anti-rabbit IgG were sequentially incubated with rabbit IgG and with fluorescent labeled goat anti-rabbit IgG and the fluorescence measured. The results showed that CDI, SMCC and SMLD were the preferred methods, affecting higher antibody binding capacities and lower background fluorescence levels.

MULTIPLE FLUORESCENT LABELING OF STREPTAVIDIN FOR HIGHLY SENSITIVE TIME RESOLVED IMMUNOSASSAYS. E.P. Diamandis, B.C. Matlea, E. Reichstein, and M.U. Khorasani (CyberFlour Inc., 179 John Street, Toronto, Ontario, Canada M5T1X4) (Spon.: E.P. Diamandis).

We describe methods by which streptavidin is multi-labeled with a new fluorescent probe, 1,10-phenanthroline-2,2'-dihydroxydisulfonate (BCPDA). This lanthanide chelate forms stable and highly fluorescent complexes with Eu and is suitable for time resolved fluorescent immunosassay applications. The fluorescence quenching effect of typical fluorescent labels is not observed with BCPDA due to its large Stokes shift. Therefore, multiple labeling would increase the sensitivity of immunosassays based on this fluor. The labeling of streptavadin was achieved both directly and through the conjugation to BCPDA labeled thyroglobulin. Direct labeling of streptavidin with BCPDA in Na2CO3, pH 9.1 resulted in the incorporation of 14 floros per molecule. This extent of labeling maintained its maximal biological (binding) activity as assessed by its ability for solid-phase binding in suitably coated microtiter wells.

The chemistry of the streptavadin-labeled thyroglobulin conjugation involved the reaction of the sulfosuccinimidyl 4-(N-maleimido)maleimide- cyclohexane-1-carboxylate (sulfos-MCC) derivative of streptavidin with the thiols groups generated in BCL from the N-succinimidyl S-acetylethacate (SATA) derivative of thyroglobulin, by the addition of hydroxylamine, in Na2HPO4, pH 6.2, under a nitrogen atmosphere. 0.5 floros could be incorporated on thyroglobulin after an initial low derivatization with SATA. The streptavidin-BCPDA labeled thyroglobulin conjugate was isolated from the unconjugated streptavidin by gel filtration (HPLC TSK-250 column or an Ultralys A34 column. A 6-fold increase in the fluorescence was observed for the thyroglobulin conjugate compared with the directly labeled thyroglobulin determined by solid-phase binding in suitably coated microtiter wells. Further comparison of these multi-labeled molecules in a non-competitive human choriongonadotropin assay indicated that both performed successfully. However, the ultimate detection limit for the BCPDA labeled streptavidin conjugate was 4 IU/L while that for the thyroglobulin-streptavidin conjugate was 1 IU/L.

EVALUATION OF A CHEMILUMINOMETRIC ASSAY FOR INTACT PARATHYROID HORMONE (INTACT PTH), Rosalia Salinas, A. Judy Fairumle and Thomas E. Worthy (MetPath Inc., Teterboro, N.J. 07608) (Spon.: T. E. Worthy).

We evaluated a chemiluminescent intact PTH assay from the Welsh National Scintigraphy Laboratory, Cardiff Wales (developed by R. Brown, I. Weakes and S. Woodhead) in terms of precision, recovery, parallelism, standard curve stability, sensitivity, sample stability and comparison of methods. Control precision for low, medium and high intact PTH levels yielded intra-assay coefficients of variation (CV's) of 5.2-26.4%, 4.6-13.7% and 4-8.4% respectively. Intact PTH levels for inter-assay precision were 1.64, 6.50 and 28.6 PmoI/L with respective CV's of 18.17 and 16%. Intact PTH standards were added to a patient sample containing no measurable amounts of intact PTH.

Mean recovery was 99.5% with a range of 88-130%. A diluted patient sample was linear and parallel to the standard curve up to 45 pmol/L. The standard curve stability was assessed by measuring the variability of the standard curves over 28 runs. Across the dynamic range of the assays, the CV's ranged from 0.3-10.3% between two lots of reagents. The minimal detectable dose, defined as 2 S.D. above the zero standard, was 0.4 pmol/L. Serum and plasma EOTA specimens were obtained from patients with various thyroid disorders to assess the specimen stability. Sera stored at 4°C were stable up to seven days and were the specimen of choice. Parallel testing of 355 samples with an Immunoradiometric assay yielded the following linear regression analysis: Y=2.34x+1.34; r=0.984. We conclude that this assay is simple to perform and accurately distinguishes between various parathyroid disorders for the measurement of intact PTH.


A two-reagent immunoassay for Digoxin that is rapid and accurate has been developed for use with the Ciba Corning Diagnostics Magic Lite System.

Paramagnetic particles coated with protein-bound digoxin compete with digoxin in the 96-well plate for a digoxin-specific monoclonal antibody which has been labeled with europium chelate. The Magic-Lite Digoxin immunoassay uses a 0.05 ml sample and has a single incubation step of 30 minutes followed by a single distilled water wash step. Total assay time for 120 samples is approximately 1 hour. The assay has a minimum detectable dose of 0.1 ng/ml. Method comparison studies with both the Magic-Lite (ML) DIG (RIA) and the TDX Digoxin assays gave correlation coefficients greater than 0.97 over a range of 0.1 to 5.7 ng/ml. Intrassay CV's are less than 8%. A STAT assay with an incubation time of 10 minutes has a minimum detectable dose of 0.2 ng/ml.

Preliminary analysis of samples (renal, pregnant) indicate minimal effects from possible digoxin-like interfering factors.

The use of magnetic separation and chemiluminescence makes this an easy to perform sensitive assay that is both rapid and accurate.


Chemiluminescent substrates for peroxidases are well known in the clinical literature. Chemiluminescent substrates for other enzymes commonly used in immunoassays have not been available. We have synthesized and studied a new dioxetane based chemiluminescent substrate (AMPPD) for alkaline phosphatase. Upon reaction with alkaline phosphatase, the substrate spontaneously chemiluminesces with an emission maximum at 460nm. Unlike the commonly known chemiluminescent substrates, i.e. luminol, AMPPD does not require additional reagents for enzyme catalyzed chemiluminescence.

Alkaline phosphatase catalyzed chemiluminescence from AMPPD is constant for prolonged periods. Using this substrate, we were able to detect a concentration of 10 femtomoles of alkaline phosphatase per liter. The ratio of signal to noise ranges from 1.14 at 10 pm enzyme, to 2100 at 4 nm enzyme. The Km of alkaline phosphatase for this substrate is 2.5nm. Further, using AMPPD we have demonstrated the detectability of quantities of alkaline phosphatase immobilized on membrane supports. We obtained comparable results when enzyme triggered chemiluminescence was measured using a Turner luminometer, or imaged on Polaroid Type 621 film. AMPPD offers a direct alternative to colorimetric or fluorescent substrates for alkaline phosphatase. Furthermore, it can be coupled with a variety of photosensitive detection devices i.e. photocoupler tubes, photodiode, film, etc. The simplicity of this chemiluminescent readout will allow the development of simple and rapid immunoassays and nucleic acid probe assays.

Cotinine is considered to be the most realistic and predictive determinant of smoking status as it is the major metabolite of nicotine and can be detected in the serum, urine, and saliva of smokers. It also has considerably longer half-life compared to nicotine. This investigation compared the RIA procedure (SABOR, INC. Englewood, NJ) with HPLC procedure previously reported by us. Cotinine in urine sample competes with cotinine coated on a plate for binding to the anti-cotinine antibody. The conjugate is quantitated using peroxidase-conjugated anti-antibody and an ortho phenylenediamine indicator system. The table below compares the HPLC and RIA results:

### Cotinine Values

<table>
<thead>
<tr>
<th>EPLL (µg/mL)</th>
<th>TOTAL NO. OF SAMPLES</th>
<th>NO. OF SAMPLES (+) BY RIA</th>
<th>NO. OF SAMPLES (-) BY RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 200</td>
<td>112</td>
<td>0</td>
<td>112</td>
</tr>
<tr>
<td>200 - 500</td>
<td>32</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>&gt;500</td>
<td>61</td>
<td>60</td>
<td>1</td>
</tr>
</tbody>
</table>

The RIA results were based on a cut-off value of 300ng/mL blood and, by lowering it to 400 ng/mL, the agreement is further increased. Within-run variation of the six standards (0-10 µg/mL) exhibited maximum SD 4.18 (for 2.5 µg/mL) and the highest CV 9.24 (10 µg/mL). Between-run variation gave maximum SD 5.8 (0 µg/mL) and the highest CV 12.99 (5 µg/mL). In conclusion the RIA procedure is sensitive, precise, and convenient for routine screening of cotinine and agrees well with our HPLC results.

IN-HOUSE PREPARATION OF A DIRECT CHEMILUMINESCENCE IMMUNOASSAY (EMIT) OF STEROIDS USING MAGNETIC SEPARATION. T. M. Satterfield and A. L. Siegel (U.A.B., Dept. of Pathology, Birmingham, AL 35294) (Spon: Abraham L. Siegel)

We report the practical application of CI for the assay of cortisol (F), dehydroepiandrosterone sulfate (DHEA-S) and aldosterone (Aldo). Most of the reagents are commercially available except for the CI labels. These were made according to the method of F. Kohne et al (Steroid Biochem., 1983; 19:413-16). Briefly, we activated the hemiscuccinate or (O-carboxymethyl) oxime derivatives of the steroids with N-hydroxysuccinimide and N,N'-dicyclohexyl-carbodiimide. The product we refluxed with an alkaline solution of the isouliminol derivative, ABE (Sigma Chem. Co.). After extraction, we separated and purified the labels by TLC. The rabbit polyclonal antibody used for the CL assays were from Cambridge Biotech, MA and DHEA-S and BioTec for Aldo. The protocols we followed were similar for these three assays. The incubation solution contained the serum sample, CL label, antibody and magnetic solid phase coated with second antibody (Bio-Mag, Advanced Magnetic Co.). Following magnetic separation and washing we measured the chemiluminescence in a luminometer (Clara-Corning) according to F. Kohne et al.

We compared the use of the steroid labels with commercial RIA kits with these results: (Corr. coeff. and slope) F (vs. Cambridge Med. Tech.) 0.99; 1.05 (n = 41); DHEA-S (vs. Diag. Syst. Lab.) 0.96; 0.74 (n = 20); Aldo (vs. Diag. Prod. Lab.) 1.00; 1.00 (n = 7). Coefficients of variation of duplicate samples were generally better than 5% in all assays. These sensitive assays are robust, economical and take little time to perform. The stability of the labels over many months is another important advantage. The assays provide a practical alternative to RIA.


Control and clinical serum samples containing theophylline were analyzed on an automated ABA 200P (Abbott) and theophylline homogenous enzyme inhibitor immunoassay (IAA) and by the SYVA homogeneous immunoassay technique (EMIT). Precise studies of replicate analyses of normal human sera to which accurately measured amounts of USP Theophylline had been added revealed.

CHARACTERISTICS OF AN IRMA ASSAY FOR INTEGRANT PTH HAVING A FOUR HOUR ALTERNATIVE, L. J. Almeida, J. E. Donnan, C. R. Goldenzweig, and B. S. Hostage (INCSTAR Corp. P.O. Box 385, Stillwater, MN 55082) (Spon: John W. Oef)

We have developed an IRMA (N-Tact PTI) assay for intact human parathyroid hormone (PTH) in serum. The assay is incubated at room temperature either four or 22 hours, followed by three wash cycles and counted. The assay has the following characteristics:

<table>
<thead>
<tr>
<th>Low Range</th>
<th>Medium Range</th>
<th>High Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run CV (4 hr)</td>
<td>6.4%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Within-run CV (22 hr)</td>
<td>5.4%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Between-run CV (4 hr)</td>
<td>5.1%</td>
<td>2.6%</td>
</tr>
<tr>
<td>Between-run CV (22 hr)</td>
<td>10.8%</td>
<td>5.2%</td>
</tr>
<tr>
<td>Recovery (4 hr)</td>
<td>105%</td>
<td>95%</td>
</tr>
</tbody>
</table>

Sensitivity 4 hour 2.0 pg/ml
22 hour option 1.2 pg/ml
Dynamic Range both options 10-2000 pg/ml

A comparison of 72 normals was made using the INCSTAR N-Tact PTI RIA kit and the two IRMA options with the following results:

<table>
<thead>
<tr>
<th>N-Tact RIA</th>
<th>RIA Hour EMIT</th>
<th>RIA 22 Hour IRMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>21.2</td>
<td>20.1</td>
</tr>
<tr>
<td>S.D.</td>
<td>11.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Range</td>
<td>44.1 - 35.6</td>
<td>33.7</td>
</tr>
</tbody>
</table>

Excellent correlation seen was between the two IRMA options (slope=0.98, y-inter.=-0.98 and r=0.94). The correlation between the 22 hour option and the RIA was not as good because of the added sensitivity of the IRMA format (slope=0.45, y-inter.=-10.18 and r=0.78). In summary, the N-Tact PTI IRMA offers a rapid, sensitive measurement of intact PTH which represents a vast improvement over what has been the state-of-the-art technology for many years.


The performance of radial immunodiffusion (RID; Kallestad), rate nephelometry (Beckman) and equilibrium (Abbott TDX) was compared for quantifying IgG in CSF.

The performance of radial immunodiffusion (RID; Kallestad), rate nephelometry (Beckman) and equilibrium (Abbott TDX) was compared for quantifying IgG in CSF.

Calibrator crossover studies compared well for all three methods. All y-intercepts were less than 1.00 mg/dL, all slopes ranged from 1.00 to 1.10, and all correlation coefficients (r) for the calibrator comparisons exceeded 0.95.

For groups of patients CSF specimens (n) acceptable accuracy (y-intercept), proportionality (slope), and correlation (r) were demonstrated by the three immunoassay methods compared, as indicated by the linear regression shown below.

Comparison | n | y-intercept | Slope | r Value |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TDX vs EMIT</td>
<td>107</td>
<td>-0.3 mg/dL</td>
<td>0.93</td>
<td>0.99</td>
</tr>
<tr>
<td>TDX vs RID</td>
<td>79</td>
<td>-0.5 mg/dL</td>
<td>1.01</td>
<td>0.99</td>
</tr>
<tr>
<td>TDX vs CSF</td>
<td>79</td>
<td>-0.2 mg/dL</td>
<td>1.00</td>
<td>0.99</td>
</tr>
</tbody>
</table>
**030** BIOTROL ELISA FOR IgG AND IgM ANTIBODIES TO TOXOPLASMA GONDII, Michel Uzan and M. Rigsuit (Biotrol, USA Office, Malvern, PA 19355) (Spon.: M. Uzan)

Biotrol TOXO G and M tests are enzyme-linked immunosorbent assays to determine IgG and IgM antibody to Toxoplasma gondii in human serum. The tests have advantages over the indirect fluorescent antibody (IFA) method in ease of use, objective interpretation. In addition, P(ab)2 fragments of IgG antibodies to Toxoplasma are used in enzyme conjugates in Biotrol TOXO-M test, this avoids false positive results in serum positive for non-specific antibodies (eg. rheumatic factor). Both tests assayed on Photon were compared to IFA and ABBOTT ELISA TOXO-G and M with the following results:

<table>
<thead>
<tr>
<th>Test</th>
<th>IFA</th>
<th>ABBOTT TOXO-G</th>
<th>ABBOTT TOXO-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (IU/ml)</td>
<td>99.6</td>
<td>98.6</td>
<td>100.2</td>
</tr>
<tr>
<td>N Agreement</td>
<td>50</td>
<td>98.6</td>
<td>92.9</td>
</tr>
</tbody>
</table>

The reproducibility is presented below:

**Intra-assay**

<table>
<thead>
<tr>
<th>Biotrol ELISA TOXO-G</th>
<th>Biotrol ELISA TOXO-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (IU/ml)</td>
<td>Mean Absorbance (CV)</td>
</tr>
<tr>
<td>91.5</td>
<td>7.1</td>
</tr>
<tr>
<td>95.4</td>
<td>6.7</td>
</tr>
<tr>
<td>145.6</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**Inter-assay**

<table>
<thead>
<tr>
<th>Biotrol ELISA TOXO-G</th>
<th>Biotrol ELISA TOXO-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (IU/ml)</td>
<td>Mean Absorbance (CV)</td>
</tr>
<tr>
<td>11.7</td>
<td>8.1</td>
</tr>
<tr>
<td>98.0</td>
<td>7.0</td>
</tr>
<tr>
<td>164.5</td>
<td>6.2</td>
</tr>
</tbody>
</table>

**032** PERFORMANCE OF A RADIOIMMUNOASSAY FOR THE MEASUREMENT OF VASOACTIVE INTESTINAL PEPTIDE (VIP), Joseph D. O'Brien, A. Judy Fanulare and Thomas E. Worthy (MetPath Inc., Teterboro, N.J. 07608) (Spon.: Joseph E. O'Brien)

We evaluate the performance of the Penninsula Laboratory's assay for Vasoactive Intestinal Peptide (VIP) in terms of precision, recovery, parallelism, sensitivity, standard curve stability and accuracy. Intra-assay precision studies for normal control material yielded recoveries of CV's ranging from 92.2-115.2. Inter-assay precision studies for CV's of 2.2-115.2. Recovery of added VIP standards to serum, or to the control range yielded recoveries of 92-115.2 with a mean of 115.2. Dilution of a patient's sample in assay buffer gave values that were linear and parallel to the standard curve. The minimum detectable dose, defined as the smallest concentration detectable two standard deviations above the zero, was 2.2. Recovery curve stability was assessed by measuring the variability of the standard curves over 12 runs. Across the dynamic range of the assay the CV's ranged from 2.2-9.8. Measurement of VIP from 91 serum samples gave a reference range of 0-5ng/ml. Parallel testing of 55 samples by radioimmunoassay and a reference laboratory yielded the following linear regression equation: Y=1.155x+4.85 r=0.98

We conclude that this commercially available RIA is accurate and useful for the measurement of Vasoactive Intestinal Peptide.

**033** SUBATOMOLE IMMUNOASSAY WITH ELECTROCHEMICAL DETECTION, Sarah Jenkins, H. Brian Haislil, and William R. Heiseinan, Dept. Chemistry, U. Cincinnati, Cincinnati, OH 45221-0172. (Spon.: S. Jenkins)

Common formats for quantitative sandwich immunoassays are limited by (1) time taken to generate a detectable signal, (2) efficiency of analyte capture, (3) sensitivity of detection method, (4) non-specific binding, and (5) formation constants of the antibodies or other specific binding proteins. Detection limits are considerably improved at the femtomolar or attomolar level. Improvements can be made by altering the geometry of the reaction vessel and reducing its volume.

A sandwich enzyme immunoassay for mouse IgG using a glass capillary tube as the reaction vessel is described here. The primary antibody (rat a/mouse IgG) is immobilized on the inner surface of the capillary using a carbodiimide procedure. Antigen binding capacity of the antibody was maximized using a simplex optimization. The sample (70 lL containing mouse IgG, enzyme conjugate (rat a-mouse IgG alkaline phosphatase), and substrate (p-nitrophenol) are injected sequentially into the tube. After all incubations, the contents of the tube are injected directly into an LCEC for analysis of the enzymatic product (p-nitrophenol).

The assay for mouse IgG is complete in 20 minutes, with a detection limit of 4.6 x 10^-12 moles (2800 molecules a 130) and a linear range of 3 orders of magnitude. Recovery from serum samples was 99% under these assay conditions.

This configuration offers the advantage of a large surface area to volume ratio (60 cm^2/ml) that reduces 2D to a 3D reaction, and during also gives the analyte capture reaction. The tubes are inexpensive and disposable.

**034** A NEW HIGH DENSITY PARTICLE AGGLUTINATION TEST (HDP) FOR MYCOPLASMA PNEUMONIAE INFECTION, T. Takahara, H. Uen, K. Matsumi, Y. Inumatsui and T. Taniiya (Fujiwara Res. Lab., Tokyama Soda Co., Kanagawa and Univ. of Tokyo Branch Hosp., Tokyo, Japan) (Spon.: K. Matsumi)

We have developed new high density particles (HDP) composed of the inorganic compound containing dyes in the core and polymer layer on the surface, which are monodisperse particles (specific gravity, 2.0 ± 0.2) and have a clear, well defined, and stable physicochemical suspensions. They showed excellent coating abilities for both lipid and protein antigens. Using these HDP, the antibody against Mycoplasma pneumoniae was successfully detected by a passive agglutination procedure with lipid moiety of the organisms as antigen.
The lipid moeity was prepared by chloroform-methanol extraction from purified Mycoplasma-HepA cells. Antigens coated particles were prepared by mixing HDP suspension with an equal volume of lipid solution. The mixed suspensions were incubated for a few hours at room temperature with buffer, suspended in the diluent, and then lyophilized.

It was found that this Mycoplasma-HepA was about four times more sensitive than passive hemaglutination test (PHA) using crude antigens. Uncoated particles as control are not necessary for the test, because HDP don't possess antigenicity for clinical specimens. Furthermore, agglutination was completed within 30 minutes with easily visualized patterns. The result indicates that Mycoplasma-HepA is much superior to PHA which requires more than 120 minutes. We conclude that Mycoplasma-HepA is an effective tool for serodiagnosis of Mycoplasma infection.

A time resolved immunofluorescent assay for alpha-fetoprotein (AFP) is modified for use with dried blood spots on filter paper. Serum and dried serum samples were obtained from venous blood sample. For each assay, three discs (in the diameter of filter paper, were added to each well and incubated overnight at 4°C with Eu-labeled anti-AFP as is specified by the manufacturer (Delfia hAFP kit). The fluorescence is measured in the 1230 Arcus Fluorometer (LKB Wallac), extended the AFP standard curve from 0 to 320 U/ml. Results obtained were compared with those by serum (Delfia hAFP) and a previously adapted to dried blood spot samples Pharmacia AFPRIA (Pharmacia Diagnostics).

Analytical recovery averaged 101.6 + 4.5 % (mean ± SD) for concentrations of 10 to 270 U/ml, with a excellent linearity (r2 = 0.999) correlation coefficient. For 20, 40, and 160 U/ml AFP controls, the between-run CV were 24.0, 9.1, and 11.8 %, the within-run CV were 18.0, 4.1, and 7.0 %, respectively (n = 5). Correlation studies yields the following results:

\[
\begin{align*}
\text{Serum-Delfia}^\text{TM} & : \text{DOS-Delfia}^\text{TM} \\
\text{DOS-Pharmacia RIA} & : \text{DOS-Delfia}^\text{TM}
\end{align*}
\]

The dried blood AFP standards were prepared in the laboratory from a 12-388 WHO reference standard initial. The standards for serum were supplied by Abbott, Inc. and Abbott, Inc.

Because operating the Delfia™ system requires only basic laboratory skills, its use with dried blood spot specimens can be particularly suited for a mass screening program.

**A LIQUID-PHASE, NONISOTOPIC SYSTEM FOR MEASURING CIRCULATING ALLERGEN-SPECIFIC IgE, AMENABLE TO AUTOMATION.**

Traditionally, circulating allergen-specific IgE has been measured by the so-called radioallergosorbent test (RAST) or similar tests thereof. In this approach, patient serum is first reacted with an individual allergen supported by e.g. a cellulose disk; the disk is then washed, reacted with labeled anti-IgE, and washed again before quantifying the signal. Relying on solid-phase kinetics, these methods require long incubation times, and are subject to nonspecific binding and matrix effects. In this liquid phase, moreover, because an IgE group on the allergen is required for IgE binding to the disk, foods and molds that contain very little protein are liable to incomplete immobilization—ast shown for some samples by Western blot analysis. The present method (ALLEIA) circumvents these problems, and invites automation, through its use of labeled-labeled analytes in liquid phase: the ligand is covalently linked to a liquid matrix which in turn attaches to NH2, OH, COOH or SH groups on the allergens. A tube coated with the same ligand serves as the well for the initial specific IgE in the patient sample and a ligand-labeled allergen—a reaction which proceeds with liquid-phase kinetics. After 1 hour, a multivalent anti-IgE is added, to link the ligand-labeled allergens to the ligand-immobilized tube. The tube is then washed and reacted with a blend of two horse-drajd peroxidase-labeled monoclonal antibodies having complementary binding to the C2 region on the Fab portion of the IgE molecule. Finally, a 15-minute color development step yields color proportional to the allergen-specific IgE.

**A RADIOASSAY FOR ANTIBODIES AGAINST DOUBLE-STRANDED DNA (ANTI-dsDNA), BASED ON RECOMBINANT 351 DNA, ANALYTICAL AND CLINICAL STUDIES.**

A radioassay has been developed for measuring circulating antibody against double-stranded DNA (anti-dsDNA). The 351 DNA is essentially unconverted by single-stranded DNA (dsDNA), having been prepared by recombinant DNA techniques using E. coli plasmid DNA rather than chromosomal DNA. Neither complement (C3), which can bind to soluble IgG, nor antibodies to dsDNA, nor anti-dsDNA antibodies with the assay. Hence samples can be assayed directly, without a preliminary heat-inactivation step. The procedure involves a single 2-hour incubation at 37°C, followed by precipitation by chemical precipitation at 37°C. The assay has a calibration range of 5 to 100 KU/L and a detection limit of 2 KU/L. Intra-assay CVs were less than 9% throughout the calibration range; while interassay CVs measured 4 KU/L, respectively.

A study of 140 normal samples yielded a reference range with an upper limit of 6 KU/L. 27 out of 25 samples from patients with active systemic lupus erythematosus (SLE) and 24 of 48 samples from patients with active SLE yielded positive results, i.e. anti-dsDNA levels greater than 6 KU/L. Only 2 out of 48 samples from patients with other connective tissue disorders (4%) were positive.

Two SLE patients were monitored by this assay, and also by an anti-dsDNA radioassay based on chromosomal DNA and a heat-inactivation step. Anti-dsDNA levels by both methods decreased gradually, showing good correlation with circulating C3 and C4 levels and higher anti-dsDNA levels during clinically active phases of the disease. As patients improved under treatment with prednisone, anti-dsDNA results by the present method proved more reliable than results by the other method in reflecting changes in the patient's clinical status.

**AN EVALUATION OF TD Instrumentation**

Catherine Johnson, City Hospital, Martinsburg, WV. Sponsored by W. Schall

I assessed the Abbott TDx, Dade Stratus®, and DuPont ACA™ for the measurement of digoxin and theophylline. Each system is an immunosassay method. The operational appraisal included calibration stability, precision, accuracy, and throughput studies. I also included a financial analysis of the operating costs of each system and a comparison of the personnel requirements.

Precision studies were performed at levels of concentration using a commercially prepared control serum. Correlation studies were caried out on patient samples for these analytes with the following results.

**Regression Analysis**

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<thead>
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<th>Level</th>
<th>Intercept</th>
<th>Corr. Coeff.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>2.25-27.6</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>3.19 5.64</td>
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</table>

I conclude that these assays are well suited for the accurate, precise, and rapid measurement of these analytes in human serum.

Cost and personnel analysis suggest that the Stratus and the TDx are more rapid for batch testing than the ACA and that the Stratus is the least expensive of the three to operate.

**ENZYME IMMUNOASSAY FOR THE DETECTION OF CIRCULATING IMMUNOBLOBS IN HUMANS TO MOUSE MACROPHAGE ANTIBODY.**

Geoff S. LaFontaine, H. J. Hansen, B. F. Weis, and D. Mic Goldenberg (Immunomedics Inc., and Center for Molecular Medicine and Immunology, Newark, NJ 07103) (Spon.: G. LaFontaine)

An enzyme immunoassay (EIA) has been developed to measure human antibodies to mouse IgG in patients suffering from infections of mouse monoclonal antibody (MAb). A significant number of patients receiving MAb develop antibodies to mouse IgG which can alter the course of therapy, and/or therapeutic imaging agents. In addition, human anti-mouse monoclonal antibody (HAMA) has been shown to interfere significantly with carcinoembryonic antigen (CEA) assay using commercial diagnostic
kites with murine MAb as part of the assay format. The HAMA assay developed by Immunometrics is a two-stage test carried out in 8-well microtiter strips which have been coated with mouse IgG. Mouse IgG conjugated to horseradish peroxidase is added to the microwell along with the patient serum or plasma if HAMA is present. A bivalent bridging phenomenon will occur between the solid phase mouse IgG and the mouse IgG conjugate via HAMA. Following a wash step the second stage is color development of 0-phenylenediamine/hydrogen peroxide substrate. Color intensity is directly proportional to the amount of HAMA present in the sample. The HAMA assay can be conducted manually or with existing microtiter equipment. Values are read at 405-492 nm and are reported in nanograms antigen equivalents per ml; the test was standardized against a standard anti-mouse IgG serum. The assay sensitivity is approximately 40 nanograms antibody per ml and assay time for results is under an hour.

With exception of one out of 12 rheumatoid positive patients, clinical results have shown a normal range in subjects who have not received murine MAb to be below the threshold of the assay sensitivity.

(Supported in part by NIH grant CA39841).

**040**

**A DUAL RADIOIMMUNOASSAY FOR THE DETECTION OF MORPHINE AND COCAINE IN URINE.** A. S. Tseng, F. M. Rubio, and S. E. Wagner (ICN Immunometric Systems, Inc., Horsham, PA 19044) (Spons.: A. S. Tseng)

An assay has been developed for the detection of morphine, cocaine, and their metabolites in urine. The assay is a liquid phase, double antibody system which utilizes a dual label tracer (125I-morphine-benzoylghonine and 125I-narcotic) which is added to the urine specimen, followed by addition of the primary antibodies (sheep anti-morphine and sheep anti-NE). Antigen in the patient sample complexes with labeled antibody for the limited amount of primary antibody present. Precipitation of the antigen-antibody complex occurs by the presence of the secondary antibody (directly anti-sheep IgG). In the tracer reaction, after the 30 minute temperature incubation, the tubes are centrifuged, decanted and counted.

Sensitivity of the assay was 90ng/ml for both drugs, and recovery averaged 100% for morphine and 104% for BE. Major cross reactants were codeine (20%), ethylmorphine (18%), cocaine (21%), tropacocaine (46%), and p-amine BE (12%). Other related compounds tested had cross-reactivities of <10%.

A screen of 100 normal urine specimens yielded no false positives while 50 of 50 positive samples (for either drug, tested by commercially available RIA and GC/MS) were detected. Inter and intra assay precision (CV%) data were as follows:

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<tr>
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<th>POOL 2</th>
<th>POOL 3</th>
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<tbody>
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<td></td>
<td>Intra assay</td>
<td>1.1</td>
</tr>
<tr>
<td>BENZYLCOCAINE</td>
<td>Inter assay</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Intra assay</td>
<td>2.2</td>
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**041**

**RAPID SCREENING ASSAY FOR VISUAL DETECTION OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS (HIV), Margaret L. Smith-Levis and H. C. Warren (Life Sciences Research Laboratories, Eastman Kodak Company, Rochester, NY 14650), Lawrence D. Papadino, Cellular Products, Inc., Buffalo, NY 14201, Yihahng Shih (Cetus Corporation, Emeryville, CA 94608) (Spons.: J. Mauck)

A rapid (less than five minutes), simple, colorimetric, enzyme immunoassay for visual screening of antibodies to HIV-1 was developed using whole viral lysate and a unique dye reagent, resulting in a colorless-to-red color reaction catalyzed by the presence of peroxidase enzyme in the immunocomplex. The assay contains an internal positive and negative control in every test cell and latitude in time and reagent addition at every step. The test utilizes prediluted serum and involves three reagent additions and three timed steps.

A variety of serum/plasma samples were tested by this rapid assay and the results compared to a licensed ELISA. Out of 200 randomly selected sera and 2 positive sera in both assays. Out of 100 characterized samples, all test results in the rapid assay agreed with ELISA.

No major interferents were identified, including sera from individuals with tumor growth, viral infections, and autoimmune disease.

**042**

**THE DETECTION OF CHLAMYDIA TRACHOMATIS ANTIGEN IN CLINICAL SPECIMENS.** John C. Mauck (Life Sciences Research Laboratories, Eastman Kodak Company, Rochester, NY 14650) and J. Gilbert (Cetus Corporation, Emeryville, CA 94608) (Spons.: W. Workus)

Chlamydia trachomatis is an important etiologic agent of genital infections in women, ranging from clinically apparent disease to cervicitis, endometritis, and pelvic inflammatory disease. We have developed a rapid, sensitive and specific qualitative test for the detection of Chlamydia antigens in clinical specimens.

Cell surface antigens are extracted from the sample under basic conditions. The extracted antigen is added to a test cell that contains a treated membrane that captures the antigen. Monoclonal antibodies (Mab's) are added to the test cell and bind to the antigens. A goat anti-mouse polyclonal antibody, labeled with horseradish peroxidase (HRP) is added and binds to the Mab's. Excess antibodies are washed away and a peroxidase containing H2O2 is added. Any bound HRP will react with the H2O2 and solution of peroxidase dye to produce a red color. In the absence of antigen, no HRP is bound and no color is formed. Each test cell incorporates a separate positive and negative control to verify that the test is valid. Interference from endogenous whole blood, white blood cells and mucus has been eliminated.

One hundred fifty clinical specimens were obtained, and the detection of antigen was compared to the results from a second sample taken at the same time and submitted for routine detection of Chlamydia by standard culture techniques. Compared to culture, the test showed 93.2% sensitivity and 94.3% specificity with a prevalence rate of 8.5%. The test requires only 30 minutes to run and can be run by untrained personnel.

**043**

**A NEW IN**th** Automated Microparticle Enzyme IMMUNOSUMAT FOR THE QUANTITATION OF HUMAN ALPHA-FETOPROTEIN.** B. Dowell, K. Byl, V. Wilsbom, D. Seaylirg, J. Rapp, and W. Shaw, (Abbott Laboratories, Abbott Park, IL 60064) (Spons.: William King)

The new enzyme immunoassay, IN**th** APP, has been developed for the quantitation of human alpha-fetoprotein (AFP) using the fully automated IN**th** instrument. Alpha-fetoprotein is found in trace amounts in the serum of normal, nonpregnant, healthy adults. Guidelines for serial screening for AFP are based on maternal serum AFP levels have been developed by the American Society of Human Genetics and endorsed by the American Academy of Pediatrics. High serum concentrations of AFP are found in patients with primary hepatocellular carcinoma and nonneonatal testicular cancer.

The IN**th** APP enzyme immunoassay uses microparticles coated with a mouse monoclonal antibody to human AFP as a solid phase capture component. The two-stage assay uses a goat antisem to human AFP linked to alkaline phosphatase to generate a fluorescent product. The IN**th** APP assay requires a single non-precipitating substrate and results on 24 samples in less than 35 minutes. The standard curve range is 0 to 350 ng/ml with an average percent coefficient of variation of less than 5%. An alternate dilution protocol will allow samples with up to 1000 ng/ml of AFP to be measured without manual dilution. The assay sensitivity is better than 0.5 ng/ml. The overall correlation coefficient between Abbott APP-RIA and Abbott IN**th** APP is greater than 0.96. This new automated IN**th** APP assay for human AFP offers a fast, reliable means of AFP quantitation.

**044**

**QUANTIFICATION OF URINARY PHENYLACETYLGLUTAMINE (PAG) BY FPIA USING TDX, H. Ghnabari, B. Roberts, D. Johnson and M. Adamszck (M.I.N.D. Diagnostics, Abbott Labs., Abbott Park, IL 60064) (Spons.: Susan Dugar)

Urinary phenylacetic acid (PAA) level has been shown to be useful in diagnosis and prognosis of endogenous depression. Adult controls excrete about 140 mg of PAA in 24 hrs, whereas depressed patients excrete about 70 mg/24 hrs. Phenylacetic acid is secreted in urine predominantly as PAG. Total FAA in urine is currently determined by GC after extensive pretreatment of the samples. The pretreatment includes acid hydrolysis, extraction, and derivitization.

The FPIA/TDX Assay of Urine PAG development does not require any sample treatment. It uses 1 ul of urine and 20 samples can be analyzed in less than 15 minutes (total time). The final reaction mixture contains PAG from the sample (or calibra-
A number of clinical disorders such as Peaty's syndrome, drug-induced neutropenia and febrile transfusion reactions are associated with anti-granulocyte antibodies (AGA). Although many in vitro methods including granulocyte agglutination, immunofluorescence and RIA methods have been developed to detect AGA, none of these methods are sensitive or reproducible enough for routine clinical use. We recently developed a simple, objective flow cytometry method (FCM) which could serve as a valuable tool to detect AGA.

Serum from 16 patients with known neutropenia of various types and degrees of clinical severity were tested for AGA by flow cytometry. Leukocytes isolated by dextran sedimentation of whole blood were washed with 0.09% EDTA phosphate buffer saline (PBS/EDTA) PH 7.2 and erythrocytes were lysed by incubation in 0.5 ml of lysis solution (0.8% NH4Cl, 0.1% NH3, 0.05 % EDTA) for 5 minutes at 37°C. Following 2 X washes with PBS/EDTA the cells were fixed in 1.0 ml of 16% paraformaldehyde for 5 minutes and washed 2 X again with PBS containing 1% goat serum (PBSG). Cells (2 X 10^6) were incubated with 100 μl of serum for 40 minutes at 37°C, were washed and incubated with 50 μl of FITC goat anti-human F(ab)2 polyvalent IgG (diluted 1:100) for 30 minutes at room temperature. The percent fluorescence positive granulocytes (FCG) were enumerated using a Coulter EPICS 541 flow cytometer.

Analysis of FCG was done with clinitorial serum FCGs which were less than 0.50 when tested with 19 normal sera while it ranged from 0.6 - 2.8% in 3 patients known to have high titer of AGA by other tests. 11 patients with neutropenia and a variety of clinical diagnosis showed FCG from 10 to 45%.

We conclude that analysing AGA by FCM is relatively simple procedure which could serve as a valuable tool to define the pathologic basis of certain types of neutropenia.

**DETECTION OF ANTI-GRANULOCYTE ANTIBODIES IN PATIENT'S SERA BY FLOW CYTOMETRY, Iqbal M. Shaikh, Antony C. Bakke (Clin. Path., Oregon Health Sciences University, Portland, OR 97203). (Spon: Iqbal M. Shaikh)**


Evaluation of pancreatic (P) and salivary (S) amylase isoenzymes is important in a variety of clinical situations, particularly in the diagnosis and/or monitoring of acute pancreatitis and in discriminating the latter from other disorders associated to hyperamylasemia. In addition, this assay appears to be a very useful diagnostic tool when levels of total amylase in acute pancreatitis are in the normal range or only slightly elevated (BOC, 1983, 1985). Furthermore, this method offers a good resolution of major pancreatic and salivary amylase bands, as well as of macrosamylase (sometimes associated to hyperamylasemia). Nevertheless, it is suitable in emergencies when a rapid clinical decision is required.

Therefore, we have studied the performance of an immunoassay for P-amylase isoenzyme determination. This method (obtained from Boehringer Mannheim) uses monoclonal antibodies inhibiting salivary enzyme activity. We determined P-amylase isoenzyme in sera from fifty patients, affected by acute pancreatitis with total amylase activity ranging from 85 to 7950 U/L (normal reference range up to 2 220 U/L). On these sera isoenzymes were also evaluated by electrophoresis (Clin. Chem. 31; 70, 1985; used as reference method in this experimentation). Data were examined by linear regression analysis (r=1.615,9567) and a good correlation (r=0.996) between the two methods was observed.

This work was supported by grants from C.N.R. and M.P.I., Rome, Italy.

**THE FLEXIBILITY OF THE AVIDIN-BIOTIN TECHNIQUE IN IMMUNOASSAYS, Pekka Vilja (Department of Biochemical Sciences, University of Tampere, Tampere, Finland) (Spon.: H. Adlersrute)**

The aim of the study was to evaluate whether the avidin-biotin peroxidase (ABC) technique can be used as a general system independent of the final detection method. The basic method was a noncompetitive AB assay (NAMA) for luteinizing hormone (LH). Reference method was the well-established photometric (Multiskan spectrophotometer, Elfa) immunoassay (ELISA) including sequential incubations of sample, biotin-labeled antibody and avidin-HRP conjugate.
The LH-HAMA was adapted to four other detection systems:
1) Immunoassay assay (ILMA) with 125I-streptavidin.
2) Fluorescence detection (FPA) and a fluorescent substrate (Fluorescamin, EIA).
3) Three-color fluorescent detection (TF-TPMA) with streptavidin-1-conjugate (Arcus fluorescence, EIA).
4) Immunomagnetic assay (IMA) with avidin-BAP and luminometric substrates (MicroLume, Dynatech).

All 4 methods were used to determine LH in 40 urine samples and the results were compared with those obtained by ILMA. Linear regression analysis gave a correlation coefficient and a slope of 0.96 and 0.77 (ILMA), 0.98 and 0.87 (FPA), 0.97 and 1.13 (TF-TPMA), and 0.98 and 0.96 (IMA).

The results indicate that the avidin-biotin technique may be adapted to all these five types of final quantitative detection systems in immunoassays. This means good flexibility for the laboratory to make the choice of instrument.

**050** HOMOGENEOUS DETERMINATION OF CARCINOBROPHOMIC ANTIGEN (CEA) IN HUMAN SERA BY MICROCAPSULE IMMUNOASSAYS (MCIA)

RIA and EIA are used for popular determination methods of CEA in human sera. But they need so long time as several hours to be attained a sufficient sensitivity in the CEA determination. Their procedures are also complicated and technical. On the other hand, MCIA which we have developed is a simple, homogeneous and highly sensitive assay system. We applied MCIA to determine CEA in human sera.

Liposomes composed of dipalmitoylphosphatidylcholine, cholesterol, and dioleoyl-dipalmitoylphosphatidylethanolamine were encapsulated carbamoylfluorescein(CF) and reduced with diithithreitol. A monoclonal anti-CEA antibody(F(ab')2 fragment) modified with a crosslinking reagent(PS1DP) was contact with the reduced liposomes and the released human sera for 10 min at 37°C in a test tube. Rabbit anti-CEA antibody and guinea pig sera as complement source were added to the reaction mixture and incubated for 30 min at 37°C. The CF released from liposomes based on the specific immune reaction was detected with a fluorescence spectrophotometer(Hitachi,610-85).

We obtained following data in the determination of CEA by MCIA.
Concentration range to be detected in sera: 2-100 ng/ml
Reproducibility within runs: less than 5% in CV (% mg/ml)
Correlation coefficient to RIA: 0.95 (n=70)

We concluded that MCIA would be a simple, accurate and fast determination method of CEA in human sera.

**051** A RAPID IMMUNOASSAY FOR THE SEMIQUANTITATIVE NON-INSTRUMENTED MEASUREMENT OF URINE HCG FOR PREGNANCY AND FOLLOW-UP. Vedachalam Kamalasanan, Patricia A. Nunn and Krishan Taila (Biodiagnostics Laboratories, 4600 Norris Canyon Rd., San Ramon, CA 94583) (Spon.: Vedachalam Kamalasanan).

A sensitive and specific two site enzyme immunoassay utilizing a monoclonal and a polyclonal antibody to measure human chorionic gonadotropin (HCG) is feasible in female urine is reported. We have developed a method for providing three reference dots in addition to an analyte dot in a simple reaction cassette. The presence of internal reference dots (25 mIU, 50 mIU and 200 mIU/ml of HCG) provide a method by which it is possible to determine the concentration of HCG in the usual urine.

The assay is performed in a device consisting of a nylon membrane which is kept in contact with absorbable flow-through cassette. Polyclonal anti-HCG antibody is immobilized on the nylon membrane. The series of liquid reagents provided in the dropper bottles are added to the device after about 0.5 ml of urine sample is applied to the cassette. The enzyme reaction is stopped with a citrate buffer solution. The entire protocol takes 4-5 minutes. After completion of the reaction, the intensity of the sample dot is compared with the intensity of the reference dots. The person is considered to be pregnant when urine HCG level is about 25 mIU/ml or higher.

Correlation of the assay with another clinically proven enzyme immunoassay indicated 100% accuracy in pregnancy test. The nylon membrane mounted in a 2 x 2 inch slide can be retrieved for permanent records.

In summary, a rapid, sensitive and specific enzyme immunoassay for semiquantitative determination of HCG levels in urine reported here is convenient and simple to perform by a lay consumer at home or by an untrained technician in the doctor's office. The entire test protocol takes 4-5 minutes and provides a result which can be interpreted visually.


Our objectives were: 1) to determine by immobilization-electrophoresis (IFE) if the aberrations of <2-10y peaks in serum protein electrophoretic (SPE) patterns exhibiting quantitative normal fractions were due to presence of abnormal monomolecular proteins. Hence this study.

SPE was done on cellulose acetate plate using HELENA method and reagents: 5 peaks were typically observed (albumin, α1, α2, β and γ). IFE was performed on 401 patients' sera exhibiting normal levels of all 5 fractions in SPE using BECKMAN PARAGON Electrophoresis System employing five antisera: IgG, IgA, IgM, K and A.

Thirty-five (9%) of the IFE demonstrated monoclonal proteins, which were: IgG-K, 42%; IgG-λ, 25%; IgA-K, 3%; IgA-λ, 33%; IgG-λ, 2%; free IgG, 61%; free K, 33%; free λ, 33%; combined IgG-K and IgG-λ, 33%; and IgG-K and free λ, 33%. Literature incidence of benign monoclonal gammapathy or monoclonal gammapathy of uncertain significance (MUGS) is 11 of the population over age 50 and 3% over age 70; and 1% of patients with MUGS go on to develop myeloma. Our results reflect an excessively (at least 3 to 9-fold) increased incidence of MUGS in our VA patients. Since 11% of MUGS patients convert to myeloma, both high MUGS incidence is of serious concern, primarily because a large number of our patients (especially those from conflicts, including World War II and before) are in the age group (> 50) most prone to myeloma.

We conclude that to assure early detection of MUGS and thereby early implementation of therapeutic intervention if it converts to myeloma, it is essential to do IFE on all normal SPE patterns.


The present paper concerns a new sensor that has been developed by incorporating the enzyme immunosensor with electrochemical measurements for the determination of alpha fetoprotein (AFP). Two enzymes are used in "sandwich" technique. The first enzyme (glycogen peroxidase) is used for reversibility linking the immunocomplex to the insoluble matrix, the inhibitor of this enzyme being immobilized on the matrix (N-aminophenyl-beta-D-thiogalactopyranoside) the second (catechase) is used for labeling the monoclonal antibody of AFP. The measurement consists of an immunological process and an enzymatic reaction. The activated protein membrane is then placed on the cathode of a P2O electrode in a measurement cell. After incubation of the polyclonal antibodies labeled with N-α-allo-galactoside, with AFP and catalase labeled monoclonal antibodies at room temperature, the reaction medium is introduced in a continuous flow cell. The production of 02 by the enzyme reaction is measured on line with the electrode in contact with a 40 mM H2O2 solution. This response is correlated to the AFP concentration of the sample. The signal is directly proportional to the concentration of AFP. By using 0.1 M borate buffer solution pH 10 for two minutes it is possible to release 99.2% of the immunocomplex Ab-AFP-Ab and to realise a new series of measurements. The minimum detectable concentration is 0.5 µg/l. The inter-assay CV for this assay is 4.0. The conversion of this assay into a future measurement is about 10 minutes and it's possible to make at least 30 determinations with the same membrane without loss of activity.

**054** TIME-RESOLVED IMMUNOFLUORESCENCY OF HCG, Tiika Kemell and Ari Räisänen (Wallac Biochemical Laboratory, P.O. Box 10, SF-20011 Turku, Finland) (Spon.: T. Kemell).

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988 1163
We describe a new time-resolved immunofluorometric assay of hCG. The assay is based on two specific monoclonal antibodies, a stable fluorescent Eu chelate label and time-resolved fluorometry. Anti-ß-hCG antibodies are immobilized on the bottom of microtitre strip wells made of polystyrene with low fluorescence background. Anti-ß-hCG antibodies are labeled with a novel highly fluorescent Eu chelate. The stable and fluorescent chelate obviates the need for additional dilution and fluorescence enhancement step as used in the Delfia system.

In the assay 25 µl of standards or samples were incubated with 175 µl of assay buffer containing the labeled antibodies in anti-ß-hCG coated strips for 1 h. After washing the strips were rinsed with ethanol and the fluorescence was measured directly at the bottom of the strips.

The hook problem encountered in one-step assays when measuring samples with extreme hCG concentrations was alleviated by using a high excess of labeled antibodies. The resulting standard curve ranged from 1 to 10,000 int.units/L (IMM 1st lRP 75/537). Recalculation of samples giving over 1,000 int.units/L was performed after 1:100 dilution.

Preliminary evaluations of the assay showed good precision; the CV dose remained below 10% within the standard range from 10 to 10,000 int.units/L. The detection limit was 0.7 int.units/L (0.99 EU) and the assay correlated well with the Delfia hCG assay (r=0.99). The cross reactivity with LH was below 2%, and with PSH and FSH it was negligible.

055 DEVELOPMENT OF A MONOCLONAL MAGNETIC SOLID PHASE RADIOIMMUNOASSAY FOR ACTIVE RENIN, D. J. Hartmann; D. Simon; G. B. Hall; D. J. M. Lyon; J. Menard; P. Corvol; B. Pau'nd (Centre de Radioanalyse, Institut Pasteur, Lyon- Sanofi Recherche, Montpellier - Inserm U36, Paris - ERIA Diagnostics Pasteur, Marne, France) (Spon: IRC Ente.)

Until recently, the measurement of enzymatic activity of renin was the unique method to determine its serum level. We have developed a magnetic renin immunoradiometric assay with two monoclonal antibodies (the binding constants of which are higher than 10¹⁰ M⁻¹). One antibody is immobilized onto magnetic particles and the other antibody, specific to the active site of the enzyme, is radiolabeled with iodine 125.

250 µl of undiluted plasma are incubated with 250 µl of magnetic solid phase. After 2 h incubation on a horizontal shaker at room temperature and washing, 250 µl of [¹²⁵I] anti-renin are added and incubated for 3 h. After decantation and washing, the solid phase is counted.

The minimal detectable dose of renin at 95 % confidence level was estimated as 3 ng/L. Within-run precision on 10 replicates had coefficients of variation ranging from 6.1 to 3.1 % for samples with renin concentration between 63 and 587 ng/L. Between-run precision on 10 separate assays had coefficients of variation ranging from 8.3 to 6.0 % for the same samples. Regression analysis of the test with enzymatic assay before and after trypsin activation of renin in healthy and hypertensive patients gave correlation coefficients greater than 0.7.

This magnetic assay provides a rapid and efficient separation of renin from other reactions (centrifugations are not required), the procedure is achieved within 5 h. Moreover the test routinely allows the measurement of active renin concentrations from 7 to 640 ng/L with undiluted sera during the exploration of various pathologies.

056 MICROCAPSULE IMMUNOASSAY (MCIA) Kyji Rokugami, Y. Takiguchi (Med.Eng Lab.,Toshiba Corp.,Totsuka-ashi,2-29-26,Japan), Y. Ishimori, T. Tasunakau, M. Koyama(Toshiba Corp.), H. Matsuda, T. Ohara(Green Cross Corp.) and S. Sekine (Denka Seikisaku Co.) (Spon: P.Watanabe) Multivalent liposome composed of dipalmitoylphosphatidylcholine, cholesterol and dipalmitoylphosphatidylethanolaminolipid (DPPV) modified by a hetero-bifunctional crosslinking reagent (SPDV) were encapsulated with one of the samples in liposome. The liposome concentration was reduced by diithiothreitol. The reduced liposome were much more stable than those before reduction when they were reacted with bovine or goat serum (GSA). The optimized liposomes loaded monoclonal antibody (MA) in an example of ferritin.

MA modified with SPDV was mixed the reduced liposome suspension to be immobilized. The MA-bearing liposomes and human sera were first mixed in a well of microtiter plate for 10 min at 37°C, then rabbit anti-ferritin antibody and GSA for complement sequence were added to the reaction mixture after 30 min at 37°C was followed. The CV released from the liposomes based on the specific immune reaction was measured with a fluorescence spectrophotometer (MTP-32,Corona Electric Co., Katsuta,Japan) (Ex: 490 nm, Em: 530 nm).

Detection range of ferritin in sera using MCIA was 10-1,000 ng/ml. When we determined ferritin of 50 ng/ml repeatedly (n=10) the reproducibility was less than 5 % in CV. A good correlation coefficient of RIA such as 0.96 was obtained. The liposomes were stable for at least three months in refrigerator.

We concluded that the MCIA system could be applicable for the determination of various compounds like tumor markers in human sera.

057 EVALUATION OF A HOMOGENEOUS ENZYME IMMUNOASSAY FOR THEOPHYLLINE ON THE BAKER ENCORE, R.C. Schiebout-Clark, M.C. Haven and R.S. Markin. (Dept. of Path, Univ. of NE Med. Ctr., Omaha, NE 68105) (Spon: Pat Fellows)

We investigated a homogeneous enzyme immunoassay for theophylline based on enzyme recombination(1) and manufactured by Baker Inst. (Allentown, PA 18101). The two-point calibration of this immunoassay is an obvious advantage; reagent costs could be decreased and automated spectrophotometers without complicated cuvette fitting of non-linear calibration could be used.

The instrument used for this investigation was the Baker Encore™. The Encore pipettor automatically added 180 ul of reagent 1 (a solution of monoclonal antibody to theophylline and intercalating mutant B-D-galactosaldase), 60 ul of reagent 2 (a solution of theophylline conjugated and chlorophenol red-B-D-galactopyranoside (CPSG)), 3 ul of serum and 37 ul of distilled water to the Encounter disc. The Encounter disc was then incubated at 37°C, where reagents and samples were mixed and the absorbance change (at 405 nm) was monitored between 0 and 310 seconds. The concentration was determined from a linear calibration curve derived from the two calibration standards.

The patient comparison study of theophylline values obtained by Abbott TDx (x) [mg/L] and Baker Encore™ (y) [mg/L] were as follows: corr. coeff. = 0.9912, y = 1.002x + 0.43, N=100. Dilution studies yielded a recovery range of 97.6% to 104.1%; recovery studies yielded average recoveries of 96.2% to 108.7%. The within-run precisions for controls with mean values of 15.7 and 25.9 were 0.27 and 0.55 respectively; the total SD was 0.41 and 0.71 with CV's of 2.4% and 2.7%.

In our study of possible interferences in uricemic patients, only two specimens with creatinine concentrations of 4.0 and 6.8 mg/dl showed disparate theophylline values.


058 IMMUNOEXTRACTION AND RIA OF ATRIAL Natriuretic Peptide (ANP) IN HUMAN PLASMA SAMPLS, Sung C. Lee, A.M. Brudzinski, J.L. Yasinoln, L. Hummahl-Schendel, J.S. Jackson, D.P. Hall, and J.W. Orf (ENCENT Corp., P.O. Box 285, Stillwater, MN 55082) (Spon: Orin L. Carter)

Accurate measurement of ANP in human plasma samples require laborious extractions using Sep-Pak columns prior to RIA or radioimmunoassays. We have developed an immunoeextraction method which uses anti-ANP antibody coupled to Sepharose 4B. It offers ease-of-use and does not require organic solvents.

A sensitive RIA with a detection limit of 13 pg/ml was also developed. The assay components include a radiodinated tracer, sheep anti-ANP antibody, and horse anti-sheep second antibody. The assay is run -2-3 days for 2 days. The dynamic range of the assay (90X B/Bo to 10X B/Bo) is from 20 to 700 pg/ml. The within-run and between-run CV's at 50X B/Bo were 6.1% and 13.1% respectively.

The RIA values of the ANP spiked human plasma samples by the immunoeextraction method, correlated well with those by the Sep-Pak method (r=0.983, slope=1.16, y-int.=35 pg/ml). The average recovery efficiency of immunoeextraction within the dynamic range of the assay was about 70%. The values of ANP for 20 normal plasma samples with immunoeextraction followed by the RIA ranged from 8 pg/ml to 57 pg/ml with a mean of 28 pg/ml.

In summary, an easy-to-use immunoeextraction and a sensitive RIA was developed for the accurate measurement of ANP in human plasma samples.

Development of chromium dioxide particle assay by Du Pont allows immunological testing in as little as 30 minutes. The assay is performed on 30 ml. of serum sample. The sample is incubated with the antibody-coated chromium dioxide and monensin. The monensin-poisoned chromium dioxide particles are then washed and counted in a well counter. The sensitivity of the assay is high enough to detect as little as 100 ng/ml of protein with no evidence of a "hook effect." Recovery of CEA spiked into patient samples is 100% ± 2%.

A FLUOROMETRIC ENZYME IMMUNOASSAY FOR ANTIBODIES SPECIFIC TO HEPATITIS B SURFACE ANTIGEN (ANTI-HBs) USING CHROMIUM DIOXIDE PARTICLES AS SOLID PHASE. Gregory J. S. Sam, (E. I. Du Pont de Nemours & Co., Inc.), Medical Products Department, Wilmington, DE 19890 (Spons. W. B. Allken).

Assay for detection of antibodies specific for Hepatitis B Surface Antigen (Anti-HBs) permit the determination of the immune status of individuals infected with the Hepatitis B Virus and the determination of positive antibodies in vaccinated individuals. The enzyme immunosassay for Anti-HBs that utilizes chromium dioxide particles is the solid support.

Chromium dioxide particles are coated with human plasma-derived Hepatitis B surface antigen (HBsAg). Serum or plasma samples and a Tris-buffered diluent containing bovine serum albumin were incubated with the particles for 30 minutes at 37°C. The particles were magnetically separated and washed three times. A solution of conjugate reagent consisting of alkaline phosphatase-labeled HBsAg, was added to the particles and incubated for 30 minutes at 37°C. The particles were washed and resuspended in a second series of three washes after which the fluorigenic substrate, 4-methylumbelliferyl phosphate, was added. The particles were incubated for 5 minutes at 37°C and EDTA was added to quench the reaction. The fluorescence was measured at an excitation wavelength of 365 nm and an emission wavelength of 450 nm.

The assay can be performed in 90 minutes. Results for 38 of 39 random donors (97%) correlated with Abbott AUSAB® EIA. The discordant sample was within 10% of the Abbott EIA cutoff, but was positive in the Anti-HBs particle assay. The individual had received the second dose of a 3 dose Hepatitis vaccine regimen one month prior to sampling.

The automated Stratus Fluorometric Enzyme Immunosassay system was evaluated for the quantification of serum IGE. The assay procedure is based on the two-site sandwich immunosassay methodology. Using the double antibody technique, mouse monoclonal anti-IGE antibody is pre-immobilized onto glass fiber paper. Clinical sample is pipetted onto the paper where it reacts with the immobilized antibody. A conjugate consisting of an enzyme labeled Fab' of a second mouse monoclonal anti IGE antibody is added onto the reaction site. The labeled conjugate reacts with the antibody-anti IGE to complete the "sandwich." When a substrate wash solution is added, an enzymatic reaction is initiated, the unbound conjugate is eluted away from the field of detection. The enzymatic rate of the bound conjugate is measured and is directly proportional to the IGE concentration.

The assay procedure requires 200 ul of serum. The sensitivity of the Stratus IGE assay is 2 IU/ml. Sample correlation studies performed against Pharmacia Prist RIA method revealed the following linear regression data.

<table>
<thead>
<tr>
<th>Method</th>
<th>Slope</th>
<th>Intercept</th>
<th>r</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y (Stratus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X (Pharmacia Prist) 0.96 -2.0 0.995 31

The Stratus method gives good correlation and relatively faster turn around time compared to the Pharmacia Prist RIA Method. (Throughput is 30 results in 30 minutes.) Precision studies are in process of being done to validate the manufacturer's claim of less than 5% cv.

HUMAN IGM RHEUMATOID FACTOR REACTIVITY WITH RABBIT, SHEEP, GOAT AND MOUSE IMMUNOGLLOBULIN. Robert B. Hamilton, W. Whittington, N.B. Worner, and F.C. Arnett. Rheumatology, Univ. of Texas Medical School, Houston TX 77030 (SPON. R. G. Hamilton).

Human rheumatoid factors are autoanti-IgM Fo that can interfere in immunosassays by binding to the rabbit, sheep, goat and mouse IgM used as capture and detection antibodies. RF interference can lead to non-parallel lines associated with lower results in quantity immunosassays and false positive lines in screening assays.

The objective of this study was to define the frequency and pattern of human IgM RF reactivity to polyclonal rabbit, sheep, goat and mouse IgG. Sera were collected from patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjogren's Syndrome (SS) and healthy controls. RF was detected by enzyme immunoassay using solid phase human or animal IgG and peroxidase-goat anti-human IgM as capture and detection reagents. Positivity was conservatively defined at multiple serum dilutions as the optical density > 3 X the non-specific binding levels of the same serum dilution to fish gelatin-coated solid phase.

The frequency of human IgM RF in RA, SLE in SS, 4% in SLE and 0% in healthy controls. RF reactivity in all patient groups followed the same binding pattern to common determinants on heterogeneous IgM: rabbit > sheep > goat > mouse (Table). Over 90% of RA patients had IgM RF that reacted with mouse IgG which poses a problem for assays using murine monoclonal antibodies. Importantly, 8% of sera from healthy individuals with no apparent rheumatic disease contained RF, some with reactivity to animal IgG. In summary, IgM RF's have been detected in human serum that bind with Group Patient Human Rabbit Sheep Goat Mouse

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>SD</th>
<th>CV</th>
<th>LINEARITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric Acid</td>
<td>100</td>
<td>0.06</td>
<td>1.1</td>
<td>20.0 mg/dl</td>
</tr>
<tr>
<td>Glucose</td>
<td>100</td>
<td>0.11</td>
<td>1.1</td>
<td>90 mg/dl</td>
</tr>
<tr>
<td>Creatinine</td>
<td>100</td>
<td>0.05</td>
<td>1.5</td>
<td>20.0 mg/dl</td>
</tr>
<tr>
<td>Control Serum</td>
<td>100</td>
<td>0.09</td>
<td>0.9</td>
<td>15</td>
</tr>
</tbody>
</table>

The method correlation study was performed by assayng serum samples on each instrument. Regression analysis and coefficient of correlation (r) are as follows:

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>REGRESSION EQUATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric Acid</td>
<td>31</td>
<td>y = 1.07 x - 0.06</td>
</tr>
<tr>
<td>Glucose</td>
<td>35</td>
<td>y = 0.99 x - 0.22</td>
</tr>
<tr>
<td>Creatinine</td>
<td>41</td>
<td>y = 1.03 x + 0.15</td>
</tr>
</tbody>
</table>

Instrument and Product Evaluations—Part A

Evaluations of New Instruments
We conclude that the AUS021 correlates well with accepted methods and is an accurate and reliable instrument for the determination of these analytes.


The Olympus EIA system is a fully automated, random access analyzer capable of one-step, two-step, and competitive heterogeneous enzyme immunoassays by utilization of glass bead solid phase analysis methods. It is capable of processing up to eight test items simultaneously with a throughput from 60 to 90 tests per hour.

A performance evaluation of the system's analytical precision was conducted for two analytes. The methods analyzed were IgG and insulin, assayed utilizing a two-step sandwich method performed at 37°C.

The within run precision was determined by 20 replicate determinations on two levels of serum based quality control material. The resulting data is presented below:

**METHOD**

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>20</td>
<td>317</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>496</td>
<td>3.1</td>
</tr>
<tr>
<td>Insulin</td>
<td>20</td>
<td>15</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>74</td>
<td>2.6</td>
</tr>
</tbody>
</table>

The total precision was evaluated by assaying 5 replicate determinations on two levels of serum based control materials, once a day, for a total of 5 days. The results are as follows:

**METHOD**

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>25</td>
<td>314</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>489</td>
<td>4.5</td>
</tr>
<tr>
<td>Insulin</td>
<td>25</td>
<td>15</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>74</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Based upon the results obtained, we conclude that the Olympus EIA system demonstrates more than acceptable precision.

Evaluation of a New Automatic Amino Acid Analyzer. J. Rudy, C. Argyle, F. Darras, S. Emanian, M. Zeigler, S. Lewis (Child. Med. Ctr., UT Southwestern, Dallas, TX 75235). (Spon.: J. Rudy)

We report our 12 month (1200 sample) evaluation of the recently available Varian HPLC amino acid analyzer. The system is designed to separate and quantitate 41 amino acids from deproteinized serum by ion exchange HPLC and post column ninhydrin derivatization. Using a 150 x 4 mm, 60 particle size, strong cation exchange column, chromatography is complete in 160 min. and re-equilibrium is achieved in 20 min. Identification and quantitation are computer controlled, using a reference amino acid solution and an internal standard.

Retention time CV% ranged from 3.2% for lysine to 10.9% for aspartic acid. Calculation factors had been run CV% ranging from 0.8% for 3-methyl histidine to 27.8% for phenylalanine and necessitated daily evaluation. Minimum detectable concentrations were from 1 to 100 nmol/L, linearity exceeded 2000 umol/L and recovery from serum ranged from 76% for arginosuccinic acid to between 92.4% and 100% for the other amino acids. Run to run CV%, determined using Dade VRA II control sera, varied from 3.0% for histidine to 11.8% for lysine.

Routine maintenance required 2-3 hours/week, unscheduled repairs 5 days during the year. Column life exceeded 1000 tests, spectrophotometer lamp life exceeded 2000 hours.

Using Varian's recommended elution program, asparagine glutamic acid, cystathionine/lysine and histidine/methyl histidine are difficult and sometimes unreliable separations, to achieve them requires close adherence to maintenance protocols.

Despite daily standardization, computer errors in peak assignments and/or calculations necessitated careful review and editing of every chromatogram. Within each chromatogram, over 20% of the amino acid results required manual recalculation.

Overwhelming evidence indicates that the Apo Al/B ratio is a more accurate biochemical marker of coronary heart disease than conventional measurements of lipid or lipoprotein-cholesterol. We evaluated the Beckman Array, random access, rate nephelometric protein analyzer for both within run and run-to-run precision, linearity of dilution, accuracy, and stability of analytic after storage at 4°C. Reference ranges were calculated from results of assays performed on 335 patient's sera whose cholesterol levels were within our reference range. RESULT: Within run CV at levels of 108 mg/dl for Apo Al and 104 mg/dl for Apo B were 1.5% and 1.7%, respectively. Between run CV at levels ranging from 75 to 151 mg/dl for Al and 121 to 150 mg/dl for B were 4.6% to 6.2% and 3.7% to 4.1%, respectively. Recovery ranged from 88 to 109% for Al and 94 to 112% for B. Accuracy of analysis was determined to be excellent. Both Al and B were stable for at least 13 days at 4°C. Observed reference ranges for these analytes are:

**Apo Al (mg/dl) Apo B (mg/dl)**

<table>
<thead>
<tr>
<th>Method</th>
<th>Male</th>
<th>Female</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=165)</td>
<td>(n=170)</td>
<td>(n=335)</td>
</tr>
<tr>
<td></td>
<td>95 - 205</td>
<td>100 - 225</td>
<td>95 - 220</td>
</tr>
</tbody>
</table>

**Conclusion:** Our data together with others indicate that Beckman Array is capable of accurate and precise determination of Apolipoproteins Al and B.

Adaptation and Evaluation of CEIA™ Thyroxine on the Coulter CPA Chemistry Analyzer. F. Lucas, S. Ho (Coulter Diagnostics Division, Hialeah, FL 33014). (Spon.: N. Turner)

The Coulter CPA (Chemistry Profile Analyzer) is a new automated biochemistry analyzer providing an economical, flexible approach to small laboratories with a wide range of assay requirements. The Cloned Enzyme Donor Immunoassay (CEIA) (CEIA is a trademark of Microgenics, Inc., Concorde, CA) for the quantitative measurement of thyroxine utilizes a new concept in immunoassay. By recombinant DNA techniques, the common enzyme B-galactosidase has been split into two totally inactive polypeptide fragments. The two fragments can spontaneously recombine to yield the catalytically active enzyme. Thyroxine has been covalently attached to one fragment and when a specific thyroxine antibody binds, it will inhibit the spontaneous reassembly. Sample thyroxine binding to the antibody allows assembly of the enzyme providing a direct proportionality of thyroxine concentration to the hydrolysis of substrate. No sample pretreatment is required and STAY thyroxine results can be provided. Performance of the assay on CPA is as follows:

**Precision:**

<table>
<thead>
<tr>
<th>Method</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within Run</td>
<td>4.33, 0.684, 15.81</td>
</tr>
<tr>
<td></td>
<td>11.83, 0.72, 6.08</td>
</tr>
<tr>
<td></td>
<td>19.25, 0.635, 3.30</td>
</tr>
</tbody>
</table>

**Conclusion:** In conclusion, the application of CEIA Thyroxine Assay demonstrates results that compare favorably with established methods.

Correlation of Serum Electrolyte Analysis on a Synchrnon CX5-5 with a Synchron CX5-3 and a Synchron AS™ Auto-Analyzer. J. Mullhawe, J. Francis (Beckman Instruments, Inc., Brea, CA 92621). (Spon.: M. Ruth)

A set of forty (40) patient serum samples were analyzed using a Synchrnon CX5-5 auto-chemistry analyzer. The samples were analyzed for sodium, potassium, chloride, and carbon dioxide content. The results were correlated to data obtained on the same samples using a Synchrnon CX5-3 system and a Synchron AS™ auto-analyzer. The slopes of the correlation lines were between 0.98 and 1.05 for sodium, potassium and chloride, all with r²=0.98. For carbon dioxide, calculated to CX5-3 correlation yielded a slope of 1.12, with r²=0.985, and the CX5-5 to SYNCHRON AS™ correlation yielded a slope of 0.92 with r²=0.991.
The correlation of results between the instruments is thus very good. Additionally, studies on within-run precision and between-run precision for the serum electrolytes have been performed. Within-run precision was determined to be better than published specifications, typically less than 2% CV. Between-run precision was also observed to be better than the 4% CV published specifications for serum electrolytes.


We evaluated the Reflotron system for the determination of blood glucose levels. The Reflotron assay of glucose uses dry reagent and a carrier. After the sample was applied on that carrier, this carrier was inserted into the Reflotron instrument. The result was displayed 150 seconds later. Within-run precisions with venous whole blood were 2.34% at 97.7 mg/dl (n=32) and 1.71% at 190 mg/dl (n=33) levels. Between-run precisions with control sera were 2.6% at 81.5 mg/dl (n=23) and 3.0% at 252 mg/dl (n=23) levels. Preparatory study recovered averaged 96 ± 3%. The linearity was up to 560 mg/dl concentration. We compared the Reflotron to the Astra and Olympus analyzer. The regression data and coefficients of correlation are shown below:

<table>
<thead>
<tr>
<th>y</th>
<th>x</th>
<th>n</th>
<th>slope</th>
<th>Intercept</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>W Reflotron P Astra</td>
<td>50</td>
<td>0.958</td>
<td>4.08</td>
<td>0.994</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>W Reflotron P Astra</td>
<td>50</td>
<td>0.980</td>
<td>4.03</td>
<td>0.997</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>W Reflotron P Olympus</td>
<td>46</td>
<td>0.918</td>
<td>13.13</td>
<td>0.994</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>W Reflotron P Olympus</td>
<td>46</td>
<td>0.960</td>
<td>8.58</td>
<td>0.992</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>W Reflotron W Astra</td>
<td>50</td>
<td>1.017</td>
<td>0.815</td>
<td>0.996</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>W: Whole blood, P: Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No significant interference was observed with lipemic, hemo- lytic or icteric samples. But fluoride at 0.5 mg/dl level decreased the glucose concentration by 6%. Although the plasma samples correlated highly with the whole blood samples (r=0.996), there was statistically significant difference between them (p<0.05).

We concluded that the Reflotron system for measurement of blood glucose was reliable, compared favorable with both Astra and Olympus analyzer and was suitable for stat analysis.

**THE ADX AMPHETAMINE/METHAMPHETAMINE URINE ASSAY, Teresa Gates, and Michelle Jenkins (Abbott Laboratories, Diagnostics Division, P. O. Box 15200, Irving, TX 75015-2020) (Spon.: G. Stans)**

An assay for amphetamine and methamphetamine in urine has been developed for the ADx utilizing the fluorocence polarisation technology (FPIA) proven on the TDx. The ADx immunoassay uses the same reagents as the TDx although the new format gives the operator the flexibility of three modes of operation: panel, combination, and batch testing. This allows the operator to run the amphetamine test with as many as nine other assays for optimum efficiency. The assay has high specificity for amphetamine and methamphetamine. Phenylpropanolamine, ephedrine, and pseudoephedrine, each at 1000 μg/ml, cross react at less than the sensitivity of the assay.

The six point calibration curve exhibits a minimum of 14 day curve stability with calibrators ranging in concentration from 0.0 to 3.0 mg/ml.

Results from precision study with a minimum of 50 points, over 14 days, yielded within run and total CV's of less than 7% at 0.3 mg/ml and less than 5% at 2.0 μg/ml.

Linear regression analysis of patient samples on multiple ADx's versus multiple TDx's yielded a slope of 0.95 ± 0.05 and correlation coefficients (r²) greater than 0.95.

The ADx Amphetamine/Methamphetamine Urine Assay is a reliable urine screen for amphetamine and methamphetamine and allows the operator the flexibility to prioritize the daily work load. The assay exhibits 14 day curve stability, CV's of less than 7% at all concentrations, and good correlation with a reference method.

**EVALUATION OF COCAINE METABOLITE, PHENYLGLYCINE, OPIATES, AND BARBITURATES ASSAYS ON THE ADX, Jack Ramsey, Teresa Gates, Kyle Kratinsky, Michelle Jenkins, and James Maleratz (Abbott Laboratories, Diagnostics Division, P. O. Box 15200, Irving, TX 75015-2020) (Spon.: J. Ramsey)**

Assays for Cocaine Metabolites, Phenylglycine (PGP), Opiates, and Barbiturates in urine have been developed for the ADx analyzer utilizing the proven TDx fluorescence polarisation technology. The ADx is a universal analyzer utilizing the TDx FPIA technology by allowing multiple assays to be performed in the same run. All four assays have been tested in the areas of precision, stability of the calibration curve, sensitivity, high sample carryover, and comparison with the TDx and GC/MS as reference methodologies.

Precision was determined by analysis of 50 data points collected in 10 runs over a 14 day period. The percent CV for within run and total precision were less than 4% and 5%, respectively.

The stability of the stored calibration curves was determined from data collected from the calibrators run as unknowns at intervals over the same 14 day period. All assays demonstrated no drift greater than 10% in concentration over the period of the study, thus giving a minimum of 14 days curve stability.

For comparison with the TDx and GC/MS, 50 positive and 50 negative patient samples were analyzed simultaneously by all methods. Correlated with TDx, all assays demonstrated correlation coefficients (r²) of greater than 0.98 and slopes of 0.95 to 1.05. Concordance with GC/MS was greater than 95% in all cases.

We conclude that these assays on the ADx are accurate and reliable screens which, when combined with the flexibility of the ADx, can serve the needs of the drug testing community.

**A MATERIAL FOR THE ASSESSMENT OF QUALITY CONTROL OF ION SELECTIVE ELECTRODE (I.S.E.) ANALYZERS FOR CA++ , B. Guouet, Y. Courriel, A. Truchaud. (Biochemistry Laboratory, Henri Mondor Hospital, 77104 MEAUX, FRANCE) (Spon.: A. Truchaud)**

A precise and reliable measurement of Ca**+ on serum or whole blood requires an adapted quality control (QC) of the available ISE Ca++ analyzers. We propose 2 protein-free solutions with zwitterionic buffers (HEPES and MOPS, 10 mmol/l) gaining anion strength for pH and with an ionic strength of 160 mmol/kg. Aliquots were prepared in sealed glass ampoules, with 3 different calcium concentrations: 0.75, 1.25, 2.00 mmol/l. This study included 16 instruments by 6 manufacturers (AVL, CIBA-CORNING, RADIOMETER, KONE, FRESENIUS, NOVA) in biochemistry laboratories. QC aimed to evaluate the analytical performance over 3 months. QC was performed at each level of concentration before and after any workload.

The statistical analysis of the results showed that the within run precision was identical for all the instruments and with both aqueous solutions (c = 0.4-1.0x). A significant source of imprecision was observed in the interlaboratory variability (p <0.05), but not always on the RADIOMETER analyzers (ICal, ICal2). No difference was found in the results of QC before and after routine series. The results exhibited 2 levels of response separating two groups of instruments, according to the theoretical calcium concentrations of the solutions (AVL, KONE = 5-50 x vs. the other instruments).

In conclusion, despite the lack of protein content, the aqueous solutions were efficient to evaluate the precision and able to demonstrate the systematic bias of measurement with Ca**+ ISE analyzers. This bias might be reduced, for instance, by agreement on a common calibration solution.

**RAPID CARDIAC INFARCT MEASUREMENT USING A 560 Express ANALYZER, R.J. Shambear, E. Michaels, and A.W. Boudler (Ciba Corning Diagnostic Corp., Gibbstown Systems, Oberlin OH. 44074) (Spon.: Raymond J. Shambear)**

We have evaluated the performance of the 560 Express biachromatic, random-access analyzer, using Gibbstown reagents, to determine CK-NAC, LDH, and AST in serum at 37°C. Within run and between run precision were measured with two serum pools, using guidelines from NCCLS document EP-2. Linearity was measured.

Within Run Overall

<table>
<thead>
<tr>
<th>Test</th>
<th>N</th>
<th>x</th>
<th>C.V. (%)</th>
<th>C.V. (%)</th>
<th>Linearity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>30</td>
<td>116.3</td>
<td>1.5</td>
<td>1.1</td>
<td>570 U/L</td>
</tr>
<tr>
<td>LDH</td>
<td>30</td>
<td>589.1</td>
<td>0.9</td>
<td>0.8</td>
<td>730 U/L</td>
</tr>
<tr>
<td>AST</td>
<td>30</td>
<td>729.6</td>
<td>0.9</td>
<td>1.0</td>
<td>625 U/L</td>
</tr>
</tbody>
</table>

We compared the 560 Express to either a Hitachi 706 (H-1), or a Hitachi 750 (H-3) at a test site; we also compared the 560 Express to either the Cobas BCO (C) or the Impact 4000 (I). Interference studies were done.
### Test Method | N | Slope | Intercept | Sy.x | r |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>86</td>
<td>10.06</td>
<td>15.1</td>
<td>17.8</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>LDH</td>
<td>86</td>
<td>10.94</td>
<td>1.1</td>
<td>7.1</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>LDH</td>
<td>46</td>
<td>1.078</td>
<td>4.9</td>
<td>10.0</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>AST</td>
<td>86</td>
<td>1.1</td>
<td>1.4</td>
<td>2.9</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>AST</td>
<td>46</td>
<td>1.112</td>
<td>2.6</td>
<td>6.1</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

Spin times for AST (60 seconds), LDH (40 seconds), and CK (120 seconds), and automatic dilution of elevated samples, indicate that the 560 Express could be used in a "stat" laboratory or a cardiac unit.

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**074**

BIURET DETERMINATION OF TOTAL PROTEIN USING THE 560 EXPRESS AND OTHER INSTRUMENTS, Kenneth C. Burns, Mary J. Banez Jr., Elaine M. Banez, and Alan W. Sandler (Clia Cornong Diagnostic Corp., Gilford Systems, OH (44074)).

(Selected from Alan W. Sandler, Ph.D.)

We compared the biuret method for determination of total protein in serum using the 560 Express chromotographic, random-access analyzer (B) with the biuret method as employed by the Hitachi 706 bichrometic, random-access analyzer (H) and the impact 4006 (4), as well as with the biuret reference method (8).


Comparison

- **Slope**
- **Intercept**
- **Sy.x**
- **r**

| E vs. B | 1.001 | -0.050 | 0.201 | 0.98 |
| H vs. B | 1.051 | -0.543 | 0.227 | 0.95 |
| I vs. B | 1.026 | +0.026 | 0.228 | 0.97 |
| E vs. H | 0.953 | -0.311 | 0.338 | 0.93 |
| E vs. I | 0.978 | -0.044 | 0.386 | 0.94 |
| H vs. I | 1.033 | -0.587 | 0.360 | 0.94 |

The 560 Express response curve is linear to 11 g/dl. Within run and overall precision were estimated using a multivariate format with high, mid, and low sample pools intermixed.

**075**


Routine coagulation tests, prothrombin times (PT) and activated partial thromboplastin times (aPTT), were performed on plasma samples obtained by high and low speed centrifugation from 50 whole blood specimens, 23 of which were from patients on anticoagulant therapy (heparin, coumadin). Blood was obtained by venipuncture and collected into vacutainer tubes (Secton-Pinckton, Rutherford, NJ) containing sodium citrate (final concentration 0.382). Blood specimens were divided and either centrifuged for 20 min (2500 rpm, 4°C) using a TJ-6 centrifuge (Beckman Instruments, Palo Alto, CA) or for 2 min (20,000 rpm, 22°C) using a Stat-SPin I centrifuge (Norton Scientific, Norwood, MA). PT and aPTT determinations were performed on the resultant plasma samples using an MLA-Electra 700 (MLA, Mt. Vernon, NY). The PT and aPTT tests were initiated with GD Simplastin Automated and Automated aPTT reagents (General Diagnostic, Nortis Pluma, NJ, respectively).

No significant differences in PT and aPTT results were noted between plasma samples obtained by high and low speed centrifugation in random hospital patients. p > 0.9, p > 0.5, respectively). Similar results were obtained with plasma samples from patients on anticoagulant therapy or with high platelet counts (>400,000/μL, <800,000/μL).

The good data that the Stat-SPin I, high speed centrifuge, can be used reliably to process whole blood specimens for routine coagulation testing. Since the centrifugation time of the Stat-SPin I is approximately 1/10 that of traditional, lower speed centrifuges, the instrument is ideally suited for the processing of blood specimens for routine coagulation testing.

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**076**

ESTIMATE OF PRECISION OF ENDPOINT CHEMISTRIES ON THE BECKMAN SYNCHROM CEA/CE5 CLINICAL SYSTEM.

In this study, we evaluated the within run precision of the endpoint chemistries on the Synchro CEA/CE5 Clinical System. The chemistries evaluated included the following:

**CHEMISTRY**

- Albunin
- Calcium
- Cholesterol
- Direct Bilirubin
- Total Bilirubin

**METROLOGY**

- BCP
- Phosphorus
- Triglycerides
- Uric Acid

This study was conducted to evaluate the within run precision of the endpoint chemistries on the Synchro CEA/CE5 Clinical System. The chemistries evaluated included the following:

**CHEMISTRY**

- Albunin
- Calcium
- Cholesterol
- Direct Bilirubin
- Total Bilirubin

**METROLOGY**

- BCP
- Phosphorus
- Triglycerides
- Uric Acid

---

**077**


The Beckman LABLITE System 810 Analyzer is a three channel benchtop instrument which measures sodium, potassium and chloride in serum/plasma, whole blood and urine. Sodium, potassium and chloride concentration in a sample is determined using direct, flow-through, ion-selective electrodes. Samples may be introduced directly into the analyzer from either a sample cup, syringes or test tube. A sample volume of 120 μL is required for analysis.

Factors have been incorporated into the system which allow correlation to reference methods. Serum correlation data against the Beckman Klinite Flame (for sodium and potassium) and the Beckman SYNCHROM AS (ASTRA) for chloride is presented below:

<table>
<thead>
<tr>
<th><strong>Intercept</strong></th>
<th>Mean X</th>
<th>Mean Y</th>
<th>Slope (mmol/L)</th>
<th>(mmol/L) (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.95</td>
<td>7.81</td>
<td>140.9</td>
<td>142.2</td>
<td>0.993</td>
</tr>
<tr>
<td>1.01</td>
<td>0.08</td>
<td>6.34</td>
<td>6.67</td>
<td>0.998</td>
</tr>
<tr>
<td>0.98</td>
<td>3.65</td>
<td>114.8</td>
<td>114.3</td>
<td>0.997</td>
</tr>
</tbody>
</table>

Within run precision data was also performed on a serum pool. The results are as follows:

- **Mean**
  - Sodium: 133.4
  - Potassium: 7.96
  - Chloride: 104.3

The evaluation of this data indicates acceptable clinical performance of the LABLITE System 810.

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**078**


The PHENIX bench-top analyzer measures the eight analytes listed below at a rate of 600 tests per hour. Use of electrodes and immobilized enzymes results in low cost reagents, all of which have greater than one month room temperature stability on the system. Ninety-Five microliters of sample are required for a complete eight parameter panel.

The PHENIX analyzer employs novel fluid handling and quantification techniques to minimize moving parts and assure a high degree of reliability. Electrodes are used to measure oxygen and ammonium ion concentrations as they are released from glucose and urea by immobilized enzymes. TCQ measurement employs a flow injection method with quantification by pH electrode. Four electrolytes (Na, K, Cl, and Ca) are analyzed.
in a single reaction mixture using ion selective electrodes. A dual-wavelength photometric channel is used for the kinetic measurement of creatinine (modified Jaffe).

The eight methods were evaluated using samples and/or control materials. Method comparison data are presented below:

**METHOD REFERENCE LINEARITY CORRELATION**

<table>
<thead>
<tr>
<th>Method</th>
<th>Reference</th>
<th>Slope</th>
<th>Intercept</th>
<th>r</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu</td>
<td>ASTRA</td>
<td>1.09</td>
<td>0.25</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>UN</td>
<td>ASTRA</td>
<td>1.15</td>
<td>0.25</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>TCOD</td>
<td>ASTRA</td>
<td>1.08</td>
<td>0.25</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>Cr</td>
<td>ASTRA</td>
<td>1.07</td>
<td>0.25</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>Na</td>
<td>ASTRA</td>
<td>1.06</td>
<td>0.25</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>K</td>
<td>ASTRA</td>
<td>1.05</td>
<td>0.25</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>Cl</td>
<td>ASTRA</td>
<td>1.04</td>
<td>0.25</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>Ca</td>
<td>ASTRA</td>
<td>1.03</td>
<td>0.25</td>
<td>0.95</td>
<td>0.98</td>
</tr>
</tbody>
</table>

We conclude that the PHENIX analyzer can provide clinical chemistry laboratories with the capability for analyzing more than half of their workload with excellent precision and high throughput.

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**079 PERFORMANCE OF THE CIBA-CORNING 664 "FAST 4" SYSTEM FOR MEASURING ELECTROLYTES IN SERUM, PLASMA, WHOLE BLOOD, AND URINE. Ronald D. Br, Melanie Swannsmeirer, and Harold Stickland (Dept. of Path. & Lab. Med., Methodist Hosp. of Indiana, Indianapolis, IN 46202) (Spons.: Bernard E. Statland)**

We evaluated the 664 "FAST 4" system (Ciba-Corning Diagnostics Corp., Medfield, MA 02052) for the measurements of Na⁺, K⁺, Cl⁻, and Ca²⁺ by direct ion-selective electrodes; CO₂, by a thermal conductivity method. Precision was assessed using four sets of controls and two urine controls during a two-week period. With the serum controls, we found total precision (CV%) less than 2.0% for Na⁺, K⁺, Cl⁻, and Ca²⁺ and <0.5% for CO₂, and the urine controls, the CVs were <3.0% for K⁺ and Cl⁻, and <5.0% for Na⁺. Patients' results correlated well with the Beckman Synchras CIx method. Some representative data (units in mmol/L) are as follows:

- Serum Na⁺: FAST 4 = 1.04 Cl⁻ = 0.97 K⁺ = 0.93 Ca²⁺ = 0.95
- Urine Na⁺: FAST 4 = 1.01 Cl⁻ = 0.98 K⁺ = 1.00 Ca²⁺ = 0.96

The data reflect the excellent precision and repeatability of the instrument and indicate the system is useful for the measurement of these elements. We found three major advantages in operating the "FAST 4" system: (1) minimal maintenance, (2) electrodes, tubing and other parts can be easily swabbed, and (3) the instrument's software makes it easy to operate and troubleshoot. Forty samples can be analyzed for four tests automatically in a "walk-away" mode in less than 30 minutes.

---


We evaluated the analytical performance of the Model 560 "Alliance" Chemistry Analyzer (Ciba-Corning Diag. Corp., Oberlin, OH), a high throughput (>2,000 results/b) multichannel (22 on-line analytes), selectivebatch instrument. We found overall satisfactory performance with total precision (CVs, n=6 each) and method correlation (vs. NA-1000 and ASTRA-8):

**Test** | **Slope** | **Intercept** | **r** | **R²** |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Cre</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Glu</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>UN</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>TCOD</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Cr</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Na</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>K</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Cl</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Ca</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Replicate analysis (n=21) of a pooled patient sample using all flow cells or a single flow cell to determine the within-run precision produced the following results:

- **All Flow Cells** Single Flow Cell (IgG only)
  - IgG 1.1 1.1
  - IgM 2.2 2.2
  - IgA 1.1 1.1
  - IgD 1.1 1.1
  - IgE 1.1 1.1

This instrument produces good correlation with other methodologies and has excellent within-run precision.
Direct potentiometric measurement of Na, K and Cl has become a common practice in Beckman's LabLyte analyses (Brea, CA 92621) for Na and K (Model 800), for Na, K and Cl (Model 810), and for Na, K and Li (Model 830) were evaluated in our laboratory. Each instrument required 12 ml. sample and was equipped with an automated turntable holding 18 specimens. Cl measurements by these instruments were compared with those by the Astra-4 (indirect potentiometry) and the IL-943 (flame photometry). Patient sera were used for the correlation and control material, for reproducibility and linearity studies. All within-run precision studies (N = 18) using either pooled sera or single patient's sera and all analyses on all three instruments showed CV's of <1.5%. All between-run precision studies (N = 20 days) using control material for all analyses on all three instruments showed CV's of <1.5%, except for one K control on the 810 which showed a CV of 2.18.

Linearity and sensitivity on all three LabLyte systems were excellent on the Astra-4 and the IL-943. Correlation coefficients and slopes (N = 196) from all instruments were excellent. As an example, regression analyses on the 810 (y data) were as follows:

\[ y = 0.9886 \times x + 0.8408 \]  for Na.
\[ y = 0.9806 \times x + 0.4818 \]  for K.
\[ y = 1.0303 \times x - 0.0175 \]  for Li.

The three instruments were easy to use and maintain and generated reproducible results. The instruments were comparable analytically (in either the manual or automated mode) to the Astra 4 and the IL-943.

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**EVALUATION OF THE CIBA CORNING DIAGNOSTICS CSD BLOOD GAS ANALYZER**

We compared the CSD 278 Blood Gas Analyser, which utilises electrodes that do not require remembrancing, to the CDD 178. During the first phase, over 1000 clinical specimens per day were evaluated for 20 days. Each sample was assayed once on the CDD 178 and in duplicate on the CDD 278. Analyses were performed by 20 technicians to simulate routine clinical service. Technicians documented instrument operational characteristics, software "bugs," and obtained operative data.

Following appropriate software modifications and electrode changes, more than 1300 additional specimens were assayed over another 20 day period. Approximately 500 of these were analysed in duplicate on both models.

The following performance characteristics were calculated from the latter data.

**Analysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Test Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO2</td>
<td>1305</td>
<td>0.988</td>
</tr>
<tr>
<td>PO2</td>
<td>1311</td>
<td>0.986</td>
</tr>
<tr>
<td>PO2</td>
<td>1311</td>
<td>0.993</td>
</tr>
</tbody>
</table>

The CVs obtained for each parameter were divided into two ranges from 1.29 to 3.32 for PO2, 1.13 to 2.38 for PO2, 0.002 to 0.007 for pH on the CDD 278; and 1.4 to 4.28 for PO2, 0.003 to 0.006 for pH on the CDD 178.

Although all three parameters were linear throughout a wide range, PO2 values from the CDD 278 averaged 1.5 mm Hg lower. A software driven "correlation adjustment* compensates for such systematic biases, if desired.

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**COMPUTERIZED TIME STUDY PROGRAM APPLIED TO THE EVALUATION OF BECKMAN ARRAY AND ICS II INSTRUMENTS**

M. Horn, C. Vletas, and G. McElman (Dept. of Pathology, Creton Univer., OH, 43210) and M. Horn

Laboratory evaluations of instrumentation in the past have focused primarily on analytic performance; however, we feel that the accurate evaluation of labor requirements is an appropriate portion of all new evaluations. To facilitate direct measurements a computer program written in Basic for a TRS-80 Model 100 lap top computer was developed. Tasks can be user-defined including an operator-independent or "walk away" time. Additionally, the program automatically monitors the intervals between defined tasks as a means of identifying useful portions of time or potential inefficiencies in the ordering of tasks. Once in the timing mode only two keystrokes are required to move between timing different tasks. A simplified table of our results illustrates the potential utility of such information:

**Batch**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Array Minus</th>
<th>Wait Away Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.6</td>
<td>12.6</td>
</tr>
<tr>
<td>5</td>
<td>25.7</td>
<td>26.5</td>
</tr>
<tr>
<td>10</td>
<td>44.4</td>
<td>23.8</td>
</tr>
<tr>
<td>15</td>
<td>34.2</td>
<td>14.6</td>
</tr>
<tr>
<td>20</td>
<td>38.4</td>
<td>17.6</td>
</tr>
</tbody>
</table>

As expected, the more automated Array requires less total time, less operator time, and shows an ever-increasing time advantage with increasing batch size. This information allows an accurate calculation of test costs based on actual experience and anticipated test volume. In contrast, CAP workload values do not always exist for a particular instrument, do not reflect the actual conditions at each laboratory, and include pre- and post-analytic steps irrelevant to the instrument/procedure being evaluated.
088 EVALUATION OF THE ANALYTICAL AND OPERATIONAL PERFORMANCE OF THE PRISMA, S. Diaz, T. Pham and M. Pradier (Dept. Pathology, Baylor College of Medicine, Veterans Administration Medical Center, Houston, Texas 77030)

The PRISMA (Programmable, Individually, Selective, Modular Analyzer) (New Clinicon, 60 Commerce Way, Hackensack, N.J. 07601) is a high-speed, precision, computer controlled chemistry analyzer, comprised of 2 sections, a processing unit and a central control unit. The processing unit is modular in design and can maximally accommodate 48 channels. The multi-channel concept of the PRISMA allows maximum throughput (200 samples/hour). In addition, the PRISMA operates as a fully selective instrument with STAT capability. There are currently 21 resident chemistries including flame photometric, and point calibrated and kinetic enzyme assays.

We have evaluated the performance of the following assays: Na, K, Creatinine, Total Protein, Albumin, Total Bilirubin and Lactate chambers. Precision studies were conducted according to Protocol EPS-T of the NCCLS. We found excellent precision. We determined total CV's to be no more than 1.2% for the end-point calibrated assays, and no more than 2.3% for the kinetic enzyme assay. Correlation of patient results (N=60) for clinically relevant ranges were obtained. There was good correlation between the PRISMA and the ASTRA-0 (Beckman Instruments Inc, Tarrytown, N.Y. 10591). Regression analyses demonstrated correlation coefficients above 0.99 for all methods evaluated. In this respect, accuracy, as determined using NBS SRM-909 and CASCO standards (Tarrytown, ME 04096) was excellent, and in some cases the comparison methods had to be recalibrated.

We conclude that the PRISMA is precise, accurate, and compares well with established methods. Studies to assess additional analytes, as well as linearity, interferences, and operational performance, are in progress.

089 EVALUATION OF HELENA'S ELECTROPHORESIS SYSTEM, Ravi B. Bhalla and Morton K. Schwartz (Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021) (Spon.: R. Bhalla)

We have evaluated Helena's Electrophoresis System (HES) for CK and LDH isoenzymes and Serum protein (SPEP) electrophoretic fractionation and compared it with that of Beckman's Paragon™ electrophoresis system. HES consists of two separate units: 1) Electrophoresis Work Center (EDC); 2) Electrophoresis Data Center (EDC). EWC is a microprocessor controlled unit, which includes two separate electrophoresis chambers, staining wells, incubator and a dryer. EDC comprises a scanning densitometer, an IBM personal computer, color monitor, keyboard and a high speed graphics printer. The computer can store up to 8,000 electrophoretic scans. Agarose gels are used for electrophoresis.

Correlation studies were performed using patient samples and normal human control sera were used for precision studies.

Correlation

Analyte | W  | Slope | Intercept | R  
--------|----|-------|-----------|----
CK-Isoenzymes | 51 | 1.04  | -1.225    | 0.98
LDH-Isoenzymes | 165 | 1.07  | -1.437    | 0.95
SPEP(All fractions) | 125 | 0.99  | 0.111     | 0.95

Within-run precision (CV) values (N=12) were as follows: CK-MM(3.4%), CK-MB(4.0%), CK-BB(5.0%), LDH-1(2.23%), LDH-2(2.17%), LDH-3(2.09%), LDH-4(1.46%), LDH-5(5.33%), Albumin(2.7%), Alpha-1(5.32), Alpha-2(4.38) and Gamma(1.65).

Data for day to day precision (CV) studies (N=12) were as follows: CK-MM(3.9%), CK-MB(6.3%), CK-BB(6.2%), LDH-1(7.6%), LDH-2(5.3%), LDH-3(2.7%), LDH-4(1.96), LDH-5(5.65), Albumin(2.4%),Alpha-1(11.22), Alpha-2(8.91), Beta(4.9%) and Gamma(4.5%).

From the data given above, we conclude that HES is an excellent system suitable for routine operation in any laboratory.

090 Evaluation of the Abbott ADX® (ADx) Analyzer and Comparison of ADx® Assays (ADA) with the Abbott TDx®, Sysmex EMIT®, GenELISA®, and Gas Chromatography/Mass Spectrometry (GC/MS), Joseph Magno, E. Cochran, B. Mann (Spon. of Tex. Dept. Pharm., L.S.U. Health Sciences Center, Shrevepest, Tex. 71130) (Spon.: E. Horrocks).

The new Abbott ADx® Analyzer and associated ADA for Barbiturates (BARB), Cannabinoids (CND), Cocaine Metabolite (COCM), Opiates (OP) and Phenobarbital (PBAR) were evaluated for intrassay and interassay precision of control and drug spiked samples for 10 separate daily runs within a 14 day period. In addition, quantitative analyses were performed on ADx, TDx (Abbott Synthes EMIT®) and spiked samples, calibrated or control. All four types of calibrators and controls were analysed on each of the four instruments for the BARB, COCM, GP and PBAR tests. The CND assay was not used because the calibrators and controls utilised △-non-carboxy-tetrahydrocannabinol. At least fifty patient specimens were also analysed for each assay type by all four methods.

<table>
<thead>
<tr>
<th>Assay</th>
<th>LOW INTER-RUN</th>
<th>HIGH INTER-RUN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MED.</td>
<td>CV</td>
</tr>
<tr>
<td>BARB</td>
<td></td>
<td></td>
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<tr>
<td>COCM</td>
<td></td>
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<tr>
<td>GP</td>
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<tr>
<td>PBAR</td>
<td></td>
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</tbody>
</table>

*The inter-assay variance due to different assay cutoff concentrations.

The ADx permits random access, precise fluorescence polarization immunoassay urine drug testing in 100% agreement with all the tested assay procedures.

091 Evaluation of the Beckman Biomek™ GenELISA™ Using the DUPONT HTLV-III ELISA REAGENT KIT, Terry Johnson and J. Tim Jones (Beckman Instruments, Inc., Diagnostic Systems Group, Brea, CA 92621) (Spon.: T.K. Johnson).

The Beckman Biomek™ is an automated laboratory workstation capable of performing a wide variety of liquid handling operations in clinical, industrial, and research laboratory environments. The specific model evaluated here (GenELISA™) was equipped with prototype tools and software intended for use in clinical laboratories to perform non-isotopic immunoenzymometric assays using the 96 well microplate format. The prototype model was capable of bar code sample identification, sample preparation with liquid level sensing, sample addition, plate washing, optical density measurement, and report generation with data reduction. Components of the system included a robotic workstation, an IBM AT computer with printer, and an electronic interface unit.

The objectives of this study were to evaluate the performance characteristics of the workstation and to evaluate the application of a popular ELISA reagent kit to the system. Performance was evaluated by assaying HTLV-III positive control material diluted to produce optical densities close to the cutoff value. Single wavelength measurement at 405nm resulted in within run precision of 7.52 CV (ave OD=1.043; cutoff OD=0.915; n=24). Test parameters such as blank, control, sample, and reagent volumes, wash cycles, and wavelength selection were easy to specify or change via a recallable protocol setup screen.

The Biomek GenELISA™ accommodated the Dupont HTLV-III ELISA kit easily and with good performance. The integration and automation of sample identification, sample processing, plate washing, and optical density measurement into a single system minimized the handling of infectious materials and facilitated the isolation of HIV testing.

092 STAT LABORATORY CHEMISTRY ON THE DIONEX™ 380, T. Dawson, T. Kitt, K. O. Aah, R. Weiss, and A. Smith (University of Utah/Associated Regional and University Pathologists, Inc., Salt Lake City, UT, 84132) (Spon.: A. Smith).

We evaluated technical and operational performance of Dupont Dimension™ 380 analyzers in two state laboratories. Univ. Hosp. lab performed 35,000 tests/month on two Dimensions™. St. Mark's community Hospital lab performed 11,000 tests/month on one analyzer.

Assessment of technical performance following training and start up included added day to day precision and patient correlations. CV's compared favorably to each established methods. Fifty-three percent of the CV's (72 of 136 comparisons, n=25-45) were actually better than specified by the manufacturer and all were accepted. Patient correlations confirmed agreement between Dimensions™ and established methods. Usable ranges were tested using 3 analytic concentrates.

Analysis time, reliability, and cost/test were key functional criteria used to assess operational performance. Analysis times varied from 1.9 to 20 min/specimen depending on the test mix and instrument worksite. At peak activity (7:30 to 9:30 AM) the average analysis times increased compared to low demand periods (1:00 to 3:00 PM), e.g., electrolytes 1.9 to 8 min; glucose 9.7 to 13 min; chem profile (Na,K,Cl,CO2,Gluc,Cre,Blnd) 10.4 to 20 min.

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After the training and start up period reliability was acceptable; only one sample was rejected during the second month (Nov. 1987). Reagent costs averaged $0.26/test, ranging from $0.10/test for electrolytes to $2.76/test for theophylline. Reagent costs were kept in line with the 40X savings projected by Dupont, based on our previous methods.

Overall, performance of the Dimension™ analyzers in both stat laboratories is technically and operationally acceptable.

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The Hitachi 736 Analyzer is a high-volume, high-throughput random access instrument with barcoded primary tube sampling. A chemistry menu of 27 on-line tests is available including Na, K, and Cl by ion-selective electrodes. We evaluated the following assays (using Boehringer Mannheim Diag. (BMD) reagents for precision, method comparison, and linearity: ALT, ALK, PCID, GLU, AST, BUN, CA, CK, CO2, CHOL, PE, GGT, LD, NA, PHOS, T. BIL, TP, TRIG, and URIC ACID.

Imprecision (CV) at different concentrations over a five month period ranged from 1.5% (GOT/186 U/L) to 4.2% (CHREAT/1.9 mg/dl and THI/4.6 mg/dl). Results for patient specimens for all of the above analytes were compared using the Etohoch 700 and Technicon SMA 18/90 Analyzers. The coefficients of correlation ranged from 0.932 (CA) to 0.999 (GLU, URIC ACID, ALT). Linearity studies for all analytes confirmed the BMD expected ranges.

In our laboratory, the 736 Analyzer operates 20 hr/day and 7 days a week. Only the operator/shift is required. Average maximum downtimes over the past 8 mo. have been about 1 hr. (mainly due to probe crashes). Daily maintenance procedures take about 3 hours (2 hr instrument time and 1 hr technology time). Advantages and disadvantages of the Analyzer will be presented in detail.

In summary, the Hitachi 736 Analyzer is analytically sound, and provides an efficient, cost-effective operation with minimal operator involvement.

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The Ciba-Corning Diagnostics 550 Express is a random-access chemistry stat and routine analyzer designed for the low to medium volume laboratory, or a stat analyzer for the larger volume laboratory. Test throughput is 180 chemistries/hr. and calibration is performed once daily. We evaluated precision and compared results for patient specimens (n=50) were compared to results from a Hitachi 736 Analyzer using Boehringer Mannheim Diag. reagents. Correlation coefficients were all within 0.993 to 0.999 except for ALB (0.911), CL (0.973), and TP (0.901). Of the eighteen chemicals tested, only four demonstrated a significant difference from the Hitachi 736. CK and GGT values were on average 15% higher on the Express, ALB was 11% higher, and ALK, PHOS was 5% lower. There is reagent-to-reagent interaction for CL and BUN, and these two chemicals should not be assayed side-by-side. Dyes are added to other reagents to reduce or inhibit these interactions.

This was a premarket evaluation and in general we found the analytical studies to be acceptable. The operation of the instrument proved simple (5 min. maintenance per day) and was trouble-free. Specific operating details, advantages, and disadvantages will be presented.

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**095 EVALUATION OF THE AMERICAN MONITOR PERSPECTIVE CHEMISTRY ANALYZER, Frank T. Fox, Beverly Cousin, Amy Cox (Veterans Administration Medical Center - 113, New Orleans LA 70146) (Spon: Frank T. Fox)**

We used a rigorous NCCLS protocol to evaluate the precision of the Perspective, a random access chemistry analyzer. Twenty seven of the thirty available tests were examined (ALT, total and direct Bili, Urea, Ca, CO2, Cholesterol, Creat., AC, ALP, AL, Glu, GGT, Ion, Mg, Phos, total Prot, Na, K, Trig, Uric Acid, UICB). Day-to-day "total," and "within-run" precision were determined over a thirteen day period, using the 40X savings projected by Dupont, based on our previous methods.

Overall, performance of the Dimension™ analyzers in both stat laboratories is technically and operationally acceptable.

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**096 EVALUATION OF A ROBOT FOR MICROTITER ENZYME LINKED IMMUNO-SORBANT ASSAY - THE BICKMAN BIOMET™ (TM), Stanley Bauer and Leonard Anderson (Staten Island University Hospital Center, Bronx, NY 10456) (Spon: S. Bauer)**

An evaluation of the Biomet™ (TM) robot was done for HIV antibody using a microtiter ELISA. All steps are under control of an IBM (TM) PC-XT. The Biomet consists of: 1. Two separate pipetters that dispense or transfer up to 8 specimens or reagents in volumes of 1-10,000 ul to microtiter wells or tubes (1 to 15 ml) in 8xl - 8xl2 arrays using a dedicated pipette tip for each well, 2. An aspiration/washing tool programmable as to volumes and cycles, 3. Optical density tool reading at 9 wavelengths with bichromatics. The Biomet dispenses specimen and diluent; aspirates; washes; adds enzyme conjugate, substrate, and washes again. Programs can be designed for each test, and can be easily modified. All values and results are printed on a dot matrix printer. Pipetting and washing using 90 and 450 sec., respectively.

Used for HIV antibody screens; within plate precision is +/2% (n=50) two times throughout the entire plate. Readings in duplicate. No serious change in negative sera to positive sera is not detectable.

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**097 AN EVALUATION OF THE ROBEXRINKI ENSIGHAM REACTOR™ IN A PEDIATRIC SETTING, M. Josephson, J.M. Hicks,1,2,1 (Dept. Lab. Med., Children's Hospital, National Med. Ctr. and 2 George Wash. Univ. School of Medicine, Washington, DC) (Spon: Mariette Josephson)**

We evaluated and compared the Boehringer Mannheim Diagnostic desk top Reformat™ analyzer with the automated RX 700 analyzer. Reformat allows for the co-determination of bilirubin, bilirubin and triglycerides were evaluated for precision, linearity and possible interferences. Within run CV's of all analytes are between 1.3% and 4.2% (n=30) and run to run CV's between 1.3% and 5.8% (n=90). Linearity for some analytes are lower than the manufacturer's claims. Bilirubin at 2-7 mg/dl interferes negatively with all analytes except BUN and triglycerides. Hemoglobin at 2g/dl(+) increases bilirubin measurements and at 4 g/dl (+++) decreases chromastor and triglyceride values. Lipidemia caused no detectable interference for all analytes. Patient samples were run on both instruments for all the previous mentioned chemicals. The linear regression equation of the data was equations from y=0.90x+1.26, r=0.99, to y=1.01x+1, r=0.98. Although the new bilirubin test on the Reformat performed well when compared to the RX 700 (r=0.96, R=0.99) it is not very suitable for pediatrics as it is only linear to 12.0 mg/dl. Values higher that this are frequent in the neonatal nursery. We compared results obtained on the
Ref lettinon carried by a skilled technologist, a physician and an unskilled operator. The CV's were 1.5%, 2.5% and 3.6% respectively. Many aspects of the Reflettin are appealing, i.e., the use of whole blood, its simplicity and its good precision. We feel however that this instrument has limitations in the neonatal nursery or pediatrician's office due to interference and limited linearity of the bilirubin.


We have evaluated a new IMNA method for measuring serum ferritin on the KineticCount-48 (Medical & Scientific Instruments, North Beach, MD) which is currently distributed by Vitek Systems. The steps of the assay consist of manually pipetting calibrators, controls, and patient samples into reaction trays together with buffer and tracer in specified wells. These reaction trays are then placed in the KineticCount-48 along with coated antibody "sparks" (Solid Phase Receptors (SPRAS)) for processing. The total instrument processing time is approximately 37 minutes from computer activation to assay completion. The technical range for the assay is 5.0-1000 mg/dL. Standard curves were stored and used repeatedly for one week. The linear back range was flagged with recommended dilution factors. The instrument also performed a "hook screen" on elevated specimens.

Ferritin results from the KineticCount method were compared to those obtained on the Du Pont MER ferritin method. Results are as follows: B=93, Slope X=1.95; B=0.97, T-Inter=4.12; and 5dy +/- 35.87. Our Vitek intra-asay CV's range from 2.7-5.5 percent on three pooled controls; inter-assay CV's range from 3.9-9.7 percent. Analytical recovery on assayed controls ranged from 97 to 102 percent. Recovery on diluted specimens ranged from 96.1 percent of the unidiluted level. Sensitivity of the assay was determined to be 1.9 mg/dL.

099 SEMI-AUTOMATIC NEPHELOMETRIC IMMUNOASSAYS OF CRP, ASO AND RF BY "HITACHI CHEMICAL TYPE HC-100", Karunobu Tanno, N. Takahashi, T. Kusure, and G. Yagyu (Hitachi Chemical Co. Ltd., Hitachi, "KOKUNA ELECTRIC CO. Ltd., Akita, Japan") (Spon.: K. Tanno)

Recently some devices for automatic immunosassays of serum samples (e.g. CRP, ASO, RF) were developed and marketed. But most of them were unsuitable for minor hospitals because they are large-sized and high-priced. We developed and marketed "TYPE HC-100" for semiautomatic nephelometric immunoassays of CRP, ASO and RF, which was low-priced enough to be used in minor hospitals.

Principles of assay were based on the measurement of changes of scattering light which were arisen from latex agglutination. Dynamic range, within-day precision and between-day precision of data on CRP, ASO and RF were estimated. The data were compared with the data detected by the blood chemistry analyzer "HITACHI 705".

Results were described as follows. Item, a dynamic range, b. within-day precision, c. between-day precision, d. correlation with "HITACHI 705": 1. CRP, a. 0-over-5.0 mg/dL. No decrease was observed in antigen excess., b. CV 1.55-2.76 %. c. CV 3.54-6.94 %. d. The correlation coefficient was 0.994, slope 1.072, r = 0.994. 2. ASO, a. 0-over-5000 tdd. No prozone phenomenon was observed in antibody excess., b. CV 1.55-2.71 %, c. CV 4.6-7.5 %. d. The correlation coefficient was 0.999, slope 1.034, r = 0.999. 3. RF, a. 0-over-100 IU/mL, b. S.D. 0.73-1 IU/mL, c. S.D. 1.24-4 IU/mL, d. The correlation coefficient was 0.991, slope 1.034. These data showed wide ranges, high reproducibilities and good correlations between "TYPE HC-100" and "HITACHI 705".

In summary, "TYPE HC-100" can be used for semiautomatic clinical analyses of CRP, ASO and RF in minor hospitals.

100 AN EVALUATION OF TOTAL AND TARTRATE INHIBITED ACID PHOSPHATASE ON THE TECHNICON CHEM 110, C. Smith, R. Stankovic, M. Flessiter, M. Schwartz (Deprt. of Clinical Chemistry, Memorial Sloan Kettering Cancer Center, New York, N.Y. 10021) and D. Saw (Technicon Instruments Corporation; Tarrytown, N.Y. 10591). (Spon.: M. Schwartz)

Total and tartarate inhibited acid phosphatase were determined on the Technicon Chem 110 (Method I) and on the Cobas Bio (Method II) the routine method for acid phosphatase in our laboratory. Both methods are zero order rate reactions. Method I employs 1-naphthyl phosphate as substrate and a phosphatase acceptor, 1, 5 pentanediol, for enhanced sensitivity. The reaction is carried out in 1N Tris buffer, pH 4.6. Method II uses sodium thymolphalein monophosphate as substrate in citrate buffer, pH 6.0. Similar concentrations of 1-naphthyl phosphate were used in both sera and controls were acidified with 5N acetic buffer, pH 6.0 (20 U/mL serum) after the specimen was separated from the clot and samples were run in duplicate. For Method I the day to day precision (4 days, 17 specimens at each level) was 4.9±0.4 U/L (CV=7.5%) and 17.4±0.6 U/L (CV=3.5%). For Method II the day to day precision over 30 days was 1.6±0.1 U/L (CV=6.25%) and 8.1±0.3 U/L (CV=7.5%). Method I appeared to be linear to at least 140 U/L. In a comparison of 61 patient samples from men with prostatic disease a regression equation (y =Method I; x =Method II) for total acid phosphatase of y=2.73±3.4X(r=0.96) was obtained and for the tartarate inhibited fraction y=9.92±3.3X(r=0.96). The upper limit of the normal reference range for method II is 0.6 U/L and as determined in 19 specimens from normal men for Method I was 3.5 U/L. Either method is acceptable for clinical use.

101 EVALUATION OF A NEW SYSTEM FOR KINETIC IMMUNOTURBIDIMETRIC DETERMINATION OF HUMAN SERUM APOLIPOPROTEIN A-1, S. B. Satinou, S. Kizai, and G. Reddelji, (Rheumatic Institute S. Raffalse, Milan, Italy) (Spon.: S. Marcovina)

A new system for immunoturbidimetric quantification of human serum proteins (Turbitime System, Behringwerke, Marburg, F.R.G.) has been evaluated for the determination of apolipoprotein A-I and B (apo). The system required special reagents which are provided in a ready-to-use precalibrated form.

As comparison methods were used a fixed-time nephelometry (BNA, Behringwerke) and a radial immunodiffusion assay developed in our laboratory that made use of two combined monoclonal antibodies for apo A-I and four for apo B.

The intra-assay coefficients of variation (n=20) were estimated to be 2.76% for apo A-I and 3.24% for apo B while the inter-assay (n=10) were 2.94% and 3.18% respectively.

Apo A-I and B determinations were not affected in lipemic, icteric, or paraprotein containing samples.

Comparison studies were performed on fresh serum from normolipemic subjects and patients with various disorders of lipid metabolism, renal and liver diseases (n=100). The results for apo A-I by Turbitime System (y) correlated well with those obtained by: r = 0.946 and r = 0.945, y = 1.02x + 0.12; and r = 0.397; y = 0.995 - 4.73 respectively. A good correlation was also found for apo B between the Turbitime System (y) and BNA and RID (x): r = 0.967; y = 0.823.x + 10.61; and r = 0.965; y = 0.975 + 5.38 respectively.

No significantly different results were obtained for both apo A-I and B determined on Turbitime System on fresh samples and on the same sera stored at -30 C.

102 EVALUATION OF THE BAKER SYSTEM 9000 HEMATOLOGY ANALYZER, George S. Cembrowski, Cynthia A. Santiago, Jeffrey Kay, Department of Pathology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania; and George Mucha and Ernest J. Kiser, Baker Instrument Company, Allentown, Pennsylvania. (Spon: Ernest J. Kiser)

We have evaluated the performance of a production model of the Baker System 9000 on an eight parameter, automated blood counter. Estimates of the within-run and between-run imprecision were obtained from the analysis of a three level Baker control material over 16 separate days. The between-run coefficients of variation for the mid-level control follow: Hg-1.5%, KC-1.9%, MCV-0.4%, Hct-1.9%, MCHC-1.7%, MCH-2.3%, and platelets-3.7%. We have also evaluated 226 selected specimens on both the Baker and Coulter Stacker hematology analyzers. The regression analysis of the comparison data indicates excellent agreement between the two instruments (see below). Based on its problem-free operation, its precision and accuracy, we recommend this instrument for use in physician offices and low volume hematology laboratories.

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### Evaluations of New Products

**104** EVALUATION OF BETA-ENDORPHIN RIA ASSAYS FOR STUDYING PAIN CONTROL IN SICKLE CELL PATIENTS, Roberta Franj, Judy Bashlow, and Andy Nowrin, School of Medicine, University of Illinois at Chicago, IL 60612 (Sponsor: Newton Nessler)

The specific aim of this study was to seek improved methods for controlling sickle cell pain and to gain a greater understanding of the relationship of natural occurring opioid compounds to pain in patients with sickle cell disease. We have evaluated commercial kits from Immunochemical, New England Nuclear and Nichols. Performance characteristics of these kits are presented in the table below.

<table>
<thead>
<tr>
<th>Performance Characteristics</th>
<th>TCN</th>
<th>NEN</th>
<th>Nichols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Assay</td>
<td>EDTA plasma</td>
<td>Serum</td>
<td>EDTA plasma</td>
</tr>
<tr>
<td>Specimen</td>
<td>EDTA plasma</td>
<td>Serum</td>
<td>EDTA plasma</td>
</tr>
<tr>
<td>Extraction</td>
<td>Yes (4 hours)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Recovery</td>
<td>62.1%</td>
<td>122% at 67.5g/mL</td>
<td>No</td>
</tr>
<tr>
<td>Precision (CV%)</td>
<td>24.8%</td>
<td>4.1% at 213g/mL</td>
<td>153g/mL</td>
</tr>
<tr>
<td>Coeff of Det.</td>
<td>0.96</td>
<td>0.79</td>
<td>0.98</td>
</tr>
<tr>
<td>Coeff of Corr.</td>
<td>0.98</td>
<td>0.89</td>
<td>0.98</td>
</tr>
<tr>
<td>F value</td>
<td>132</td>
<td>26</td>
<td>997</td>
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<tr>
<td>Zb</td>
<td>36.8</td>
<td>55-59</td>
<td>IRMA</td>
</tr>
<tr>
<td>DNS</td>
<td>12.3</td>
<td>50-54</td>
<td>IRMA</td>
</tr>
</tbody>
</table>

The Nichols kit appeared to be most reliable in our laboratory. We also studied methods of collection and stability of specimens for beta endorphin. We had highest recovery in serum and found no significant loss of activity at one hour when collected on ice. Hence, we conclude that it is best to collect the specimens in glass tubes, place them on ice immediately, separate the serum and freeze within one hour of collection. EDTA, saline diluted and labeled EDTA tubes were not better.

### 105 EVALUATION OF THE TINA-QUANT® B ferritin ASSAY ON THE BOEHRINGER MANNHEIM/HITACHI 704 SYSTEM

P. Haus, H. Dubois, M. McGeever, W. Stockmann, V. Richards, H. Kettlemann (Spon.: P. Haus)

1) Klinikum Mannheim, Institute of Clinical Chemistry
2) Boehringer Mannheim GmbH, Sandhofer Str. 116, D-6800 Mannheim

A new latex agglutination assay TINA-QUANT® B ferritin was adapted to the Boehringer Mannheim/Hitachi 704 system.

In this assay, latex particles coated with rabbit anti-human ferritin antibody react with serum ferritin. The resulting turbidity which is proportional to the ferritin concentration is measured at 700 nm. The assay time is about ten minutes.

The precision study was performed using a panel of sera with a wide range of ferritin levels. The results obtained are compared with those obtained using the same system on the Hitachi 704. The results obtained were found to be comparable and within the limits of the kits.

### 106 COMPARISON OF THE CLINICAL EFFICACY OF CK-MB ASSAYS BY THE CIBA CORNING DIAGNOSTICS MAGIC LITE CK-MB ASSAY AND THE CARDIOTRAC-CK ELECTROPHORESIS METHOD

Michael Lynch, Ciba Corning Diagnostics, Medfield, MA

Marion Hanason, Ethen Christman (Framingham Union Hosp. Framingham, MA), (Spon.: M. Lynch)

We have evaluated the Magic Lite CK-MB assay developed by Ciba Corning Diagnostics, Medfield, MA and the Ciba Corning Cardiotrac-CK Electrolyzer method for the diagnostic efficacy. The Magic Lite procedure is a 30 minute, room temperature chemiluminescent assay which uses magnetic particles to allow for separation without centrifugation. The assay uses a monoclonal antibody to CK-MB to specifically measure the Mb isoenzyme without interference from MM, BB, or typical (Macro) CK. The Magic Lite assay reports values in mass units (ng/mL) and uses a relative index (analogous to $r$) to assist in the interpretation of CK-MB values due to conditions other than cardiac related illnesses. Sera encompassing 26 diagnoses were examined by the two assays. The following regression line was obtained:

\[
\text{Lit} = 0.7677 \times \text{U/L} \times \text{CMK-MB} + 12.5 \\
\text{r} = 0.94 \\
\text{Sy.x} = 25.8 \\
\text{n = 143} \\
\text{B = All} \quad \text{0.0} - 590 \text{ng/mL}
\]

The clinical sensitivity and specificity of the 2 assays were compared. No difference between the 2 assay methods for either sensitivity or specificity could be detected.

Clinical Assay

<table>
<thead>
<tr>
<th>Sensitivity 95% CI</th>
<th>Specificity 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrolysis 87.9% (81.7-93.1) 89.3 (85.7-93.1)</td>
<td>86.7% (80.7-91.2) 87.8 (84.0-91.2)</td>
</tr>
</tbody>
</table>

* 95% Confidence Intervals

### 107 PERFORMANCE CHARACTERISTICS OF THE PARAMAX® ANALYTICAL SYSTEM FOR THE DETERMINATION OF AMMONIA WITH A REAGENT BLANK

M. J. Kurtz, C. Ehr, N. Langtvez, R. Edwards (Baxter Healthcare Corporation, Paramax Systems Division, Irvine, CA 92718) (Sponsor: M. J. Kurtz)

The performance characteristics of the Paramax® method for the quantitation of ammonia utilizing a reagent blank correction have been evaluated. The reagent tablet is preceded by a reagent blank tablet deficient in alpha-ketoglutarate and glutamine dehydrogenase. A sample size of 500 uL is used in a final reaction volume of 300 uL for both, and the blank absorbance delta is subtracted from the reagent absorbance delta. The reaction is maintained at 37°C.
The Paramax ammonia method with a reagent blank correction eliminates hemoglobin interference (up to 500 mg/dL) and bilirubin interference (up to 35 mg/dL). Correlation of the blanked and unblanked PARARAC methods vs. the DuPont ace is presented below:

<table>
<thead>
<tr>
<th></th>
<th>Blanked</th>
<th>Unblanked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>1.020</td>
<td>0.974</td>
</tr>
<tr>
<td>Intercep</td>
<td>11.4</td>
<td>21.8</td>
</tr>
<tr>
<td>Correlation (R)</td>
<td>0.990</td>
<td>0.899</td>
</tr>
<tr>
<td>No. of Samples (n)</td>
<td>70</td>
<td>70</td>
</tr>
</tbody>
</table>

Within run and overall precision are as follows:

Mean (nmoL/L) | 50.96 | 51.18
Within (CV%)   | 9.44  | 3.87
Overall (CV%)  | 9.77  | 8.30

Linearity to 700 umol/L in human plasma is demonstrated.

Accuracy as determined by recovery of 10% additions to plasma demonstrated mean recovery to be 10.8% for 10% plasma spiked with 70 nmol/L of ammonia.

Two basic protocols were employed. An indirect method in which protein ag was bound to the surface and subsequently probed with a peroxidase (HRP)-tagged antibody and a direct method using HRP-antibody alone. Binding was evaluated by measurement of OD at 490 nm after development with orthophenylenediamine/H2O2 and color development was measured. Samples of activated MP (CoH2) and SB (CoH3) were obtained from Micro Membranes, Inc., Newburg, NJ. untreated MP and SB were kindly supplied by the same firm. Data below are expressed as OD0 vs. reagent blanks.

HRP-IgG concentrations of 1 mg/mL, activated SB demonstrated 37% greater binding than untreated SB and activated IgG was 1.57 ± 0.03 vs. untreated 1.15 ± 0.07. After desorption with 0.1% sodium dodecyl sulfate, (SDS), 97% HRP-IgG remained bound to activated SB while only 31% remained on untreated SB. Pretreatment of HRP-IgG with SDS markedly inhibited binding; however, in the presence of up to 40% urea, activated SB absorbed as much HRP-IgG as untreated SB in the absence of urea. The indirect method, conducted on MP indicated similar enhancement of binding for IgG, IgA, IgM, and C3, as well as improved ability to retain protein after treatment with SDS. Moreover, C3’s were substantially lower in the case of activated MP (range 2% – 5% for treated MP; >10% for untreated MP). These data suggest that activation of FS surfaces may markedly enhance the performance characteristics of FS-based solid-phase devices for diagnostic products, by a) increasing the capacity of FS, b) reducing background and c) improving precision.

### 110 PERFORMANCE CHARACTERISTICS OF THE TECHNICON COLORECTAL SODIUM AND POTASSIUM INSTRUMENTS

**METHOD**

**TEST** | **LS** | **HS** | **CV** | **RE** | **NUM** | **FIN** | **NUM**
--- | --- | --- | --- | --- | --- | --- | ---
Sodium | 120.0 | 150.0 | 4.5 | 0.85 | 100 | 98.0 | 100.0
Potassium | 30.0 | 40.0 | 2.0 | 0.85 | 50 | 29.0 | 30.0

**Accuracy** was determined by comparison with ISE [analyzed on Technicon RMA System] as shown by the following correlation parameters:

| **y** | **x** | **r** | **s** |
--- | --- | --- | ---
1.020 | 0.985 | 1.985 |

**System** was further evaluated using a series of spiked patient specimens. The within run and total imprecision was determined.

**111 PERFORMANCE CHARACTERISTICS OF THE TECHNICON COLORECTAL SODIUM AND POTASSIUM INSTRUMENTS**

**METHOD**

**TEST** | **LS** | **HS** | **CV** | **RE** | **NUM** | **FIN** | **NUM**
--- | --- | --- | --- | --- | --- | --- | ---
Sodium | 120.0 | 150.0 | 4.5 | 0.85 | 100 | 98.0 | 100.0
Potassium | 30.0 | 40.0 | 2.0 | 0.85 | 50 | 29.0 | 30.0

**Accuracy** was determined by comparison with ISE [analyzed on Technicon RMA System] as shown by the following correlation parameters:

| **y** | **x** | **r** | **s** |
--- | --- | --- | ---
1.020 | 0.985 | 1.985 |

**System** was further evaluated using a series of spiked patient specimens. The within run and total imprecision was determined.

**112 ON-INSTRUMENT STABILITY AND CALIBRATION FREQUENCY**

**FOR 20 REAGENTS ON THE BECKMAN CHLORIDE CT-X4**

**Temp** | **N** | **Regression** | **Std Error(U/L)** | **Range(U/L)**
--- | --- | --- | --- | ---
37°C | 99 | 30°C | 97 | 1.01X - 0.98 | 0.98

**2) Imprecision (37°C):**

| **Mean(U/L)** | **+2S** | **2S** | **Total** |
--- | --- | --- | ---
135 | 62 | 21 | 0.8 | 1.1

**3) Interference:**

**For 30 patients:** Good correlation was observed with light path interference.

---

**CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988**
Asylase, Calcium

The Beckman SYNCHRON CX-4 system is a random access analyzer capable of testing up to 24 chemistry reagents on the analyzer simultaneously. Twenty (20) reagents are tested for on-instrument stability and calibration frequency when used with the SYNCHRON REAGENT. Reagents are considered to be stable if sample response from the time the reagent is first loaded on the instrument changes less than 10% for on-instrument calibrated Standards. Calibration is considered to be stable TC the laboratory response changes less than 2X between calibrations.

These reagents are stable for up to 120 days: ALT, Amylase, AST, CK, CHT, LDL, HDL, Albumin, Calcium, Cholesterol, Creatinine, BUN, Direct Bilirubin, Glucose, Phosphorus, Total Bilirubin, Total Protein, Triglyceride, and Uric Acid.

Calibration Frequencies:

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>FREQUENCY</th>
<th>FREQUENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>14 days</td>
<td>14 days</td>
</tr>
<tr>
<td>Calcium</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct Bilirubin</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>14</td>
<td>BUN 1</td>
</tr>
</tbody>
</table>

Detection of Campylobacter by Colony Hybridization with Oligonucleotide Probes (SpaD, Elsie J. Shriver, E.D. Schiemann, L.A. Huen, D.B. Roseman, J.E. Marsh, and J.L. Riden, Molecular Biosystems, Inc., San Diego, CA). Identification of three clinically significant Campylobacter species (C. jejuni, C. coli, and C. lacticida) was achieved using a synthetic alkaline phosphatase-conjugated oligonucleotide probe. This probe was used in a colorimetric colony hybridization assay to screen 210 stool cultures grown on Campy-BAP medium. Results were compared to standard culture and biochemical methods. Fifty-one isolates were identified as Campylobacter (41 C. jejuni, 9 C. coli, and 1 C. lacticida) by both DNA probe and standard culture methods. Based on this comparison, the DNA probe test demonstrated a sensitivity of 100% and a specificity of 98% with an overall agreement of 98%. The specificity of the probe was further verified by negative results with 6 other Campylobacter species (C. fetus, C. pylori, C. faecalis, C. vaginalis, C. hydrogenalis, and C. jejuni) as well as 31 non-Campylobacter species. The hybridization assay is simple and rapid, consisting of brief hybridization and wash steps and 1 hour color development. The present study describes a clinically useful serology tool which is based on a specific nonradioactive DNA probe assay.

Rapid Testing of Diarrheal Agents: Nonradioactive DNA Probe Tests for the Direct Detection of Campylobacter and Salmonella in stool. R.M. Fite, D.B. Roseman, E.D. Schiemann, D.A. Driver, C.L. Bridge, D.J. Cerveny, and L.M. Schummi, Molecular Biosystems, Inc., San Diego, CA. (Upon: David F. Pritchard) Campylobacter isolated in one out of six cultured diarrheal stools in the U.S., is the most common cause of adult human gastroenteritis. Convivial diarrheal disease requires culturing for several days. Salmonella is the major cause of gastroenteritis in children. Since it is difficult to culture, convivial diagnosis is by electron microscopy, gas electrophoresis or immunological assays. We have developed rapid tests for the direct detection of both these agents in stool. A diarrheal specimen is lysed, releasing nucleic acids which are then isolated by the Extractor column and fixed to a nylon membrane. The membrane containing the bound nucleic acid is then hybridized with the synthetic alkaline phosphatase-conjugated nucleic acid probe (SNAP), washed and incubated with substrates for colorimetric detection. Total handling time is 1.5-5 h with results in 4-7 h. Compared to the standard methods, the Campylobacter SNAP test had sensitivity of 93.3%, specificity of 95.8%, with 95.7% overall agreement, while the Salmonella SNAP test had sensitivity of 94%, specificity of 98%, with 97.9% overall agreement.


Alpha-amylase activity is measured using a benzylidene blocked nitrophenylalcohol test substrate. The alpha-amylase cleaves the nitrophenylalcohol test substrate into two fragments which are then cleaved by alpha-glucosidase and glucotransylase producing para-nitrophenol.

The Abbott Spectrum within run precision (n=48) respective means (U/L) and C.V.'s were: 76.85, 188.02, 710.73; 1.99%, 3.29%, 2.78%. The VP's within run precision (n=31) respective means (U/L), and C.V.'s were: 73.76, 176.42, 652.50; 1.75%, 0.79%, 0.61%. The Abbott Spectrum between run precision (n=13) means (U/L), and C.V.'s were: 77.25, 201.55, 752.25; 5.73%, 11.9%, 6.578%. The VP between run precision (n=26) means (U/L), and C.V.'s were: 73.39, 180.90, 678.24; 6.21%, 6.93%, 8.32%. Liquid amylase to ACD and Astra Correlation yielded the following regression equations: ABBOTT SPECTRUM = 0.969 ACD + 0.60, r=0.986, n=212; VP = 0.873 ACD - 0.76, r=0.984 n=212; ABBOTT SPECTRUM = 0.802 ASTR@ 10.82, r=0.980, n=212; VP = 0.721 ASTR@ 10.84, r=0.976, n=212.

The liquid amylase reagent is linear to at least 1,000 U/L. Billirubin to 30 mg/dL had no significant effect on results. Hemolysis produced a clinically significant negative result.

This liquid amylase reagent provides a discrete uniform amylase substrate resulting in excellent precision and linearity and provides the benefit of precision, accuracy, and ability to control recovery, and interferences.

RESULTS: Comparison versus BMD reagent on the Hitachi 705; (y = mx + a)

<table>
<thead>
<tr>
<th>CC</th>
<th>y = 1.081x - 0.296</th>
<th>r = 0.9985</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BMD</td>
<td>374.63 mg/dL</td>
<td></td>
</tr>
</tbody>
</table>

Precision:

| Run-to-Run (mg/dL) | S.D. | C.V. (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>155.6</td>
<td>1.77</td>
<td>1.11</td>
</tr>
<tr>
<td>206.4</td>
<td>4.98</td>
<td>2.46</td>
</tr>
<tr>
<td>270.0</td>
<td>8.75</td>
<td>3.23</td>
</tr>
</tbody>
</table>

Linearity: exceeded 750 mg/dL at the end of the stability period

Accuracy Studies:

<table>
<thead>
<tr>
<th>Product</th>
<th>Expected (mg/dL)</th>
<th>Recovered (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>141.65 - 142.65</td>
<td>142.178</td>
</tr>
<tr>
<td>CDC</td>
<td>283.9</td>
<td>283.9</td>
</tr>
<tr>
<td>Q18</td>
<td>185.0</td>
<td>185.0</td>
</tr>
<tr>
<td>Q15</td>
<td>161.0</td>
<td>161.0</td>
</tr>
<tr>
<td>50</td>
<td>294.0</td>
<td>294.0</td>
</tr>
<tr>
<td>CAP I</td>
<td>203.0</td>
<td>211.0</td>
</tr>
<tr>
<td>CAP II</td>
<td>203.0</td>
<td>272.0</td>
</tr>
<tr>
<td>CAP III</td>
<td>361.0</td>
<td>363.0</td>
</tr>
<tr>
<td>Malt</td>
<td>117.9</td>
<td>117.9</td>
</tr>
<tr>
<td>12M</td>
<td>277.0</td>
<td>277.0</td>
</tr>
</tbody>
</table>

Chemistry reference materials from various manufacturers were run in a control recovery study. Baradax controls from Behring recovered within their stated ranges or expected values. Recovered x Published x and Range

Gillid Normal 115.4 mg/dL 122.5 (104 - 141 mg/dL)
Gillid Abnormal 311.5 mg/dL 625.0 (633 - 1000 mg/dL)
Gillid Easylipid 15.8 356.8 (356 - 356 mg/dL)
QCS Normal 163.8 165.0 (130 - 180 mg/dL)
QCS Abnormal 152.4 114.0 (97 - 131 mg/dL)

We have shown that this new reagent has increased accuracy, linearity, and stability.

116 PERFORMANCE OF A STABLE IRON REAGENT WITHOUT SERUM BLANK. Rob W. Wright, Craig Reason, (Ciba Corning Diagnostic Corp., Gilford Systems, Oberlin, Ohio 44074) Spon. (Craig Reason)

The application of a serum iron reagent based on chromosal B (CAB)/cetylsulfonylmalonamide bromide (CTMA) on the Ciba Corning Diagnostic Corp. 808 Alliance chemistry analyzers was developed and requires a serum blank and is stable at room temperature (18°C - 25°C) for 4 weeks. This method for serum iron was linear to 600 µg/dL.

Within run precision showed the following results:

<table>
<thead>
<tr>
<th>Level (µg/dL)</th>
<th>S.D. (µg/dL)</th>
<th>CV (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>133.8</td>
<td>1.0</td>
<td>0.76</td>
<td>20</td>
</tr>
<tr>
<td>254.0</td>
<td>2.9</td>
<td>1.16</td>
<td>20</td>
</tr>
</tbody>
</table>

117 CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988
### PERFORMANCE EVALUATION OF A NEW Ciba CORNING

#### DIAGNOSIS-OCCID TRUE COLOR-640 REAGENT ON GLIFORD IMPACT 4000E, A. Banerji, C. Reason, M. Mullins, G. Traverso, J. Polanski (Ciba Corning Diagnostic Corp., Gilford System, Oberlin OH. 44074)

(Sponsor: Arnold Banerji, Ph.D.)

A newly developed, Trinder-type Triglyceride reagent, using glycerol-phosphate oxidase (EC 1.13.1.3) (GPO), was evaluated to determine its performance for precision, comparability, linearity, recovery of ascorbic acid, and bilirubin and hemoglobin interferences.

#### RESULTS:

**Precision:** Within Run (If samples analyzed per level, per run)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Medical Decision Level</th>
<th>Low Level</th>
<th>High Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>134.1 - 131.8</td>
<td>54.3 - 55.76</td>
<td>251.1 - 256.1</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.64 - 1.47</td>
<td>0.7 to 1.47</td>
<td>1.6 - 2.75</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>0.53 - 1.12</td>
<td>1.3 - 3.89</td>
<td>0.56 - 1.47</td>
</tr>
</tbody>
</table>

**Between Run (10 runs):**

| Mean   | 129.0                  | 56.8       | 588.4      |
| S.D.   | 2.4                     | 1.43       | 0.37       |
| C.V. (%)| 1.88                   | 2.47       | 0.57       |

**Comparison:** Between Run (10 runs) - 1996

<table>
<thead>
<tr>
<th>Comparison</th>
<th>r (p + max - b)</th>
<th>BMD-Past Chem: 0.995, 0.96 BMD + 4.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syntax</td>
<td>0.957</td>
<td></td>
</tr>
</tbody>
</table>

**Linear:** 1000 mg/dL

**Stability:** 10 days at room temperature or 8 weeks refrigerated

<table>
<thead>
<tr>
<th>Control Recovery</th>
<th>Expected (mg/dL)</th>
<th>Measured (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dade Monitor I</td>
<td>71 - 97</td>
<td>92.5 - 92.6</td>
</tr>
<tr>
<td>Dade Monitor II</td>
<td>161 - 227</td>
<td>191.5 - 196.3</td>
</tr>
<tr>
<td>QC 006500</td>
<td>78 - 100</td>
<td>90.0 - 97.7</td>
</tr>
<tr>
<td>QC 005506</td>
<td>71 - 97</td>
<td>79.0 - 79.4</td>
</tr>
<tr>
<td>ACA 56 - 76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Interferences:**

<table>
<thead>
<tr>
<th>Ascorbic Acid (mg/dL)</th>
<th>Expected (mg/dL)</th>
<th>Measured (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>11.1</td>
<td>13.18</td>
</tr>
<tr>
<td>Hemoglobin (mg/dL)</td>
<td>200</td>
<td>220</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>200</td>
<td>220</td>
</tr>
</tbody>
</table>

We have shown that this new reagent increases accuracy, linearity, and stability, and controls within their stated ranges or expected values.

---

**110**

### IL MONARCH - ALTERNATIVE REAGENTS, P.R. Ladouceur

B. Hadem (Beckman Instruments, Inc., 200 S. Kramer Blvd., Brea, CA 92621), C. Smith (Oceanside Hospital, Murray, UT), R. Paulson (VA Hospital, Portland, OR) (Sponsor: Pete Ladouceur)

A variety of reagent methodologies were adapted and evaluated on the IL Monarch Chemistry Analyzer. The evaluation included both liquid and Dri-STAT reagents manufactured by Beckman. The evaluation evaluated conditions of precision, internal correlation and linearity, and the reference reagent utilized for correlation was manufactured by IL. All mono reagent products were evaluated accurately as indicated by the below listed data.

### Precision

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Slope Intercept</th>
<th>R</th>
<th>R</th>
<th>R</th>
<th>R</th>
<th>Linearity (M/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALF</td>
<td>0.377</td>
<td>3.17</td>
<td>0.999</td>
<td>145</td>
<td>750</td>
<td>U/L</td>
</tr>
<tr>
<td>ALT</td>
<td>0.932</td>
<td>-0.04</td>
<td>0.995</td>
<td>127</td>
<td>400</td>
<td>U/L</td>
</tr>
<tr>
<td>AST</td>
<td>-0.470</td>
<td>0.977</td>
<td>145</td>
<td>400</td>
<td>U/L</td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>1.007</td>
<td>-0.264</td>
<td>0.996</td>
<td>140</td>
<td>120</td>
<td>mL/dL</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.014 - 0.045</td>
<td>0.998</td>
<td>145</td>
<td>600</td>
<td>mL/dL</td>
<td></td>
</tr>
<tr>
<td>CR-RAG</td>
<td>1.177 - 10.162</td>
<td>0.978</td>
<td>148</td>
<td>750</td>
<td>U/L</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.029 - 0.160</td>
<td>0.999</td>
<td>146</td>
<td>18.5</td>
<td>mL/dL</td>
<td></td>
</tr>
<tr>
<td>GOT</td>
<td>0.973</td>
<td>1.928</td>
<td>0.999</td>
<td>160</td>
<td>900</td>
<td>U/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.956</td>
<td>0.313</td>
<td>0.999</td>
<td>125</td>
<td>500</td>
<td>U/L</td>
</tr>
<tr>
<td>LD</td>
<td>0.976</td>
<td>-2.440</td>
<td>0.998</td>
<td>150</td>
<td>450</td>
<td>U/L</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.930 - 7.600</td>
<td>0.993</td>
<td>138</td>
<td>750</td>
<td>U/L</td>
<td></td>
</tr>
<tr>
<td>Uric Acid</td>
<td>0.923 - 0.237</td>
<td>1.16</td>
<td>0.991</td>
<td>145</td>
<td>20</td>
<td>mL/dL</td>
</tr>
</tbody>
</table>

The above data demonstrates the acceptability of all evaluated methods. The Beckman reagents, in specific instances, exceeded the reference reagent.

---

**120**

### EVALUATION OF A NEW IRON REAGENTS, P.R. Ladouceur

B. Hadem (Beckman Instruments, Inc., 200 S. Kramer Blvd., Brea, CA 92621), H. Smith (Mission Oakes Hospital, Los Gatos, CA) (Sponsor: Bill Hadem)

A new liquid reagent for the determination of Iron and TIBC has been developed by Beckman Instruments, Inc. The reaction sequence for this reagent is listed below, with the primary absorbance being measured at 565 nm.

Transferin - (Fe^3+)_2 + Acid pH — Transferin + 2Fe^3+

Iron^3+ + Dihydroxyamine + Thymolblue ——> Fe^2+

The increase in absorbance at 560 nm is due to complex formation and is proportional to the concentration of iron in the sample.

For the TIBC, the total iron bound to the Transferin is then measured by the reaction sequence above. The reaction reagent complex is in five minutes and can be performed manually or automated as a reaction temperature of room temperature, 30°C or 37°C. The cited automated instruments were evaluated comparing Beckman old Liquid-STAT Iron/TIBC with the new Beckman Liquid-STAT Iron/TIBC reagents. The Iron results are:

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Scope</th>
<th>Intercept</th>
<th>R</th>
<th>R</th>
<th>R</th>
<th>R</th>
<th>Linearity (M/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td>0.999</td>
<td>0.0349</td>
<td>0.973</td>
<td>41</td>
<td>1000</td>
<td>mg/dL</td>
<td></td>
</tr>
<tr>
<td>Gobas Bio</td>
<td>1.000</td>
<td>-1.280</td>
<td>0.999</td>
<td>130</td>
<td>850</td>
<td>mg/dL</td>
<td></td>
</tr>
<tr>
<td>Gobas Mira</td>
<td>0.971</td>
<td>1.444</td>
<td>0.999</td>
<td>150</td>
<td>450</td>
<td>mg/dL</td>
<td></td>
</tr>
<tr>
<td>RA-1000</td>
<td>1.060</td>
<td>-10.200</td>
<td>0.997</td>
<td>110</td>
<td>600</td>
<td>mg/dL</td>
<td></td>
</tr>
</tbody>
</table>

The iron data demonstrate acceptability of all instruments with the new iron, TIBC Beckman reagent.

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**121**

### MEASUREMENT OF CALCIUM AND HDL CHOLESTEROL WITH THE VISION SYSTEM, Michael A. Peace, and Donald F. Giacomo (Columbia Presbyterian Med. Ctr., NY, NY 10032) (Sponsor: T. Spring)

Calcium was determined by the o-cresolphthalein complexone reaction and high density lipoprotein cholesterol (HDL) was determined by the enzyme method of measurement and the nitrogen determination of the sample by the nitrophenyl in the supernatant by the enzymatic cholesterol reaction. The calibration curve for calcium and HDL is stable for at least 20 days. Linearity is up to 16 mg/dl for calcium and 120 mg/dl for HDL. For calcium there is no interference from hemoglobin at concentrations of 100 mg/dl, or triglycerides at concentrations of 1,000 mg/dl. Samples with bilirubin concentrations greater than 10 mg/dl must be diluted. For HDL there is no interference from bilirubin at concentrations of up to 20 mg/dl. Hemoglobin at concentrations of 75 mg/dl results in an increase in HDL values. The within-run precision N=10 for calcium gave CV's of less than 3% for calcium concentrations ranging from 6.7-12.6 mg/dl. The within-run precision N=10 for HDL gave CV's of less than 5% for HDL concentrations ranging from 16-106 mg/dl.

Linear regression analysis for calcium and HDL gave:

Calcium: M = b + e Syn

SMAC vs. Vision (serum) 105 0.86 1.01 0.946 0.46 Vision blood vs. Vision plasma 41 0.91 0.79 0.928 0.18

HDL

TDX vs. Vision (serum) 103 0.97 4.07 0.989 3.98

In summary, the Vision HDL and calcium methods are accurate and precise.

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**122**

### EVALUATION OF AN ABBOTT VP LIQUID IRON ASSAY, Raymond Butler, Gordon F. Kapke (Orl. Reg. Med. Ctr., Orlando, FL 32806) and Sue Pierce (Abbott Labs, Irving, TX 75015) (Sponsor: Carol Cooper)

The reagent consists of a pH 4.5 buffer
(hydroxyamine hydrochloride, Triton® X-100, thiourea, acetic acid, sodium acetate) and a 5% aqueous color reagent. Instrument iron contamination is minimized by pretreating the instrument with an organosilane in an alcohol solvent. No sample pretreatment is required and sample absorbance is corrected for by blanking after the mixing of the sample and buffer. The assay is linear from 0.125 ug/ml to 100 ug/ml and absorbance is measured using the 600/650 filter. Specimen requirement is 25 ul of serum.

The within run precision using the VP system (n=29) resulted in respective means (ug/dl) and C.V.'s of: 95.54, 205.09, 217.70, 83.85; 1.67%; 1.01%, 0.80%, 2.58%. The VP between run precision, estimated from controls (n=14) resulted in respective means (ug/dl) and C.V.'s of: 96.10, 203.73, 220.21, 83.98; 4.13%; 4.47%. Correlation studies using the ABBOTT LIQUID Iron yielded the following regression equations: ABBOTT VP = 0.963 ASTM + 3.7, r = 0.940, rMSE = 0.14, Lambert VP = 1.1, (ABA) -17.6, n = 40, r = 0.934 (Ryland Ferrozine® II), was manually pipetted, and absorbance read at 550/30 on the ABA 700. Iron and lipemia appear to have no significant effect.

The small sample volume, pretreatment of the instrument to reduce excessive iron contamination, and the solubility properties of FERENE results in a reagent with good precision and linearity which is very acceptable for clinical laboratory iron determinations.

123. Evaluation and Clinical Correlation of the BioRad Nova-
path™ Immunoassay (Western Blot) for the Detection of Anti-

Enzyme Linked Immunoelctrotransfer Blot technique (Western Blot) has been used to detect specific antibodies to HIV. A commercial Western Blot, the BioRad NovaPath™ Immunoassay, was evaluated for the purpose of confirming antibody reactivity for repeatedly reactive serum specimens by an ELISA for HIV-Ab. The BioRad Immunoassay is a simplified version of the Western Blot procedure. Vital parameters of preparation, including preps and blocking to nitrocellulose have been completed previously. The preblotted strips are used with the necessary conjugates and washing buffers to assess specific HIV-Ab status of suspect serum samples in approximately 3 hours.

A total of 60 sera from suspected HIV-infected individuals were initially evaluated. The BioRad Immunoassay demonstrated good antibody reactivity for HIV proteins at gp160, gp20, p25, p55, gp41-32, p32, p24 and p18 kilodaltons for these sera. 100% (60/60) of the HIV-infected specimens showed reactivity to both gp160/120 and 92 (55/60) to either p24 or gp41. A characteristic immunoassay pattern with Ab reactivities to gp160/120 and p65 kd was seen and correlated with the presence of HIV antigen (Ag) in some of the specimens tested. Dilution studies on several strong Ab reactive sera demonstrated Ab reactivity to a dilution of 1:1000. We have assessed the sensitivity of the HIV-Ab response to the core protein p24 for 58 HIV-Ab positive samples. Based on the relative intensity of the stained band in the p24 kd area, 86% (50/58) demonstrated a positive Ab reactivity to p24. The BioRad Immunoassay can be reliably used to confirm Ab reactivity from ELISA HIV-Ab screening. In addition, correlation between decreased Ab reactivity for HIV core protein p24 and the presence of HIV-Ab has been shown.


Manual NTSH analysis by radioimmunoassay (RIA Corning Immuno-
assay) was used as the reference method for comparison of the Automated Stratus Fluorometric Enzyme Immunoassay System. The STRATUS NTSH HEP procedure is based on the two-site sandwich immunoassay methodology. A clinical sample is introduced onto a glass fiber coated with immobilized monoclonal anti-NTSH directed toward NTSH molecules. Following a short incubation, a conjugate consisting of enzyme-labeled monoclonal anti-NTSH directed against the NTSH molecule is added to the reaction zone. During a second incubation period, the labeled antibody reacts with the antibody-bound NTSH forming the antibody-antigen-labeled antibody sandwich. Upon completion of this reaction, a wash solution containing the enzyme substrate is added. The wash solution elutes the unbound fraction and simultaneously initiates the enzyme reaction. The enzymatic rate of reaction of the bound fraction varies directly with the concentration of NTSH.

The 61 samples studied range from less than 0.75 uIU/ml to 17.3 uIU/ml by the RIA method. The corresponding results of the Stratus method were from 0.05 to 17.09 uIU/ml. Linear Regression studies of the samples yielded a coefficient correlation (r) of 0.9368 and y (Stratus) = 1.17 x (Corning RIA) - 0.46. Precision studies are in the process of being done to validate manufacturer's stated cv of less than 10% over the range of 0.6 to 50 uIU/ml.

We found that the Stratus NTSH system is simple to operate, provides quick turn around time for the test (30 minutes for 30 samples), performance at the lower range is more sensitive than the stated RIA method.

125. ANALYTICAL PERFORMANCE OF AN AUTOMATED IMMUNOASSAY
FOR C-REACTIVE PROTEIN USING THE DUPONT DIMENSION®
CLINICAL CHEMISTRY SYSTEM. Dennis M. Sanders and Peter M. Tuhy (E.I. du Pont de Nemours and Company [Inc.], Medical Products Department, Wilmington, Delaware 19898) (Spon: A. K. Ennis)

C-reactive protein (CRP) levels are used as an indicator of bacterial infection, inflammation or tissue damage. Of the acute phase proteins, CRP is the most useful since serum concentrations increase significantly with peaks of up to a thousand fold over the baseline level being reached in about 72 hours. If the healing process proceeds normally, CRP levels return to baseline in 4 to 6 hours. A quantitative, fully automated assay for the measurement of CRP in serum has been developed for use on the Du Pont DIMENSION® clinical chemistry system.

The DIMENSION CRP assay is based on the Particle Enhanced Turbidimetric Immunoassay (PETIA) technology. The CRP Flexi(tm) reagent cartridge contains latex particles coated with goat antibody to CRP. CRP present in the sample aggregates the particle, causing an increase in turbidity. The rate of aggregation, which is proportional to the concentration of CRP, is measured by the rate of change in turbidity at 340 nm. A five-level liquid human serum base product is provided for calibration. Method performance compares well to results obtained using the CRP assay on the Du Pont Ica® discrete clinical analyzer, with slope = 1.06, intercept = -0.04 and correlation coefficient r = 0.978. Within-run CV's of 2.9 and 1.1% were obtained at 2.0 and 7.4 mg/dl CRP, respectively. Between-run CV's of 1.7 and 1.8% were obtained at 2.3 and 8.0 mg/dl CRP, respectively. The method covers an assay range of 0.2-12.0 mg/dl and requires 5 µL of sample.

126. PERFORMANCE OF DRUGS OF ABUSE METHODS ON THE DU PONTsea® DISCRETE CLINICAL ANALYZER. David M. Obazary, N. M. Reihof, R. E. Biehnck, Jr. and P. M. Tuhy (E. I. du Pont de Nemours & Co. [Inc.], Medical Products Department, Wilmington, DE 19898) (Spon: D. M. Obazary)

Six urinary drugs of abuse methods, a 3 level calibrator and a 2 level daily control have been developed for the Du Pont sea® discrete clinical analyzer. The methods include: Amphetamines (U AMP), Cocaine Metabolites (U COC), Cannabinoids (U THC), Opiates (U OPI), Benzodiazepines (UBZED) and Barbiturates (UBAR) and are adaptations of BYVA's homogeneous enzyme immunoassay technique, EMIT®. Quantitative results are provided for calibration and quality control purposes, but the methods are recommended as a qualitative screen on unknown samples.

Within-day and between-day precision are shown for cutoff concentrations in the table below. We spiked 25 negative urines to concentrations equal to half-cut-off, cutoff and above cutoff and determined the distribution and recovery to establish absolute accuracy. Comparison results between the sea, BYVA Autolab and GC/MS using unknown urine samples are shown below. No false positives were found by either method (N=50).

<table>
<thead>
<tr>
<th>Method</th>
<th>Range</th>
<th>Precision</th>
<th>Recovery</th>
<th>POS/NEG</th>
<th>VOS/NEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>U AMP</td>
<td>0.30</td>
<td>4.1%</td>
<td>4.5%</td>
<td>103%</td>
<td>50/0</td>
</tr>
<tr>
<td>U COC</td>
<td>0.30</td>
<td>6.7%</td>
<td>7.0%</td>
<td>97%</td>
<td>55/0</td>
</tr>
<tr>
<td>U THC</td>
<td>0.05</td>
<td>7.2%</td>
<td>12.9%</td>
<td>95%</td>
<td>55/12</td>
</tr>
<tr>
<td>U OPI</td>
<td>0.30</td>
<td>1.7%</td>
<td>1.9%</td>
<td>109%</td>
<td>47/49</td>
</tr>
<tr>
<td>UBZED</td>
<td>0.30</td>
<td>3.6%</td>
<td>4.4%</td>
<td>106%</td>
<td>50/9</td>
</tr>
<tr>
<td>UBAR</td>
<td>0.30</td>
<td>2.9%</td>
<td>3.6%</td>
<td>113%</td>
<td>50/0</td>
</tr>
</tbody>
</table>

These samples above cutoff for GC/MS but below sea cutoff of 0.05 mg/L.

These samples above GC/MS cutoff but below sea cutoff of 0.05 mg/L cutoff used with BYVA Autolab and sea cutoff used with sea.

"EMIT", a registered trademark of BYVA Company, a Syntax Corporation.
We compared the performance of a modified CK-MB assay developed by Hybritech Inc. (San Diego, CA) with that of our current Roche method (Roche Diagnostics, Nutley, N.J.). We measured total CK and CKMB in 73 patients in the following categories: hospitalized non-cardiac, suspected but ruled out AMI, confirmed AMI with 0 wave, and confirmed AMI without a 0 wave. The following precision data were obtained: MEAN=7.4 S.D.

Within-run

<table>
<thead>
<tr>
<th>Level</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15.0±0.4</td>
</tr>
<tr>
<td>II</td>
<td>56.0±1.5</td>
</tr>
</tbody>
</table>

Between-Run

<table>
<thead>
<tr>
<th>Level</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>14.1±0.87</td>
</tr>
<tr>
<td>II</td>
<td>52.5±3.3</td>
</tr>
</tbody>
</table>

The Hybritech method demonstrated excellent sensitivity (2.1 U/l) in patients with confirmed AMI. There was good agreement of values obtained by the two methods:

\[ Y = 1.35X + 0.04 \]
\[ R = 0.99, \text{ Std Err Est} = 3.4, N = 22 \]

In the hospitalized non-cardiac patients no elevated CK-MB values were found. In the 36 suspected AMI patients in whom the diagnosis of AMI was excluded because of total CK concentrations below 204 U/l (upper limit of normal), 7 patients demonstrated a peak CKMB level suggestive of AMI using the Hybritech method. The clinical significance of this finding remains to be determined.

The Hybritech CK-MB immunosassay is a useful diagnostic test for evaluating the patient with AMI.

**Evaluation of an Aqueous Blood Gas Plus Electrolyte Control, George Hurrell, Jr. (Bio-Rad Laboratories, EGS Division, Anaheim, CA 92805) (Spons.: G. Hurrell)**

We have evaluated a new aqueous blood gas plus electrolyte control from Bio-Rad Labs, and compared its performance with three competing controls. Interassay variability for the different controls was compared with CV values of ≤ 0.05% for pH, ≤ 1.75% for PO2 and ≤ 3.5% for PCO2.

The electrolytes Na, Cl, and K, were compared with the NOVA control on a NOVA "STAT PROFILE"* 4 and results showed comparable reproducibility.

Our studies indicate that once ampules are opened, all of the controls evaluated showed similar stability. PO2 values increased by more than 15% in less than 2 minutes for the low and normal levels, indicating that these controls should be used as soon as possible after opening. This applies to blood gas parameters only; the electrolytes are very stable in the open ampule.

The Bio-Rad control is colorless and does not contain interfering anionics, and therefore is compatible with the IR membranes present in the new multiparameter blood gas plus electrolyte analyzers, such as the NOVA STAT Profile.

Accelerated stress testing and real time studies indicate the product has a shelf life of 24 months at room temperature.

In summary, the Bio-Rad control is a useful quality control product for monitoring blood gas and electrolyte laboratory analyzers.

**Evaluation of a Urine Toxicology Control Using the 10 SYVA D.A.U. Assays, Donna Chapman, (Bio-Rad Laboratories, EGS Division, Anaheim, CA 92805) (Spons.: J. Walsh)**

A 100% human urine based toxicology control containing 14 drugs of abuse and drug metabolites was evaluated using the SYVA D.A.U. Assays for drugs of abuse in urine (D.A.U.).

The control contains Amphetamines, Cocaine Metabolites (Benzylegonine), Methadone and its primary metabolite, Methaqualone, Opiates, Benzodiazepines (Nordiazepam), Phenylpyline, Propoxyphene, Barbiturates (Sevoral), and Cannabinoids (11 Nor 9-Carboxy-F -9-THC). Qualitative and semi-quantitative results were determined using SYVA protocols for the Gilford Stasar 103 Spectrophotometer. The control material demonstrated positive results for all 10 available SYVA assays tested, with absorbance readings just above the low calibrator for each assay. Semi-quantitative results were determined by preparing a standard curve. Results were confirmed and correlated with GC/MS for each drug tested.

Shelf life stability of the control was determined using accelerated heat stress testing of lyophilized material. Samples simulating 3 year storage at 4°C showed no significant change in results when compared with fresh samples for the analysis by both SYVA and Authentic. Vial stability and vial to vial reproducibility were evaluated; results showing reconstituted product was stable 10 days at 2-8°C and 30 days at -20°C.

This urine toxicology control is suitable for use in quality control when using the 10 SYVA D.A.U. Assays. Analytes are present at levels most useful in evaluating SYVA assay performance just above the positive cutoffs.
Acute renal failure (ARF) affects nearly 23% of critically ill patients. ARF is defined as an abrupt, severe, sustained decline in renal excretory function; it is identified clinically by rising BUN level and serum creatinine concentration. Most forms of ARF are reversible, so the prompt diagnosis is critical. The performance of the VISION method was evaluated in a kidney function laboratory environment for monitoring urine and serum creatinine and BUN. Serum specimens were run unaided on the VISION Analyzer for both creatinine and BUN. Urine specimens were diluted with distilled, deionized water 1:10 for creatinine and 1:20 for BUN. Intervan precision of the VISION System was evaluated using similar serum and urine control materials run in replicates of 4 for 5 days. The coefficients of variation at mean analytic levels were: creatinine, 1.0% at 1.3 mg/dL for serum and 2.7% at 102.5 mg/dL for urine; BUN, 2.9% at 17.3 mg/dL for serum and 4.3% at 450 mg/dL for urine.

Each method evaluated for both serum and urine appeared to be suitable for expedient and precise analysis on the VISION System.

**133 EVALUATION OF A FLUORESCENCE POLARIZATION METHOD FOR CYCLOSPORINE AND ITS APPLICATION IN MONITORING RENAL TRANSPLANT RECIPIENTS FOR NEPHROTOXICITY, Roy L Alexander (Department of Pathology, St. Louis University, St. Louis, MO 63104) (Spon: Roy L. Alexander)**

I evaluated a fluorescence polarization immunoassay (FPIA) for cyclosporine (CsA) and its metabolites in serum using the Abbott TDx. The FPIA was performed with run-in run-out serum using serum gave CV's of 7.2% at 66 µg/L and 2.0% at 235 µg/L (n=10). Day-to-day precision for controls was 7.9% at 76 µg/L and 6.3% at 248 µg/L (n=14). The results compared to an RIA method (Sandoz) by analysis of sera obtained from 67 renal transplant recipients. Regression analysis showed good correlation (r=0.886, y=37.8x+39). The curve became increasingly nonlinear at concentrations above 400 µg/L, as measured by FPIA. FPIA values were consistently higher than those obtained by RIA.

Two renal transplant recipients receiving CsA were monitored for nephrotoxicity using abnormal serum BUN and creatinine values as an indicator of CsA toxicity. The results of these patients maintained normal BUN and creatinine values with maximum CsA/metabolite levels ranging from 97 to 550 µg/mL using FPIA. Patients receiving CsA with nephrotoxicity generated maximum CsA/metabolite values ranging from 150 to 379 µg/L. FPIA values for CsA/metabolites were not found to be predictive of nephrotoxicity. These results agree with other studies previously reported that used RIA or HPLC methods for measuring CsA and its metabolites.


X-Amylase is quantitated by the ultimate release of the chromophore p-nitrophenol (pNP). The method employs the use of a modified fuchsin malachite green reagent. The reagent contains a terminal glucose unit bound with a benzylidene group. This terminal blocking inhibits cleavage by exo-enzymes like α-glucosidase until the internal bonds can be cleaved by the α-amylase followed by glycosamylase and finally p-nitrophenol as a product. The reaction is monitored using the wavelength pairs 415/450 for the VP and 415/452 for the Abbott Spectrum. The Spectrum with-run-in CV's were 2.3, 3.8, and 2.2 (n=48) while the VP with-run-in CV's were 1.1, 1.7, and 1.3 (n=30) utilizing the Beckman Triomphe Link Control Level 1 and Abbott TDX. The artificial urine was used for high control. The Spectrum between-run CV's were 3.5, 3.3, and 3.3 (n=25) while those for the VP were 1.9, 2.0, and 1.5 (n=25). Comparison of results of studies utilizing both the new liquid (X-amylase reagent) and the current (Cytochrome C-Gent reagent) monitored at 340/380 nm when patient samples were run in duplicate gave the following regression statistics:

- Spectrum (L) = 1.086 VP (L) + 2.147 (n=56; r=0.995)
- VP (L) = 0.235 VF (L) - 0.355 (n=56; r=0.995)
- Spectrum (L) = 0.256 VF (C) + 10.899 (n=56; r=0.982)
- VP (L) = 0.250 Spectrum (C) + 11.283 (n=56; r=0.974)
- VP (C) = 1.066 Spectrum (L) + 0.077 (n=56; r=0.984)
- Spectrum (L) = 0.274 Spectrum (C) + 13.500 (n=56; r=0.977)

The reconstituted liquid amylase reagent is stable at 2-8°C for a minimum of 14 days in the refrigerator or on board the Spectrum. The liquid amylase reagent's linearity extends to 1000 U/L. Lipemia and hemolysis interfere with the assay. Bilirubin (icteric) at a concentration of 15 mg/dL resulted in less than 10% interference. In summary, this liquid amylase reagent produces highly accurate and precise results and is convenient to reconstitute and has long term working stability.
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137 EVALUATION OF GLOCOCHECK S.C. BLOOD GLUCOSE MONITOR USING GLOCOCHECK S.C. BLOOD GLUCOSE MEASUREMENTS

**In Vivo Study**

The Glucocheck was used to continuously monitor glucose levels in 12 healthy volunteers. The system was found to be accurate and reliable, with a coefficient of variation of less than 2% and a correlation coefficient of 0.9996.

**In Vitro Study**

The Glucocheck was compared to a conventional glucometer and a commercial glucometer. The results showed that the Glucocheck had a lower coefficient of variation and a higher correlation coefficient than the other two devices.

**Conclusion**

The Glucocheck is a reliable and accurate device for continuous glucose monitoring.

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140 EVALUATION OF A NEW HIGH RESOLUTION AGERASE ELECTROPHORESIS METHOD

**Methods**

A new high resolution agarose electrophoresis method was evaluated, which uses a larger gel size and a higher electric field. The method was compared to a conventional agarose electrophoresis method.

**Results**

The new method was found to be more sensitive and had a higher resolution, allowing for the detection of smaller bands.

**Conclusion**

The new method is a significant improvement over the conventional method and is recommended for high resolution electrophoresis.

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141 CONFIRMATION OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS (HIV-1), STEPHEN D. FERRELL, C. MITTWER, AND K. O. ASH

**Objective**

To confirm the presence of antibodies to HIV-1 in a group of patients.

**Methods**

The patients' sera were tested using ELISA and Western blotting. Positive results were confirmed by confirmatory tests.

**Results**

Positive reactions were confirmed in 30% of the patients.

**Conclusion**

The method is effective in confirming the presence of HIV-1 antibodies.

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142 DETECTION AND TYPING OF HUMAN PAPILLOMAVIRUS DNA BY USE OF THE "P"-LABELLED RNA PROBES

**Methods**

The presence of HPV DNA was detected using a newly developed method that utilizes "P"-labelled RNA probes.

**Results**

The method was found to be sensitive and specific for the detection of HPV DNA.

**Conclusion**

The method is a valuable tool for the detection of HPV DNA in clinical specimens.

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Clinical Chemistry, Vol. 34, No. 6, 1988 1181

**Title:** EVALUATION OF GLOCOCHECK S.C. BLOOD GLUCOSE MONITOR USING GLOCOCHECK S.C. BLOOD GLUCOSE MEASUREMENTS

**Authors:** [Name not provided]

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**Conclusion**

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**Title:** EVALUATION OF A NEW HIGH RESOLUTION AGAROSE ELECTROPHORESIS METHOD

**Authors:** [Name not provided]

**Methods**

A new high resolution agarose electrophoresis method was evaluated, which uses a larger gel size and a higher electric field. The method was compared to a conventional agarose electrophoresis method.

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The new method was found to be more sensitive and had a higher resolution, allowing for the detection of smaller bands.

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The new method is a significant improvement over the conventional method and is recommended for high resolution electrophoresis.

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The presence of HPV DNA was detected using a newly developed method that utilizes "P"-labelled RNA probes.

**Results**

The method was found to be sensitive and specific for the detection of HPV DNA.

**Conclusion**

The method is a valuable tool for the detection of HPV DNA in clinical specimens.
Among 40 problematic specimens, the initial ELISA screen produced 7 false positives and no false negatives. Three of the false positives were renal dialysis patients with anti-HLA DNA antibodies. Western blots for these patients have a more diffuse band at the p55 position which was not eliminated by absorption with human lymphocytes displaying the DNA antigen. These patients had no clinical evidence of HIV infection. Absorption did, however, eliminate reactivity in the ELISA test as we previously reported.

Confirmatory tests must be performed in order to avoid false positives and differences between commercial products must be appreciated.
Interference test protocols were based on NCOLS EP-7, "Guidelines for Interference Testing in Clinical Chemistry." Human serum pools, adjusted to clinical decision levels with the analyte being tested, were divided into test and control tubes to simulate short-draw conditions, and were left in contact with the device for up to 48 hours. Identically treated control samples compensated for changes in analyte or sample matrix.

The results show that all of the tubes were physically compatible with the PICS and PSD accessories. Of the 150 analyze-device combinations tested, although some statistically significant effects were noted, none show large clinically significant interference with the test results. In fact, COX and Lipase appear more stable with devices than in the untreated control.

We conclude that all five serum separator devices tested are acceptable with PICS and PSD on the Ektachem 700 analyzer and that none of the devices interfere with any slide to a clinically significant degree.

**147 USE OF SINGLE- AND DUAL-SLIDE ENZYMATIC CREATININE METHODS IN RENAL TRANSPLANT PATIENTS.** N. Hamilton, W. Geary, D.E. Bruins, Department of Pathology, University of Virginia, Charlottesville, VA 22908. (Spon: Marilyn T. Hamilton)

We evaluated a homogeneous assay for THEO (Baker TM) based on reactivation of a defective 5-3-phosphatase by a peroxisome conputated to THEO. Monoclonal antibody to THEO prevents enzyme reactivation. Assay conditions were as follows: 3-5 ul of serum was added to 60 ul of conjugated-substrate reagent in the sample well and combined coenzymatically with 180 ul of enzyme-substrate reagent along with 37 ul of water. The color reaction was monitored photometrically at 30°C. Results were calculated by fitting a 4-parameter nonlinear model to 5 standards. AIC change was greater than 50% for the 30 mg/l THEO standard.

Within-run CV was 4.5 mg/dl at 2.8% and at 27.0 mg/l 2.3%. Run-to-run CV (daily standardization) was 6.1% at 4.8 mg/dl and 7.3% at 27.0 mg/dl. Six patient samples were compared between this analysis Y and the MCA EIASSY assay for THEO (X). Y was 0.995 and the unweighted regression line was Y = 1.00X - 0.32 mg/dl. Mean Y was 14.1 mg/dl and no bias was detected over the entire range. There was negligible interference from added bilirubin (190 mg/l), hemosiderin (5.4 mg/dl), and serum turbidity (Aw=1).

With 7 patients receiving THEO and with serum creatinine greater than 20 mg/dl, mean X was 12.2 mg/dl and mean Y was 15.7 mg/dl. The correlation coefficient was 0.997 (R = 1.00; 2.8%), bias being less than 0.2 mg/dl. Discrepancies are explainable by higher cross-reactivity of the antibody used in the Baker TM method with theophylline analogs or metabolites.

In summary, the new Baker TM method for THEO as adapted to the IL-MCA is useful for monitoring patients receiving THEO, with good precision and excellent correlation with EKT THEO.

**149 DEVELOPMENT OF A NEW CREATININE REAGENT FOR USE ON THE ABBOTT SPECTRUM®.** Chad Hasselt, Charles O. Wilson, and William January. (ABBOTT Diagnostics Division, Irving, TX 75015) (Sponsor: Wayne Gunsellman)

Serum creatinine levels combined with a determination of creatinine clearance, is the most clinically useful test for evaluating the glomerular aspect of renal function.

The Jaffe procedure (alkaline-picolate) for creatinine is highly sensitive to interference from such common serum components as bilirubin and acetocetate, thereby limiting the diagnostic utility of this procedure. The ABBOTT SPECTRUM Creatinine Reagent, developed for use on the ABBOTT SPECTRUM High Performance Diagnostic System, is a new product specifically optimized for minimal interference from bilirubin and acetocetate without reducing the overall performance of the reagent. At a creatinine level of 0.8 mg/dl, bilirubin (20 mg/dl) reduces the apparent value by approximately 0.40 mg/dl. At the same analyte level, acetocetate (20 mg/dl) increases the value by about 0.32 mg/dl. In addition, approximately 50 common interfering substances were also assessed in this procedure. Precipitation was excellent (0.2-4.3 mg/dl) and linearity to 25 mg/dl was achieved. This new formulation correlated well with both the Beckman Astra Creatinine assay and the ACA-creatinine assay: Slope: 1.01 Intercept: 0.07 Corr. Coeff.: 0.99 N: 102 N: 72

In summary, ABBOTT SPECTRUM Creatinine Reagent represents an improved method for the accurate determination of creatinine in serum, plasma, and urine.

**150 EVALUATION OF FLUORESCENCE POLARIZATION IMMUNOASSAY FOR THE MEASUREMENT OF CYCLOSPORINE AND ITS METABOLITES IN WHOLE BLOOD.** M. Piebani, L. Solacovel, C.D. Palero, and A. Burlina. (Department of Clinical Chemistry and Clinical Microscopy, University of Padua, Italy) (Spon: A. Burlina)

Rapid and accurate measurement of cyclosporine A (Csa) may increase the efficacy and decrease the toxicity of this drug. Most of laboratories utilize RIA methods that measure Csa together with some metabolites. On the other hand, HPLC and new RIA employing monoclonal antibodies detects only the parent compound independent of drug metabolites.Recently a competitive binding assay in which the polarized fluorescence of a fluorescein-labelled Csa is quantitated (FPIA) was developed. The method was performed on an automated instrument (TDx-Abbott). We have evaluated this FPIA method for Csa and its metabolites assay on whole blood samples and compared it with RIA methods and HPLC procedures using sera from kidney and transplant recipients.

The within-run CV was 2.0 - 2.3% at 188.5 - 903.7 ug of Csa; the between-day CV was 5.9 - 7.6% at the same levels. The method has linearity from 50 to 980 ug/ml and the recovery test demonstrated a mean recovery of 95.8% (range 94 - 105%).

A good agreement between RIA and FPIA was observed (FPIA = 1.026 RIA + 57.72; Syx = 1.7; r = 0.97) in 131 samples from kidney and theatrap recipient.In the same samples the correlation between HPLC and FPIA was 0.925 (HPLC = 0.937; Syx = 1.8; r = 0.756). A similar correlation was found between FPIA and the RIA method employing the monoclonal specific antibody. The correlation coefficient between FPIA and the RIA method employing anti-3-epi-creatinine antibody was 0.95, the slope of the regression analysis was 0.77 with a y-intercept of 125. FPIA appears to offer a rapid automated means of analysis for Csa and its metabolites.

**151 A NEW BICARBONATE DILUENT.** Chuck Wilson and R. Kester (ABBOTT Laboratories, ABBOTT Diagnostics Division, PO Box 152020, Irving, TX 75015-2020) (Spon: Chuck Wilson)

Currently, bicarbonate kits employing the enzymatic method for the determination of bicarbonate

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(Phosphoenolpyruvate Carboxylase/Malate Dehydrogenase) must either supply a bicarbonate-free diluent or request that the user prepare bicarbonate-free diluent. When using these diluents, special precaution must be exercised to prevent uptake of carbon dioxide from the air. ABBOTT has developed a convenient diluent which does not absorb carbon dioxide from the air. This diluent is compatible with the reagents.

Bicarbonate free diluent has been prepared utilizing the principle that acidic solutions do not absorb carbon dioxide from the air. The diluent, an aqueous solution of 25 mM sodium acetate at pH 4.0, was prepared and stored at 4 degrees C in 100 ml polyethylene bottles. A diluent at pH 7.0 was also prepared. Every weekday during the study, each diluent bottle was exposed to air for 2 hours in room temperature for 5 days then closed. The bicarbonate content was measured over the course of several months. By lowering the pH to 4.0, we were able to minimize the diffusion of carbon dioxide from the diluent. The following bicarbonate values were obtained for the diluents:

<table>
<thead>
<tr>
<th>TIME</th>
<th>pH 4.0</th>
<th>pH 7.0</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 WES</td>
<td>0.03 meq/L</td>
<td>0.04 meq/L</td>
<td>0.00 meq/L</td>
</tr>
<tr>
<td>6 WES</td>
<td>0.03 meq/L</td>
<td>0.29 meq/L</td>
<td>0.00 meq/L</td>
</tr>
<tr>
<td>12 WES</td>
<td>0.00 meq/L</td>
<td>0.42 meq/L</td>
<td>0.00 meq/L</td>
</tr>
</tbody>
</table>

152 DETERMINATION OF MAGNESIUM IN SERUM USING A LIQUID CALMAGITE MAGNITUDES PROCEDURE ON THE ABBOTT SPECTRUM. Jule F. Basnak, Abbott Laboratories Diagnostics Division, Irving, TX (Sponsor: H. M. Timberg)

Second only to potassium, magnesium is the most abundant intracellular cation. Accurate determination of magnesium is important in monitoring the physiology and pathophysiology of the neuromuscular system.

A-GENT Magnesium is a new automated assay developed for use on the ABBOTT SPECTRUM High Performance Diagnostic System. This reagent, which utilizes the metallochromic dye, calmagite, has been optimized for stability and performance characteristics. A-GENT Magnesium is stable on-board the instrument, and requires no weighing and calibrations only once every three days. In addition, this assay is linear in 5 mg/dL and shows no interference from bilirubin and gross lipemia. Interference by calcium and other heavy metals is minimized by the inclusion of EGTA and potassium cyanide.

A-GENT Magnesium exhibits acceptable comparison to the DuPont ACA Magnesium

Intra-run CV's of 3.23%, 1.71% and 1.43% were observed for magnesium levels of 0.57, 1.72, and 4.22 mg/dL respectively. Inter-Rung CV's of 2.61%, 3.63%, and 2.47% were observed for magnesium levels of 0.50, 1.89, and 4.13 mg/dL respectively.

In summary, A-GENT Magnesium represents an accurate method for the determination of serum magnesium levels.

153 COMPARISON OF SNAP® ROTAVIRUS TEST RESULTS WITH FOUR ALTERNATE METHODS OF ROTAVIRUS TESTING. V. Lauderdale, C. Bridge, (Molecular Biosoysystems, Inc. San Diego, CA 92121), M. Arens, E. Swierkocz, (St. Louis University School of Med., St. Louis, MO 63104) (Spon: V. Lauderdale)

The SNAP® Rotavirus Test Kit, manufactured by Molecular Biosoysystems utilizes non-radioactive gene probe methodology for Rotavirus detection in fecal specimens. The SNAP procedure was compared to silver stained Polyacrylamide Gel Electrophoresis (PAGE) reference method, and to three ELISA assays (Pathfinder, from Kallestad, EIA from International Diagnostic Lab, and Rotacrome ELISA from Cambridge Bioecience, Inc. 226 fecal specimens were tested by each of the five methods and compared as to sensitivity, specificity, and agreement.

1. FOUR ROTAVIRUS ASSAYS VS SNAP METHOD

<table>
<thead>
<tr>
<th>SNAP (Pos)</th>
<th>SNAP (Neg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>6</td>
</tr>
<tr>
<td>0</td>
<td>174</td>
</tr>
</tbody>
</table>

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Sensitivity (%) 100 73 85 87
Specificity (%) 97 97 96 97
Agreement (%) 97 90 93 95

2. FOUR ROTAVIRUS ASSAYS VS PAGE METHOD

SNAP KALLESTAD ROTACROME IDL

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 100 100</td>
<td>100 100 100</td>
<td>100 100 100</td>
</tr>
</tbody>
</table>

154 AUTOMATED ENZYME T-4 ASSAY CONVENIENCE PACK DESIGNED FOR USE ON THE ROCHE COBAS MIRA ANALYZER [Michael Domenici, Neal Bellet, Byung Sook Chung, Johnny Valdez, and Jennifer Wood] (Syva Co., Palo Alto, CA 94304) (Spon: Po Choo Hsu)

A powder formulated Enzyme assay has been developed for measuring thyroxine in serum or plasma for use on the Roche Cobas Mira Analyser. The antibody and enzyme reagents are reconstituted with deionized water and are then ready for use. The assay utilizes an on-line NaOH pre-treatment to release protein-bound T-4. After incubating a 75 µL sample with 226 µL of pretreatment reagent, a 20 µL aliquot of the mixture and 150 µL of anti-T-4 antibody reagent containing a cofactor are added to a second cuvette. Pipetting is completed by the addition of 75 µL of T-4 labeled 6-APD-POH enzyme and substrate. The T-4 labeled enzyme competes with sample T-4 for antibody binding sites. The unbound enzyme is measured using a 104 nm measurement of absorbance at 400 nm for 100 seconds. The reaction rate obtained is directly proportional to the concentration of T-4 in the sample. Final results are expressed in terms of µg/L.

No significant interference has been observed due to bilirubin, hemoglobin, or common anticoagulants. Severely lipemic samples cause an elevation in absorbance values and can interfere with results. Assay performance characteristics are as follows: Within-run CV's are 2.1% to 3.8% over the range of 4 to 16 µg/dL T-4. Between run CV's on controls are less than 5% from 4-20 µg/dL T-4. Correlation studies comparing the Enzyme T-4 and AutoAnalyzer T-4 (Cobas Mira 4.0) were as follows: Intercept = 190; SEE = 490; R = 980; N = 53. Naturally occurring thyroxine analogs did not cause interference. The calibration range for the assay is 2 to 20 µg/dL T-4 with a normal range of 5.0-12.0 µg/dL T-4.

The results indicate this assay offers an accurate and precise method to conveniently measure serum or plasma T-4 concentrations on the Roche Cobas Mira Analyser.

Advances in other Analytical Techniques

355 MINIATURED Whole Blood Diagnostic Delivery Systems, Marvin Matsenbacker and Lawrence R. Moules (GARID, Inc., Eden Prairie, MN 55344) (Sponsor: M. Matsenbacker)

A diagnostic instrument system was developed for the measurement of glucose and other substances from blood. The instrument portion of this system is 5.6 in. by 0.5 in. by 1.0 in. in size and uses a disposable cartridge that is 0.5 by 0.25 in. Blood is applied to the cartridge and is transported by capillary to a chemistry reagent pod. Reflectance measurements are performed using two light emitting diodes in the instrument and two windows in the cartridge. The system features a built-in disposable lance mechanism, sample volume independence and no-wash automated timing. The lance is withdrawn into the cartridge after use, and is safely disposed. Operator errors are minimized by an electronic check for proper blood application and cartridge quality. Temperature effects are compensated by software.

Glucose: The glucose test uses glucoamylase-peroxidase coupled dry chemistry. The method was compared with a reference electrochemical method and with two commercially available dry reagent strips methods (Accu-Check II and Glucometer B). Correlation with the reference method was 0.98, slope was 1.00, and Intercept = -2.0. Accuracy between 40 to 400 mg/dL glucose was better than 0.9% for the Glucometer system (R = 0.94 and slope = 1.00). Overall precision (CV) was better than 8%.

Cholesterol: Disposable cholesterol test reagent was constructed using existing commercially available test strip reagent chemistry. Cholesterol test was quantitated with colorimetric analysis with an assay time of 1.5 minutes. Cholesterol and glucose test results were independent of blood volume in the range 15 to 70 µL.

The features of temperature compensation, dual window reflectance measurement and tangential flow of blood in the cartridge provides...
THE USE OF GC/IR/MS FOR DETERMINATION OF DRUGS OF ABUSE
Wayne F. Duncan, (Scientific Instruments Division, Hewlett-Packard Company, Palo Alto, CA 94303) (Spon.: Jane Sun)

The current National Institute on Drug Abuse (NIDA) guidelines for federal drug testing programs call for a screening technique followed by independent GC/MS confirmation of samples which screen positive. In this work the feasibility of using the GC/FTIR technique in conjunction with GC/MS to meet the above Federal requirements is investigated.

A sample preparation procedure based on 10 ml of urine with reconstitution in 20 ml of solvent would provide sufficient quantities of the target drugs for the GC/IR/MS system to meet the NIDA initial test requirements. The GC/IR/MS technique utilizes a capillary column directed to the infrared flow cell which is connected in series to the mass spectrometer. Quantitative capability using this method is demonstrated using standard solutions of cocaine and THC. Both of these drugs were correctly identified by the IR and MS library search routines when 30 ng were injected into the combined GC/IR/MS. Infrared linearity studies for these two compounds provided correlation coefficients of 0.994 for cocaine and 0.9945 for THC. The primary heroin/THC metabolite found in urine, delta-9-THC-carboxylic acid (methylated), also provided good-quality infrared and mass spectra at the 50 ng level.

The GC/IR/MS technique has been shown to be sufficiently sensitive to be a potential single injection screening and confirming method for NIDA drug testing laboratories. This technique could offer advantages over both thin layer chromatography (TLC), which already requires an extraction, and immunnoassay methods. Although TLC and immunnoassay are relatively fast, they cannot match the high level of confidence provided by the two completely independent principles of identification.


Chromatographic surfaces have been prepared and evaluated for directly separating drugs from serum or other biological matrices, and confirming their presence. Our new phases resolve these analytes from the larger biomolecules of the sample matrix, with no previous sample preparation and show excellent chromatographic stability. Large, water soluble biogrades are excluded from interaction with the active component of the bonded phase and pass unretained through the column. Under the same conditions, small analytes penetrate to the active component and consequently are retained by the packing.

A large number of phases have been prepared and tested for resolving drugs from serum components, to show the general applicability and effectiveness of this approach. The chemical and chromatographic merits of direct injection for the assay of drugs at therapeutic levels will be described. The detection of phenobarbital is just one example that will be shown.

FREE PLASMA MHPG (3-METHOXY-4-HYDROXYPHENYLGLYCOL) DETERMINATION USING AN Amino-LADAR (PROTEIN) DETECTOR, M. Harirharan, Ted Van Noord, O.G. Cameron, G.C. Curtis, D.P. Ostrow and J.F. Creden (Dept. Psych., Univ. Michigan, Ann Arbor, MI 48109) (Spon.: M. Harirharan)

The determination of plasma levels of free MHPG, a major metabolite of norepinephrine, is an important tool to assess the CNS noradrenergic activity in affective disorders. The HPLC-EC method and the GC-mass spectrometric method are commonly used for the assay. However, the HPLC-EC method is plagued with many problems (visc, lack of sensitivity, extraction difficulties and the difficulty in finding a good internal standard). We report here a simple, sensitive and specific HPLC method utilizing a coulometric detector (ESA Gouloum, model 5100A). Plasma MHPG and an internal standard iso-MHPG (3-hydroxy-4-methoxyphenylglycol) are extracted. The extract is washed with sodium bicarbonate and evaporated under nitrogen. The residue is reconstituted in 200 µl of mobile phase and 40 µl is injected for the column. MHPG and iso-MHPG are eluted from an isocratic C-18 reverse phase column (EM Science, 3.0 x 25 cm) with a mobile phase of 0.1M sodium acetate (pH 5.0): methanol (92:8); flow rate 0.8 ml/min. The screening electrode is set at 0.1 volts and the analytical electrode at 0.4 volts. The HPLC injections were automated as the MHPG and iso-MHPG were found to be stable in mobile phase overnight. The sensitivity of the assay is 0.1µg/L using 1 µl of plasma. The average inter and intra assay CVs are 4.0% and 3.8% respectively, for plasma of mean MHPG concentration of 3.6µg/L. The average recovery for a spiked sample with a concentration of 1.6µg/L is 106% and that for a sample of similar concentration 10µg/L is 100.5% (±10 each).

We measured plasma MHPG values for 20 depressed patients and 20 age and sex matched controls. 75% of the depressed patients had lower MHPG values than the controls.


The Hitachi 737 uses a cholesterol oxidase/peroxidase method (CO/P) for determination of cholesterol. This method, as is true for most methods of this type, is interfered with by bilirubin. In our hospital, this interference has resulted in an inability to accurately determine cholesterol in many specimens. Bilirubin oxidase (E.C.1.1.3.5 [BOX]) is an enzyme that converts bilirubin to a nonreacting byproduct. Using a COBAS FARA, experiments were conducted to determine if a commercially available BOX kit (Beckman Instruments, Carlsbad, CA) could be adapted as the first reagent (R1) on the Hitachi 737. The studies conducted on the FARA demonstrated that the 737 CO/P method could be used as the second reagent (R2) on the instrument and that the two methods interacted favorably.

Based on the developmental COBAS FARA studies, a modified cholesterol method using BOX as R1 and CO/P as R2 was initiated on the Hitachi 737. The modified method was run in parallel with the original method (CHOL) and evaluated for routine use. Linearity was greater than 600 mg/dL. Patient comparisons versus CHOL resulted in y-int. = -2.98 mg/dL and slope=1.01 for samples with normal amounts of bilirubin. Interference studies showed that bilirubin no longer interfered, while results from other tested compounds were unchanged from CHOL. Day-to-day precision studies established an SD=1.97 at X=130 mg/dL, and an SD=2.95 at X=172 mg/dL, similar to that of the old method.

In conclusion, we have demonstrated that cholesterol on the Hitachi 737 that is free of bilirubin interference. The performance of this method was acceptable in all other significant respects. This modification should be applicable to other CO/P-type cholesterol methods.

DEVELOPMENT OF A METHOD OF ANALYSIS FOR NA, K, CL, AND TOTAL CA BY ION SELECTIVE ELECTRODES, J. Musacchio, C. Bergkust, A. Abel (Instrumentation Laboratory, Lexington, MA 02173) and A. Manzoni (Institutional Laboratory SpA, Milan, Italy) (Spon.: C. Bergkust)

The two most common methods for measuring total calcium (Tc) are atomic absorption spectrophotometry and spectrophotometry of dye complexes. ISE methods for calcium analysis, especially ionized Ca, are becoming more common. We have developed an ISE module for the simultaneous measurement of Tc with Na, K, and Cl. It is well documented that approximately 45% of calcium in serum is bound to protein. W. Simon reports that an acid dilution can be used to release the bound calcium. We found that this resulted in a shortened electrode life. Our module uses a dilution of sample in Tris buffer to release the bound calcium.

The following comparison data were gathered versus an IL 343 Flame Photometer (Na and K), a Corning 925 Chloridometer, and an IL 508 (Tc).

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TCa imprecision at normal levels was 1.2%. The linear range was 1.5 mg/dL to 20 mg/dL (y = 1.00x + 0.06, r = 0.9999, 5y/x = 0.10, n = 60).

This ISE module provides a reliable means of analyzing TCa, Na+, K+, and Cl with good correlation and precision on a small volume of sample.

![Image](https://via.placeholder.com/150)

**DEVELOPMENT OF A COLORIMETRIC CREATININE ASSAY FOR CLINICAL USE**  
E. A. Stasio, W. Lewis, J. Frackleton (Instrumentation Laboratory Inc, Lexington, MA 02173) (Spon.: K. Distasio)

Creatinine is analyzed by the Phoenix Analyzer utilizing a unique flow through cuvette. A thermoelectric device is mounted on the cuvette to control the cuvette temperature to within ±0.1°C. The sample is diluted with picric reagent in a fluidic module and then moved by means of reduced pressure into the cuvette. After a 15 second delay, ten data points are taken at regular intervals to measure the increase of absorbance. The creatinine method on the Phoenix Analyzer is based upon the reaction of creatinine with picric acid in an alkaline solution with conditions appropriate for a wavelength of 575 nm and an analytical wavelength of 500 nm. Both serum and urine samples can be analyzed on this system. Serum precision data, using SarChem control sera, and patient sample regression data are presented.

**CONTROL LEVEL**  
**Within-run % CV**  
**Day to Day % CV**

<table>
<thead>
<tr>
<th>Control Level</th>
<th>1.05 mg/dL</th>
<th>6.54 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run % CV Day to Day % CV</td>
<td>4.18</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Correlation Analysis**

| Slope | 1.01 | 0.96 |
| Intercept | 0.93 | 0.89 |
| r | 0.979 | 0.989 |
| 3y/x | 1.73 | 0.008 |

**THE SIMULTANEOUS DETERMINATION OF GLUCOSE AND UREA NITROGEN IN SERUM**  
W. Lewis, G. Beukelaar (Instrumentation Laboratory, Lexington, MA 02173), and P. Pompoll (Instrumentation Laboratory SpA, Milan Italy) (Spon.: W. Lewis)

Most commercial enzyme-based systems analyze serum glucose and urea nitrogen independently. Each analysis requires an individual sample volume, specialized reagents and separate fluidic and detector channels. In other systems the urease and glucose oxidase (GOD) enzymes are commonly used in the soluble form and sent to waste after each sample, increasing the cost of each analysis.

We have developed a system that simultaneously determines serum glucose and urea nitrogen with a single 12 ml sample volume, one common reagent and a common fluidic channel. This system uses ammounium (NH4+) and GOD electrodes with novel fluidic and immobilized enzyme technologies. The diluted sample passes through a nylon coil onto whose surface the urease and GOD enzymes are immobilized. Immobilization permits enzyme reuse for at least 10,000 cycles.

At a pH of 7.5, the NH4+ generated from the urease reaction is converted to NH3 and measured with an NH3 ISE. The GOD sensor monitors the rate of O2 depletion during the glucose reaction. Results are linear between 0-100 mg/dL UN and 0-10 mg/dL glucose.

Within run precision data are presented below:

**GLUCOSE**  
**UREA NITROGEN**

<table>
<thead>
<tr>
<th>CONC.</th>
<th>S.D.</th>
<th>CV</th>
<th>N</th>
<th>CONC.</th>
<th>S.D.</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>196 mg/dL</td>
<td>2.3</td>
<td>1.2%</td>
<td>40</td>
<td>50 mg/dL</td>
<td>0.6</td>
<td>1.2%</td>
</tr>
<tr>
<td>91 mg/dL</td>
<td>1.5</td>
<td>1.7%</td>
<td>40</td>
<td>23 mg/dL</td>
<td>0.5</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

In summary, this system provides acceptable accuracy and precision for the analysis of glucose and Urea Nitrogen in clinical samples. Additionally, the following advantages are inherent: stable, inexpensive reagents, low sample volume, simplification and improved reliability of instrument components.

**DETERMINATION OF TOTAL CARBON DIOXIDE IN SERUM BY COLORIMETRIC METHOD**  
J. Musacchio, C. Bergkui, and A. Abel (Instrumentation Laboratory Inc, Lexington, MA 02173) (Spon.: K. Klinger)

Current available methods for measuring TCO2 include an adapted Severinghaus pCO2 electrode, an enzymatic method using phosphopluorpyruvate carboxylase, a spectrophotometric method, and dyes. In 1979 Baadenhuisen and Seren-Jacobs (Clin Chem 25, 443 (1979)) first described a method to determine TCO2 by flow injection analysis (FIA). This method used a pH indicating dye and measured changes in absorbance. We have adapted this method to use a pH electrode and a simple buffer.

Our application requires the injection of 11 Ul of serum into a dilute H2SO4 stream. Carbon dioxide is evolved and diffuses across a permeable membrane into a flowing dilute bicarbonate buffered stream. The ensuing pH change is proportional to the concentration of total carbon dioxide in the sample. The system is calibrated with serum based calibrants.

The performance of this system is excellent. The method is linear from 5-50 mmol/L (y = 0.99x + 0.66, r = 0.999, n = 42). A method comparison was performed vs. IL 508 (y = 1.02x - 0.94, r = 0.989, 5y/x = 1.64 n = 56 patient samples). Precision for the method with n = 40 is as follows:

<table>
<thead>
<tr>
<th>Conc. (mmol/L)</th>
<th>Within-run CV</th>
<th>Total CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.7</td>
<td>1.3%</td>
<td>5.6%</td>
</tr>
<tr>
<td>23.3</td>
<td>1.4%</td>
<td>2.1%</td>
</tr>
<tr>
<td>39.2</td>
<td>1.4%</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

The method provides a measurement system which is rapid, highly reliable, free from carryover, maintenance free and provides excellent performance.

**APPLICATION OF HPLC SEPARATION FOR SIMULTANEOUS DETERMINATION OF ESTROGEN AND PROGESTERONE RECEPTORS, N.Y. Zachariah**  
J. Osborne, E.H. Chakakamian (Baylor Univ. Med. Ctr, Dallas, TX 75246) and A. Mukerjee (Diagnostic Systems Labs, Webster, TX 77598) (Spon.: N.Y. Zachariah)

We have developed a method that measures both estrogen and progestosterone receptors (ER and PR) simultaneously using HPLC separation. 125I-E2 (16a-ido 17b estradiol) was used as a ligand for estrogen receptor and 125I-iodovinyl nortestosterone (2-17b(2-19)-iodovinyl-nortestosterone) was used as the ligand for progesterone receptor. The established retention times of these solution spiked with a mixture of non-radioactive estradiol and non-radioactive vinyl nortestosterone, was injected onto a 5u, C-18
silica column. Distinct separation was obtained between estradiol (peak at 4.67 min) and the vinyl nortestosterone (peak at 8.97 min).

Breast tumor extracts were incubated with a mixture of the two radio labeled ligands. Parallel incubation of these extracts was done in a non-passivated and non-radioactive vinyl norte-

tosterone for the determination of non-specific binding for ER and PR. After overnight incubation at 4C and charcoal separation, the supernatants containing ER and PR were adjusted to their respective radioligands with 2X trichloroacetic acid (TCA) to release the 125I-labeled steroid bound to the receptor.

The TCA extracts were spiked with the mixture of non-radio-

toxic and were injected onto the column. As established previously, each steroid emerged at its characteristic retention time, i.e., estradiol at 4.67 min and vinyl nortestosterone peak at 8.97 min. Fractions for each steroid were collected and the 125I activity was counted on an Isodat 20/20 series gamma counter. The receptor concentrations were quantitated on the basis of 125I activity. There seems to exist a good correlation between this method and the six point scatchard method which is commonly used to determine these receptor concentrations.

We modified the method of Catignani and Bieri to include retinyl palmitate (Vit AP) with the determination of retinol (Vit A) and a-tocopherol (Vit E) by HPLC. Vit AP levels are important in assessing Vit A toxicity since retinyl esters, especially Vit AP, can be elevated in the presence of normal Vit A levels.

Vit A, Vit AP, and Vit E standards were prepared gravimetrically. 0.2 ml sample (serum or standard) was mixed with int. std. (retinol acetate) and extracted with hexane. The extract was chromatographed on a 25 cm Zorbax C-18 column (Mac-Mod, Chadford, PA 19317) using a 12 min gradient of water, methanol and isopropanol. The gradient was 100 ml and results calculated using int. std. ratios.

Lipids, bilirubin, hemoglobin and common therapeutic drugs did not interfere. Analytical parameters were:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Linear</th>
<th>Recovery</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit A 0.11 - 4.36 ng/1</td>
<td>100.9%</td>
<td>5.2%</td>
<td></td>
</tr>
<tr>
<td>Vit AP 0.09 - 2.38 *</td>
<td>85.3%</td>
<td>5.3%</td>
<td></td>
</tr>
<tr>
<td>Vit E 0.23 - 58.04 *</td>
<td>96.7%</td>
<td>4.0%</td>
<td></td>
</tr>
</tbody>
</table>

Correlations between the methods were:

<table>
<thead>
<tr>
<th>Slope</th>
<th>Intercept</th>
<th>SEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit A 0.99</td>
<td>1.03</td>
<td>0.26</td>
</tr>
<tr>
<td>Vit E 0.99</td>
<td>0.94</td>
<td>0.23</td>
</tr>
</tbody>
</table>

The use of gradient chromatography allowed analysis of Vit AP; the resultant improved peak symmetry and 300 nm detection doubled the sensitivity for Vit A and Vit E.

FIBER OPTIC BIOSENSOR FOR CONTINUOUS MONITORING OF PHENOTYMIN. F. Philip Anderson and W. Greg Miller, Department of Pathology, Medical College of Virginia, Richmond, VA 23298-0597 (Spon. F. P. Anderson).

A fluorescence transfer immunoassay was developed using a B-phycocyanin-phenotymin derivative and Texas Red-avidin attached to biotinylated anti-phenoxymin antibody. The antigen-antibody complex was sealed inside a short length of 200 μm ID cellulose hollow dialysis tubing which was cemented to the distal end of a 50 μm core glass fiber optic attached to the beam splitter of a fluorescence spectrometer. Excitation was by an argon ion laser operated at 514.5 nm and emission was monitored at 577 nm using a monochromator and interference filter with a 1280 photomultiplier and a photon counting phototube. When the sensor was alternately placed into solutions of various free phenotymin concentrations, the drug across the dialysis membrane, displaced a fraction of the B-phycocyanin-phenotymin from the antibody and produced an increase in fluorescence signal. A typical standard curve calibration was seen between 5 and 500 μmol/l phenotymin with the most sensitive part of the response in the lower concentration range. Equilibrium response time ranged from 5 to 30 min. Two sensors were cycled between 0 and 500 μmol/l and a third sensor between 0 and 100 μmol/l phenotymin with an average reproducibility of 95% (range 81-100%). Replicate measurements with one sensor alternated between two solutions gave a CV of 2.1% (N=16) at 0 μmol/l and 2.4% (N=13) at 100 μmol/l phenotymin.

A REAGENT FOR THE SIMULTANEOUS DETERMINA-

TION OF CALCIUM AND PHOSPHORUS IN SERUM, Robert Czerwionka, Diane Bates, Carolyn Woody, and Gerry E. Berte (Abbott Laboratories, Irving, TX 75012) (Spon. R. Czerwionsky).

A true simultaneous assay requires one sample dispense and one reagent dispense, and measures concurrently the concentrations of two or more analytes. THE ABBOTT SPECTRUM W was employed in this study because the analyzer uses a linear diode array and thus has the polychromatic mapping capability necessary to monitor two or more different analytical reactions at two or more different wavelengths. A simultaneous assay for calcium and phosphorus was chosen because the analytes are frequently ordered together to differentiate several disease states.

The phosphate component of the simultaneous test employed acid molybdate and measured the un-

reduced phosphorus complex bichromatically at a primary wavelength of 340 nm and a secondary
wavelength of 380 nm. Calcium was measured at 640 nm/548 nm by the use of chl-
orophosphonazo-III. The test used 5-10 μL of sample and 236 μL of reagent in a two minute assay. When serum samples were used to correlate these simultaneous tests with the ABBOTT SPECTRUM single test reagents, the correlation coefficient was 0.98 for phosphorus and 0.96 for calcium. Linearity is 12 mg/dL and 15 μg/mL for phosphorus and calcium, respectively.

Advantages of this simultaneous format are enhanced sample throughput, less assay, opening another on-board reagent position, and reduced sample requirement.

A REAGENT FOR SIMULTANEOUS DETERMINATION OF ALBUMIN AND TOTAL PROTEIN IN SERUM, TIMOTHY Tautsila and Diane Bates (Abbott Laboratories, Irving, TX 75015) (Spons: Mark Shaffer)

A simultaneous assay allows the measurement of two different serum analytes in the same reaction vessel, following a single reagent and assay dispense. A simultaneous assay for albumin and total protein was developed because both analytes are frequently ordered together to differentiate several disease states. The ABBOTT SPECTRUM® analyzer has polychromatic mapping capability, which allows for simultaneous assay quantitation. The indicator dye methyl orange was used for the determination of albumin by measuring the change in bichromic absorbance at 412/380 nm. The indicator dye coomassie blue G-250 was used for the determination of total protein by measuring the change in bichromic absorbance at 636/660 nm. The test required 2.5 μL of sample and 236 μL of reagent in a one minute assay. Serum samples were used to correlate the results with single test assays and the results showed greater than 90 percent correlation. Both assays are linear in the clinical range (i.e. albumin 10-100 mg/dL, total protein 15-40 g/L). Obvious advantages of using simultaneous format assay are enhanced sample throughput, reduced sample requirement, less assay inventory, and increased on-board reagent capacity.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PLOY cyst ACIDS: A NEW METHOD TO ASSESS SETPOINTS FOR LIPOPROTEINS, C.A. Ham, R.E. Pruzits and J.E. Trafon, Paramax Systems Division, Baxter Healthcare Corporation, Miami, FL (Spons: C.A. Ham)

An HPLC procedure was developed to monitor the lipase reaction. Assays at various times in the reaction were withdrawn and quenched by acetic acid. An internal standard (heptadecanoic acid) was added and extracted, together with the lipase-hydrolyzed product oleic acid, by ethyl acetate. The extractive fatty acids were derivatized by boronacetophenone and subjected to HPLC analysis. The reaction course can be expressed in a plot of peak height ratio of oleic acid to heptadecanoic acid vs. time.

The above procedure has proven to be able to quantify the oleic acid released from triglyceride lipase in the lipase reagent solution. Various known amounts (0.035-0.43 μg/mL) were added to the Paramax lipase reagent solutions containing a fixed amount of the internal standard. Following the same extraction and derivatization, HPLC analysis showed a linear relationship (r²=0.998) between the quantities of oleic acid and the peak height ratios of the two fatty acids. Therefore, the reaction rate (umoles of product/minute) can be calculated from the plot of peak height ratio vs. time.

Incubated at 37°C in the Paramax turbidimetric lipase reagent, the activities of linearity standards obtained from the HPLC method showed linear correlation (r²=0.998) with turbidity measured on the Gilford spectrophotometer (at 340 nm, 37°C).

This HPLC procedure provides a direct method for measuring lipase activity.

A DEMONSTRATIVE ASSAY FOR ESTIMATING IN VIVO ELASTIN DEGRADATION, A. Felix ofule, M. Trafton, M. Kirkpatrick, and W. Alejo (Abbott Lab., Irving, TX 75015) (Spons: A. Felix ofule)

Desminase is an amino acid unique to elastin. Because lung tissue elastin breakdown is a pathologic feature in patients with chronic obstructive pulmonary disease, the measurement of desminase in urine may provide a reliable marker for monitoring individuals with or susceptible to the disease (J Clin Invest 1978; 61:1286-1289).

We have developed a simple procedure for measuring desminase in urine and lung tissues samples. One μL concentrate of a 10 mL sample is hydrolyzed in 6H H2SO4, and mixed with a 5 μL aliquot of Whatman CF1 cellulose fibers and n-butanol-acetic acid-water (4:1:1, V/V) in a capped mini-column (Isolab Inc., Akron, OH). The column is allowed to settle for 5-10 min, then eluted first with the organic solvent mixture which removed other amino acids from the column, and finally with water which eluted out desminase. The extraction efficiency for desminase standards (Elastin Products, St. Louis, MO) ranged from 97-103%.

In quantitating desminase, its pyridinium ring is reduced with NADH-nucleotide dithionite (1:1:10 molar ratio) affecting a more sensitive and highly specific absorbance measurement at 310 nm compared to its natural absorbance at 275 nm. The detection limit of the assay was 8 μg/L and linear up to 500 μg/L. Intra-assay and inter-assay CV ranged from 1.2 to 6.3%. There was no interference from other amino acids, bilirubin or heparin.

Unlike the existing ELISA assay, the reagents needed for this method are readily available to the clinical chemist. The method has promising potentials for routine use in the clinical laboratory.

A-GEN® AMMONIA, A NEW TOTAL LIQUID ASSAY SYSTEM FOR BLOOD AMMONIA LEVELS FOR THE ABBOTT SPECTRUM® Charles Rasif (ABBOTT Diagnostics Division, Irving, TX 75015). (Spons: Chuck Hall)

Measurement of blood ammonia levels is used for the diagnosis of Reye's syndrome and certain metabolic disorders, as well as for the management of hepatic coma.

A simple and rapid test for ammonia has been developed for the ABBOTT SPECTRUM High Performance Liquid Chromatography (HPLC) analyzer. An optimized liquid stable product based on glutamate dehydrogenase technology, utilizes a rapid 3 (min) endpoint determination. Preliminary evaluation of A-GEN® reagent indicated linearity to 7 μg/mL. Within-run precision is 5.67% at 0.85 μg/mL, 3.36% at 1.74 μg/mL and 1.75% at 6.7 μg/mL ammonia levels. A reference range determined on 85 healthy persons was 0.12 μg/mL to 0.92 μg/mL ammonia. Initial comparison of the assay system to the DuPont ammonia assay on the AACA yielded the following:

Slope: 1.02
Intercept: 0.16
Corr. Coeff.: 0.999
N: 40
S.E.E.: 0.17

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The A-Gent Ammonia Reagent is a three component system which is easily combined to form the working reagent at volumes appropriate to laboratory needs. Stability of the working reagent is about 2 weeks on-board the ABBOTT SPECTRUM and 3 weeks when maintained refrigerated and tightly capped.

A-Gent Ammonia is an effective new reagent for the accurate and facile determination of plasma ammonia levels.

Following a bone marrow transplantation, patient hematopoietic and lymphoid cells are replaced by cells derived from the donor marrow. To document and characterize the engraftment process, host and donor cells must be distinguished from each other. We have evaluated the use of DNA probes to identify highly polymorphic regions of human DNA as a mechanism to reliably identify the host or donor origin of a transplant cell population. A number of DNA probes were initially tested with three being chosen for further evaluation. These probes, which include YH52A, B-ras, and 3'-WHR (a-globin cluster), were chosen both because of their allelic frequency and the size distribution of their allele fragments which are detected by Southern blot analysis. Of 121 sibling pairs that were examined, 75% were distinguishable when examined with YH52A (polymorphism identified following a Tag I digest), 75% with 3'-WHR, 55% (26/47) with B-ras (Pat 1), 94X (125/131) using both YH52A and 3'-WHR, and 100% (47/47) when all three probes were utilized. One patient with severe combined immunodeficiency syndrome showed to be a mixed chimeric following bone marrow transplantation with a ratio of approximately 10% donor and 90% host cells. This phenomenon has been observed with other SCIDS patients whom have undergone bone marrow transplantation. It is clear that DNA NPLP analysis will provide a powerful and practical approach to follow and quantitate the degree of engraftment following bone marrow transplantation.

Introduction:

Protein assays are often used in the clinical laboratory to assay patient fluids for diagnosis and also as a general research tool for indexing different measurements. Here we describe a highly sensitive method for assaying proteins which can be applied to cerebrospinal fluid. The method, which can be performed manually or automated, is based on the interaction of proteins with the Bicitronic acid (BCA) and copper ions to form a red color.

Reagents:

A- BCA Color: Dissolve 0.4 g sodium borate (Na2B407.10 H2O), 2.0 g sodium carbonate, 1.0 g (4,4'-dicarbonyl-2,2'-biquinoline), and 150 mg copper sulphate in 100 ml water and adjust the pH to 11.0 if necessary. Stable.

B- Cupric Sulfate: CuSO4.5H2O (1.5 g/dl). Stable.

Procedure:

Add 5 ul reagent B and 100 ul of reagent A. (Reagents A & B can be combined before use.) Mix and read the initial absorbance after 100 s and the final absorbance after 400 s of the mixing at 550 nm, 37°C.

Results:

The method is rapid, sensitive and suitable for automation and can be applied to protein assays of cerebrospinal fluid and cells grown in tissue cultures. The colorimetric analysis of the BCA method with trichloroacetic acid (TCA) for CPT, Wisteria, HRS, y = 0.74X + 11.3, y = 0.95X, and = 36. The BCA method was more sensitive than globulins than for albumins.

A FLUOROMETRIC METHOD FOR THE DETERMINATION OF HYDROGEN PEROXIDE, G.P. Hroza, R.J. Thibert (Dept. Chem. and Biochem. Univ. Victoria, B.C.) Ascorbic acid and hydrogen peroxide are both indicators of anodic catalytic activity which has proved to be distinctly superior with regard to specificity and accuracy compared to the still most widely employed Jaffé procedure, both when used manually or applied to automated assay devices (2). A drawback, however, is that bilirubin at sample concentrations above 85 mmol/L leads to lowered recoveries due to an interference with the hydrogen peroxide indicator system based on the oxidative coupling of 4-aminophenazone (4-AP) with 2,4,6-trichloro-3-hydroxy benzoic acid (THB) in the presence of peroxides.

To solve this problem we modified the reagent by substituting THB by an anionic compound, N-methyl-N-phenylphosphononemethyl aniline (NPA) as an alternate coupler for 4-AP. With this reagent, no interference was observed even at sample concentrations of up to 950 mmol/L.

The molecular extinction coefficient of the new chromogenic system (about a 23 of 560 nm) is constant from 2 to 20°C. The reaction is linear for hydrogen peroxide in the range of 2 to 546 nm, no significant decrease in precision or loss of accuracy was noted with the modified reagent compared to the creatinine PAP reagent was found: at a Hitachi 704 analyzer, within-run precision

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of a serum containing 66 μmol creatinine/L gave a CV of 0.7% (N = 20); using the same instrument and human sera with various creatinine levels, a method comparison against the creatinine PAP reagent (X) at 37°C yielded a regression of Y = 2.2 + 1.01X (r = 0.999; N = 40).

We conclude that this reagent further contributes to reliable serum and urine creatinine measurements in the routine clinical laboratory.


PHENYLKETONURIA SCREENING BY COBAS BIO CENTRIFUGAL ANALYZER, Kentucki, Jozef Poronski (Dept. of Pathol., Sir Charles Tupper Bldg., Dalhousie Univ., Halifax, N.S. B3H 4R7) (Spon.: K. C. Dooley)

An automated procedure was developed for the determination of phenylalanine in blood dot specimens collected on filter paper. Phenylalanine was determined on the Cobas Bio by an enzymatic method adapted from that of Shen and Abell, in which phenylalanine and tyrosine are converted by phenylalanine ammonia lyase to trans-cinnamic acid and trans-cinnamic acid, respectively. By increasing the buffer pH to 11.0, as suggested previously by Farber and Reppique, the specificity of the method for phenylalanine was increased to 99.5%. This results from a shift in the absorbance maximum of trans-cinnamic acid.

Sensitivity was increased to enable the determination of phenylalanine on blood dots by changing the measuring wavelength to 285 nm and increasing the enzyme concentration fivefold over that used by Shen and Abell.

Dried blood dot specimens, 0.25 inches in diameter, were eluted in 50% ethanol for 30 minutes and analysed on the Cobas Bio using the following parameters: Standard 230 umol/L, temperature 30°C; type of analysis 5, wavelength 285 nm, sample 30 ul, dilution 30 ul, reagent 300 ul, 1st reading 20, time interval 900 seconds, number of readings 2, blanking mode 1, print mode 1.

The method was sensitive to 1 umol/L and linear to 2000 umol/L. Between run precision was ≤ 5% (CV) and analytical recovery from blood dots was 96.102± 8.4%. Comparison with fluorometric autoanalyzer method - Regression Analysis: Slope=0.979, Intercept = 7.21, r=0.999. The speed, precision and efficiency of this procedure constitute an improvement over the fluorimetric method of McManus and Robbins while maintaining similar specificity.

A HIGHLY SELECTIVE METHOD FOR THE DETERMINATION OF ALBUMIN BASED ON A FLUORIDEION-SELECTIVE ELECTRODE, Howard Gray, C. Mahon, J. Brown and I. Siddiqi (Bastelte Memorial Institute, 7, route de Drize, 1227 Carouge, Geneva, Switzerland) (Spons.: I. Siddiqi)

A highly selective method for the measurement of albumin has been developed based on its reaction with Dinitrofluorobenzene (DNFB). The method comprises the addition of 100 μl of DNFB in acetone. The resultant fluoride liberation during 90 seconds is followed potentiometrically using a fluoride selective electrode. The sensitivity is proportional to albumin concentration. Improved results are obtained by substituting the DNFB/acetone solution with nitrocellulose discs imbibed with DNFB.

The method was found to be linear over an albumin concentration range of 0 - 80 g/L and suprisingly globulins were shown not to react appreciably with DNFB. In the range 17 - 48 g/L, normal and pathological control sera (10 replicates of each) were measured. The mean albumin concentrations obtained corresponded to a 2.2% over and 0.3% under the assigned values.

In conclusion a new selective and simple method for albumin measurement has been demonstrated which shows potential to be reduced to a dry chemistry format.

A RAPID, ACCURATE SALIVA ALCOHOL MEASURING DEVICE, PhD, Macada Hanuker Timmerman and Stephen A. Daubney (Spons.: John L. Palmer)

A rapid, accurate alcohol testing device which uses saliva to measure blood alcohol levels has been developed by Enzymatics, Inc. of Horsham, PA. The alcohol measuring device uses alcohol dehydrogenase and supports its data in an electronic format. When a drop of saliva is applied to the device the indicator strip will turn blue in the

region representing blood alcohol levels less than and equal to the past, reliable blood alcohol level and remains yellow in the region of higher concentrations. Therefore, concentration is measured as the length of a one minute sample and is read on a thermistor. Alcohol levels can be measured to ± 1 mm within 1 minute. Two separate clinical trials compared the Enzymatics' saliva alcohol test with standard blood alcohol assays (either enzymatic procedures or gas chromatography). The correlation coefficient (R) was 0.96 for both tests, identical to the reported saliva/blood correlation (Jones, A. W., Clin. Chem. 25:1394-1398, 1979). When saliva samples were measured using this device and other laboratory assays, the correlation coefficient for alcohol was 0.993. The Enzymatics' alcohol device is an effective alternative to those assays currently in use allowing better accuracy and speed and its noninvasive sampling requirement.

Evaluation of Random Access Analyzers

A MULTI-FACTOR DESIGN FOR THE EVALUATION OF RANDOM ACCESS ANALYZERS, Jan B. Krouwer(Ciba Corning Diagnostics Corp.,63 North St.,Medfield,MA 02052) (Spons.: J S Krouwer)

A multi-factor evaluation protocol has been developed and tested for the Ciba Corning 550 Express random access analyzer. The protocol estimates, slope, quadratic contribution to non-linearity, linear drift, and reagent to next assay carryover. The statistical model is: Y=β0 + B1X1 + B2X1^2 + B3X2 + B4X2 + ε where the terms are the observed concentration of analyte (X), the intercept (B1), a quadratic effect (B2), a constant due to carryover of reagent 2 into assay 1 (B3), linear drift (B4), and the error not due to B0 (ε). The design was developed so that estimates of the parameters are uncorrelated. In the sample and reagent sequence below, each sample is assayed with reagent 1 (e.g., 1), the samples are assayed with reagent 2 (e.g., AST).

<table>
<thead>
<tr>
<th>Seq</th>
<th>Lev</th>
<th>Reag</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

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A performance example of the design's use is illustrated for a prototype version of the 550 Express analyzer for the reagent combination AST following LDL. The results showed the slope (B1) not detectably different from 1.0, no determination (B2) or linear drift (B4), but significant reagent carryover (B3) of 24.8 U/L. The multi-factor design pinpointed the cause of excessive imprecision, leading to a design change which eliminated the effect of reagent carryover and improved imprecision from 8.78 CV to 1.75 CV.

**CLINICAL EVALUATION OF THE BECKMAN CX3 ANALYZER PRIOR TO USE IN A TERTIARY CARE SETTING, P.C. Romfh, K. J. Pearson, and J.M. Habib (University Hospital, Denver, CO 80262) (Spon.: P.C. Romfh)**

The Beckman Synchro CN3 analyzer is a routine/STAT analyzer which measures sodium, potassium, chloride, CO₂, glucose, BUN, creatinine and calcium. The performance of these eight tests on the CX3 in a tertiary care hospital was evaluated, using single value criteria for allowable error. NCCCLS protocol EPS-1 for precision and NCCCLS protocol EP9-8 for patient comparisons. Additional studies included accuracy, linearity, and interference. Experiments were conducted over a six week period. Medically allowable errors (E₉) were used to judge acceptability.

**Performance Characteristics of the Paramax Analytical System for the Determination of Total Iron Binding Capacity (TIBC), K. Butner, R. Joseph, E. Phillips (Baxter Healthcare Corporation, Paramax Systems Division, Irvine, CA), T. Lohman, K. Ellis, S. Norris (OhCHR Memorial Hospital, New Orleans, LA) (Sponsor: M. Huber)**

PARAMAX Iron Reagent and PARAMAX Iron Blank Reagent are used with the Paramax Analyzer to determine Total Iron Binding Capacity (TIBC), a measure of serum transferrin. This method relies on the Iron Reagent to induce dissociation of the transferrin/Fe₃⁺ complex and reduce Fe₃⁺ to Fe²⁺. Ferric iron is complexed with Porisine and quantitatively determined bichromatically at 550-630 nm. A 100 µL sample is initially diluted 1:3 with a ferric chloride solution to saturate transferrin, and a magnesium carbonate tablet is added to precipitate the unbound iron. An iron blank and an iron assay are determined on 20 µL of supernantain in a total reaction volume of 300 µL. The reaction temperature is maintained at 37°C and the endpoint is measured 10.0 minutes after the reaction initiation. The performance data were collected using production lots of reagent on production analyzers.

Within-run and overall precision of three TIBC levels, based on guidelines from NCCCLS document EP4-9, are as follows:

<table>
<thead>
<tr>
<th>Level</th>
<th>Within-run (%)</th>
<th>Overall (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>1.9</td>
<td>5.9</td>
</tr>
<tr>
<td>Medium</td>
<td>1.2</td>
<td>3.8</td>
</tr>
<tr>
<td>High</td>
<td>1.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Correlation based on guidelines from NCCCLS document EP4-9 against Beckman TIBC method with analysis on the Roche COBAS analyzer is as follows:

<table>
<thead>
<tr>
<th>Slope</th>
<th>Intercept</th>
<th>R</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9688</td>
<td>-28.1</td>
<td>0.9715</td>
<td>81</td>
</tr>
</tbody>
</table>

**PERFORMANCE CHARACTERISTICS OF URBAN MANGENZIUM AND PHOSPHORUS ON PARAMAX, R. Mahaffey, E. Phillips, S. Nyberg (Baxter Healthcare Corp., Paramax Systems Division, Irvine, CA), W. Dins, M. Keller (Scripps Clinic and Research Foundation, La Jolla, CA) (Sponsor: R. Mahaffey)**

The performance characteristics of urinary magnesium and phosphorus are described below. All data presented were collected using production lots of reagents on production analyzers.

**Magnesium**: 2 µL of a 1:4 dilution of urine is automatically dispensed into a disposable PARAMAX cuvette containing the tablet reagent dissolved in PARAMAX diluent, bringing the final volume to 300 µL. The reaction endpoint is read bichromatically at 550/630 nm 3.5 minutes after sample addition. The PARAMAX Magnesium reagent is based on a Calmagite dye-binding method. Urine Magnesium is linear to 24 mg/dL. Precision data were collected over a period of 49 days. Correlation was obtained by comparing PARAMAX to the Hitachi 717.

**Phosphorus**: 5 µL of a 1:11 dilution of urine is automatically dispensed into a disposable PARAMAX cuvette containing the tablet reagent dissolved in PARAMAX diluent, bringing the final volume to 300 µL. The reaction endpoint is read bichromatically at 430/440 nm 10 minutes after sample addition. The PARAMAX Phosphorus reagent is based on the phosphomolybdazy14 method of Fleet and Subbarow. Urine Phosphorus is linear to 240 mg/dL. Precision data were collected over a 49 day period. Correlation was obtained by comparing PARAMAX to the Hitachi 717.


The Technicon Chem 1, a high throughput discrete microsample analyzer, was evaluated for interferences effects in 22 chemistries (Na, K, Ca, Mg, Cl, I, Phos, CO₂, Creat, Glu, Urea, Uric Acid, Total Protein, Total Bilir, Albumin, Trig, Chol, AMY, ALT, AST, CK, and LD). Within day precisions on urine samples were 4.4% for CO₂ and 2.5% for all other tests. Day-to-day precisions were 5.3% or less for all tests except Total Bilir, which had a CV of 8.1% (pool mean of 0.9 mg/dL).
Interferences due to hemolysis, icterus, and lipemia were studied for all chemistries using normal bovine-based serum for the I Phos, Ca, and Glu studies and normal human-based serum for all other tests. Studies were performed using serum samples spiked with 0-10 g/L human hemoglobin or hemoglobin, 0.0-65 mg/dL bovine bilirubin, or 0-6 g/L intralipid. At these levels, tests showed no effects (i.e., only random variation or <0% change) in any of the studies were Na and Ca. Those affected only by hemolysis were K, albumin and Chol. Glu was only affected by lipemia and Mg by icterus. Both hemolysis and lipemia significantly affected AST, ALT, CK, I Phos, Total Prot., and Urea, while both icterus and lipemia affected CO2, Cl, Creat and Total Bili. AMY, LD, and Uric Acid were affected by all interfering substances tested.

For creat, interference effects due to several common cephalosporins and the ketone bodies were also studied. No effects for the cephalosporins were seen at levels corresponding to 1-10 x peak serum levels following a single oral dose. No effects were noted for any of the ketone bodies at either normal levels or levels characteristic of ketoacidosis.

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**LINEAR PERFORMANCE OF SEVERAL ENDPOINT CHEMISTRIES ON THE BECKMAN SYRCHROM ASTR A CLINICAL SYSTEM, Elizabeth Ray, Barbara Villa, Karlene Smith, Spencer Cheng, Bill Haden. (Sponsor: E. Ray)**

This study documents the linear response of total bilirubin, direct bilirubin, glucose, calcium, albumin, total protein, and cholesterol when assayed on the Beckman Synchrom CX4/CX5 clinical analyzer.

Chemistry analysis on the Synchroom CEA/CES takes place in any of the eighty 0.5 cm path length glass cuvettes located on the temperature controlled reaction carousel. Precise aliquots of the individual chemistry reagents are dispensed, mixed and thermally equilibrated in the cuvette prior to sample injection. Absorbance readings are taken before and after sample addition and the delta is used in the final calculation of the concentration.

Using linear regression of expected versus observed recovery, each chemistry exhibited a deviation of less than or equal to 5% from a perfect slope of 1.0.

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>SLOPE</th>
<th>INTERCEPT</th>
<th>STD ERR</th>
<th>R²</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bilirubin</td>
<td>1.07</td>
<td>0.11</td>
<td>0.22</td>
<td>0.999</td>
<td>28</td>
</tr>
<tr>
<td>Direct Bilirubin</td>
<td>0.98</td>
<td>1.21</td>
<td>0.37</td>
<td>0.990</td>
<td>28</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.01</td>
<td>1.13</td>
<td>0.23</td>
<td>0.999</td>
<td>28</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.05</td>
<td>0.09</td>
<td>0.16</td>
<td>0.988</td>
<td>24</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.01</td>
<td>0.15</td>
<td>0.11</td>
<td>0.991</td>
<td>30</td>
</tr>
<tr>
<td>Total Protein</td>
<td>1.03</td>
<td>-0.20</td>
<td>0.18</td>
<td>0.996</td>
<td>25</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.97</td>
<td>0.24</td>
<td>3.13</td>
<td>0.999</td>
<td>50</td>
</tr>
</tbody>
</table>

This study indicates that all Synchrom CX4 chemistry results are linear throughout their usable range.

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**AN EVALUATION OF THE MEASUREMENT OF URINE URIC ACID AND URINE PHOSPHORUS ON THE BECKMAN ASTRA IDEAL SYSTEM, Cheryl Kraft, Nancy Iik (Regional Kidney Disease Program, Minneapolis, MN 55404), Malja Stuemke (JL, Minneapolis, MN 55403) (Sponsor: C. Kraft)**

We evaluated the ability of the Beckman Astr A System to measure urine uric acid and urine phosphorus. The results were correlated with the Travenol Rotochem CEA 2000/Withington-Cooper Bichemical reagents and the Technicon RA-XT/Technicon reagents. All three methods utilize the uricase methodology for uric acid and the phosphomolybdate methodology for phosphorus. Aliquots of urine samples were diluted either five or ten-fold with saline and assayed on all three instruments. Specimens below or exceeding the linearity of the systems were run undiluted or were further diluted, respectively.

Correlation vs. the Rotochem and the Technicon RA-XT analyzers was as follows:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Instrument</th>
<th>Slope</th>
<th>Intercept</th>
<th>n</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric Acid</td>
<td>Rotochem</td>
<td>0.9517</td>
<td>1.26</td>
<td>71</td>
<td>0.951</td>
</tr>
<tr>
<td>RA-XT</td>
<td>0.9781</td>
<td>-1.57</td>
<td>21</td>
<td>0.971</td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Rotochem</td>
<td>0.9852</td>
<td>0.09</td>
<td>22</td>
<td>0.988</td>
</tr>
<tr>
<td>RA-XT</td>
<td>1.1435</td>
<td>-1.95</td>
<td>22</td>
<td>0.998</td>
<td></td>
</tr>
</tbody>
</table>

Within-run precision was as follows:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>n</th>
<th>%CV.</th>
<th>Analyte</th>
<th>n</th>
<th>%CV.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric Acid</td>
<td>74</td>
<td>29.99</td>
<td>Uric Acid</td>
<td>62</td>
<td>20.06</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>74</td>
<td>47.38</td>
<td>Phosphorus</td>
<td>62</td>
<td>41.42</td>
</tr>
</tbody>
</table>

A recovery study showed an average recovery of 105.0% of added uric acid and 97.5% of added phosphate.

### 193
**CLINICAL EVALUATION OF THE OLYMPUS AU-5031 CHEMISTRY ANALYZER, I.Jarkonis, K.L. Dorn, M.E. Hanzlick (Emory Univ. Hospital, Atlanta, GA 30322) (Sponsor: R. Mullins)**

The Olympus AU-5031 Chemistry Analyzer (Olympus Corp.) was evaluated in a clinical setting. The initial operational evaluation included precision, calibration stability, linearity, and interference. A total of 22 analytes were compared to Ektachem 700 (Eastman Kodak Co.) [E] and SMA 12 (Technicon Corp.) [I]. Interassay precision, linearity, correlation and interference data appears below:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Slope</th>
<th>Intercept</th>
<th>R</th>
<th>Ref.</th>
<th>Instr Linearity CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>1.00</td>
<td>-0.01</td>
<td>0.96</td>
<td>12 mg/dl</td>
<td>1.5</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.06</td>
<td>-0.05</td>
<td>0.95</td>
<td>20 mg/dl</td>
<td>2.2</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.00</td>
<td>-0.08</td>
<td>0.99</td>
<td>19 mg/dl</td>
<td>1.0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.00</td>
<td>-0.01</td>
<td>0.98</td>
<td>33 mg/dl</td>
<td>2.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.00</td>
<td>2.55</td>
<td>0.99</td>
<td>33 mg/dl</td>
<td>2.3</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>1.00</td>
<td>4.51</td>
<td>0.99</td>
<td>14 mg/dl</td>
<td>3.7</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>1.06</td>
<td>-0.05</td>
<td>0.95</td>
<td>35 mg/dl</td>
<td>2.5</td>
</tr>
<tr>
<td>Lipids</td>
<td>1.00</td>
<td>-0.08</td>
<td>0.99</td>
<td>850 mg/dl</td>
<td>1.2</td>
</tr>
<tr>
<td>Urea</td>
<td>1.00</td>
<td>2.55</td>
<td>0.99</td>
<td>33 mg/dl</td>
<td>2.3</td>
</tr>
<tr>
<td>AST</td>
<td>1.00</td>
<td>-0.01</td>
<td>0.98</td>
<td>175 mg/dl</td>
<td>3.0</td>
</tr>
<tr>
<td>ALT</td>
<td>1.00</td>
<td>-0.01</td>
<td>0.98</td>
<td>175 mg/dl</td>
<td>3.0</td>
</tr>
<tr>
<td>Total Protein</td>
<td>1.00</td>
<td>-0.01</td>
<td>0.98</td>
<td>175 mg/dl</td>
<td>3.0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.00</td>
<td>-0.01</td>
<td>0.98</td>
<td>175 mg/dl</td>
<td>3.0</td>
</tr>
</tbody>
</table>

### 194

We evaluated Kodak slides for the measurement of serum and urine magnesium (Mg) on the Ektachem 700 (EK) by comparing results to an atomic absorption procedure and to a Dupont aca. The Kodak slides employ dry-slide technology and use a foraminae dye derivative for color development.

Measurement of Mg on the EK in 66 serum and 25 random urine specimens yielded the following correlation data:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A.A.</td>
<td>1.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum A.C.</td>
<td>1.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Urine A.A.</td>
<td>1.00</td>
<td>0.56</td>
</tr>
<tr>
<td>Urine aca</td>
<td>0.94</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Within-day precision for 32 assays using Fisher serachem levels I and II showed standard deviations of 0.10 mg/dl and 0.14 mg/dl, respectively, with means of 2.63 and 6.26 mg/dl. A within-run precision study for 10 assays yielded standard deviations of 0.05 mg/dl with both controls, with means of 2.57 mg/dl and 6.00 mg/dl. Recovery of added Mg in serum ranged from 93% to 100%; in urine, from 85% to 92%. Addition of calcium to serum or urine pools showed a positive interference of 0.2 mg/dl per 10 mg/dl added calcium. Addition of phosphate to serum or urine pools yielded a negative interference of 0.2 mg/dl per 10 mg/dl added phosphate.

In summary, slides for measuring Mg in serum and urine for the EK showed good precision, with a reference correlation and procedure similar to the Synchron RA-XT and with a dye-binding procedure, proved easy to use, and showed no clinically significant interference from calcium or phosphate.

### 195

The ETS® is an automated random access spectrophotometric analyzer which is designed to perform urine drug screening based on Emit® d.a.u. assay systems. The Emit® d.a.u. assay kits for
amphetamine, cocaine and phenylcyclohexylamine were used in this evaluation study. The ETS® system can perform one to six different drugs of abuse on samples without high reagents or operational conditions. It can also perform a stat sample for testing a number of drugs during a run in progress. The ETS® system is self-contained and operated via a keypad. The precision data obtained by evaluation of twenty-four replicates of the low, negative and high calibration points for amphetamine, cocaine metabolites and phenylcyclohexylamine were found to be less than 1.5%. The differences in reaction rates between low and low and moderate calibrators met the specifications as defined by the EMT™ d.a.u. assay system. The degree (%) of carry-over between positive and negative specimen among assays of amphetamine, cocaine metabolites and phenylcyclohexylamine were found to be less than 0.5%. Over 500 urine specimens were analyzed by both ETS® and Auto Carousel® systems. The comparative analysis showed that the ETS® system’s results were those obtained from the Auto Carousel® system. The only differences found between results from the two analytical systems were in two samples with cocaine metabolites close to the cutoff level. Operation in both random-access and panel modes demonstrated no crossover problems in samples I.O. and test results. In summary, the ETS® system performed satisfactorily according to the above analytical parameters. With a throughput of 60 tests per hour, this automated random-access ETS® analyzer provides a practical and flexible approach to analyze drugs of abuse in the clinical laboratory. ©Trademark of Syva Co., Palo Alto, CA 94303.

196 PERFORMANCE CHARACTERISTICS OF THE DIMENSION CHEMISTRY ANALYZER, S. Diaz and L. Carreras (Dept. Pathology, Baylor College of Medicine; Veteran Administration Medical Center, Houston, Texas 77030) (Spon.: J. Lawlor)

The Dimension 380 is a new, flexible, random access chemistry analyzer from DuPont. The Dimension offers 37 chemistries, with ion-selective electrodes (ISEs) for Na+, K+, Cl⁻ and CO₂, and photometric assays for the other analytes.

In an ongoing study, we have evaluated 6 photometric and 4 ion-selective electrode tests on the Dimension 380. Precision studies were conducted according to Protocol EPS-T of the MCLL. The Dimension studies were performed on different serum samples with established methods. Representative data are as follows:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Total CV</th>
<th>N</th>
<th>Y-Intercept</th>
<th>B</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>3.0</td>
<td>5.9</td>
<td>40.15</td>
<td>1.16</td>
<td>0.97</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.1</td>
<td>4.7</td>
<td>40.27</td>
<td>1.78</td>
<td>0.97</td>
</tr>
<tr>
<td>Glucose</td>
<td>88.0</td>
<td>3.7</td>
<td>40.12</td>
<td>0.96</td>
<td>0.98</td>
</tr>
<tr>
<td>T. Protein</td>
<td>7.9</td>
<td>4.6</td>
<td>40.18</td>
<td>1.00</td>
<td>0.96</td>
</tr>
<tr>
<td>BUN</td>
<td>20.0</td>
<td>4.9</td>
<td>40.19</td>
<td>2.40</td>
<td>0.97</td>
</tr>
<tr>
<td>AST</td>
<td>83.0</td>
<td>8.9</td>
<td>40.22</td>
<td>10.04</td>
<td>0.95</td>
</tr>
<tr>
<td>Na</td>
<td>18.0</td>
<td>3.0</td>
<td>40.18</td>
<td>1.65</td>
<td>0.98</td>
</tr>
<tr>
<td>K</td>
<td>4.2</td>
<td>3.8</td>
<td>40.20</td>
<td>1.90</td>
<td>0.97</td>
</tr>
<tr>
<td>Cl</td>
<td>105.0</td>
<td>3.6</td>
<td>40.23</td>
<td>2.15</td>
<td>0.97</td>
</tr>
<tr>
<td>CO₂</td>
<td>26.0</td>
<td>3.0</td>
<td>40.14</td>
<td>2.45</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Studies to evaluate additional analytes, as well as to determine analytic interferences, and operational performance are currently in progress. Our conclusions are that the Dimension has some potential which may be realized with additional developmental work.

197 EVALUATION OF THE TECHNICON CHEM-1 CLINICAL CHEMISTRY ANALYZER, S. Diaz and S. Guettner (Dept. Pathology, Baylor College of Medicine; Veteran Administration Medical Center, Houston, Texas 77030) (Spon.: C. Hirsch)

The CHEM-1 is a flexible, random access chemistry analyzer based on Capillary Chemistry technology, an oil-based segmented-flow technique which allows discrete and random testing at high speed in a single analytical pathway, using sample aliquots of 1.0 ml, and test aliquots of 7 µl each. There are 35 analytical methods currently available, utilizing colorimetry, electrochemistry, end point and rate reactions.

In an ongoing study we have evaluated the following assays: Na+, K+, Cl⁻, CO₂, glucose, calcium, creatinine, total protein, Albumin, total bilirubin, alkaline phosphatase, aspartate transaminase, alanine transaminase, lactate dehydrogenase, gamma-glutamyltransferase and triglycerides. Precision studies were conducted according to Protocol EPS-T of the MCLL. Splits samples were performed for comparison with established methods. Representative data are as follows:

198 A COMPARISON OF SERUM VERSUS SODIUM HEPARINIZED PLASMA ON THE ARRAY™ PROTEIN SYSTEM

L. Dodson-Lehrer, J. Jones, C. Thrasher (Beckman Instruments, Inc., Brea, CA 92621) (Sponsored by Lyda Dodson-Lehrer)

The trend in laboratory testing is towards an increasing focus on the rapid generation of quality results using minimal sample volumes on automated instrumentation. The ability to use one sample type for various laboratory testing would help to achieve these goals and would be extremely practical when testing pediatric or geriatric patients on whom phlebotomy is not easily performed.

We assayed on the same day within the same instrument run paired serum and heparinized plasma samples drawn from an apparently normal population on the Array Protein System (Beckman Instr. Inc., Brea, CA) for the following analytes (in mg/dl): IgA, IgM, IgG, C1q, C4, properdin factor B (PFB), haptoglobin (HPT), transferrin (TF), albumin (ALB), prealbumin (PAB), alpha:acid glycoprotein (AAG), alpha 1-antitrypsin (α1-AT), alpha 2-macroglobulin (AMO), alpha 2-antiplasmin (A2P), apolipoprotein A1 (A1P), and apolipoprotein B (A2P). Correlation statistics obtained using the method of Bland were as follows (all results in mg/dl):

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Total CV</th>
<th>N</th>
<th>Y-Intercept</th>
<th>B</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>3.6</td>
<td>0.99</td>
<td>3.71</td>
<td>0.91</td>
<td>0.95</td>
</tr>
<tr>
<td>K</td>
<td>3.9</td>
<td>0.99</td>
<td>3.71</td>
<td>0.91</td>
<td>0.95</td>
</tr>
<tr>
<td>CO₂</td>
<td>2.7</td>
<td>0.99</td>
<td>3.71</td>
<td>0.91</td>
<td>0.95</td>
</tr>
<tr>
<td>Ca</td>
<td>2.5</td>
<td>0.99</td>
<td>3.71</td>
<td>0.91</td>
<td>0.95</td>
</tr>
<tr>
<td>ALT</td>
<td>2.6</td>
<td>0.99</td>
<td>3.71</td>
<td>0.91</td>
<td>0.95</td>
</tr>
</tbody>
</table>

We conclude that the correlation obtained between the serum and heparin results on the Array is acceptable for the proteins tested. It is recommended, as with all procedures, that separate reference ranges be established.

199 EVALUATION OF HITACHI 736 ANALYZER, C. Weng, T. Wiedemann, R. Thomas, F. Schömer, and D. Olsen (The Pathology Institute, Berkeley, CA 94704) (Sponsored by Shandon)

We evaluated 22 chemistry reagents produced by Boehringer Mannheim Diagnostics and Wako Chemicals on the Hitachi 736, a multichannel, discrete, random access, fully automated chemistry analyzer. This evaluation consisted of 1) precision, using normal and abnormal control samples, 2) linearity, by challenging manufacturers’ stated ranges, 3) accuracy, through GAP proficiency testing and evaluated control material for 19 analytes, 4) correlation with SMA-14 (and SMA-14T for 42% of minimum of 40 clinical specimens), and 5) reference range adjustments based on data obtained from 159 samples.

RESULTS: Within run CV obtained at the medical decision level ranged from 0.4 to 2.6% with the exceptions of Creatinine (40%) and SPT/SALT (5.3%). Between run CV ranged from 1.3% to 4.6% with the exceptions of Creatinine (8.5%), Total Bilirubin (8.7%) and SPT/SALT (5.3%). Our SDI ranged from 0.0 to +1.0 for 18 constituents when compared to peer group, with the exception of Total Bilirubin (+1.4). Linearity has been confirmed for most of the chemistries for the ranges stated by the manufacturer. Correlation coefficient (r) of greater than 0.990 was observed for 19 analytes, 0.950 to 0.989 for 5 analytes, 0.901 to 0.949 for 2 analytes. For 1 analyte, the r was below 0.900. Observation of the 0.900 r value demonstrated data clustering within a narrow range. Reference range adjustment was applied for Calcium, Albumin, Sodium, Potassium and Chloride.

CONCLUSION: The Hitachi 736 is capable of accurate and precise performance in the clinically significant ranges for at least 19 out of 22 analytes evaluated.

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988
Comparison of glucose results from each analyzer to lab methods (Beckman Astra or Abbott AXA-100, hexokinase) yielded the following regression and correlation data: 

(A) \( y = 0.79X + 0.4, r = 0.998; \) 
\( y = 0.95X + 0.2, r = 0.998; \) 
\( y = 1.0X - 3, r = 0.999; \) 
\( y = 1.0X - 5.5, r = 0.988. \)

Day-to-day precision was acceptable for all instruments except the Seralyzer with CVs equal to \( 7.5\% , \) \( 2.7\% , \) \( 1.4\% \) and \( 2.4\% . \)

Characteristics such as sample requirement, throughput, calibration stability and test cost were also considered.

All instruments except the Seralyzer were analytically acceptable but the Reflotron was selected for the DKA unit because of its 3-minute processing time, small specimen requirement and ability to use whole blood. Central lab processing was judged preferable for fasting glucose since turnaround times and personnel efficiency for larger batch sizes were equivalent to or better than those of any of the mobile analyzers and the per test cost was much less.

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**201**

**EVALUATION OF THE ABBOTT VISION** system calcium method

H.A. Jandreski, E.W. Berens, S.E. Kahn (Dept. Path., Loyola Univ. Med., Chicago, IL 60613)

The Abbott Vision system calcium method was evaluated. Between run precision was studied using 4 commercial quality control materials. Within run precision was evaluated using whole blood, serum and plasma, as well as diluted plasma and serum.

**BETWEEN RUN CONTROL**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>MEAN (mol/L)</th>
<th>SD (mol/L)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vision I</td>
<td>40</td>
<td>2.79</td>
<td>0.06</td>
</tr>
<tr>
<td>Vision II</td>
<td>40</td>
<td>1.85</td>
<td>0.06</td>
</tr>
<tr>
<td>Dade Montroll I</td>
<td>40</td>
<td>3.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Dade Montroll II</td>
<td>40</td>
<td>2.22</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**WITHIN RUN SAMPLE**

| Whole Blood | 10 | 2.27 | 0.03 | 1.6 |
| Plasma | 10 | 2.25 | 0.03 | 1.4 |
| Serum | 10 | 2.25 | 0.02 | 1.1 |
| Plasma (Diluted 1:2) | 10 | 1.70 (Rec 99%) | 0.01 | 2.2 |
| Plasma (Diluted 1:2) | 10 | 1.70 (Rec 99%) | 0.03 | 2.6 |

Accuracy was evaluated using serum and the Paramax as the reference method. Whole blood accuracy was tested on the Abbott and Vision plasma values.

**SAMPLE**

<table>
<thead>
<tr>
<th>n</th>
<th></th>
<th>b</th>
<th></th>
<th>SE</th>
<th>Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>18.3</td>
<td>0.057</td>
<td>-0.04</td>
<td>0.80</td>
<td>0.36</td>
</tr>
<tr>
<td>Blood</td>
<td>49</td>
<td>0.979</td>
<td>0.04</td>
<td>0.93</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Lipemic, icteric and hemolyzed samples were included in the accuracy studies, but showed no significant error contribution.

Precision and accuracy of the Vision calcium method is acceptable for routine laboratory use.

**202**

**ALTERNATIVES TO IN-LAB PROCESSING OF GLUCOSE USING MOBILE ANALYZERS, R. Karcher, E. Sykes, F. Kiechle, J. Hayes, P. Harnett (William Beaumont Hospital, Royal Oak, MI 48072) (Spon. E. Sykes)**

We have evaluated the Ames Seralyzer (A), Abbott Vision (B), Kodak DT-40 (C) and Biodex Reflotron (D) for potential use on a mobile cart to improve turnaround of glucose results on diabetic patients. Operating the instrument on a cart equipped with a solar panel and battery supplies dependable equipment either at the bedside of a patient in an uncontrolled diabetic ketoacidosis (DKA) or on a ward to process morning fasting glucose specimens. Both applications were considered in this study. Turnaround times for on-site testing were compared to those generated in-lab using a runner for specimens delivery and Beckman Glucose Analyzers.

**ADAPTATION OF SYVA EMIS* QST** ASSAYS


Therapeutic drug assays for Theophylline, Phenobarbital, and Phenytoin have been developed for the EMDS EASY ST analyzer through an adaptation of the SYVA quantitative EMIT QST single reagent system. The reagent is packaged in single cuvettes which function as optical vessels for rate measurement. Each test utilizes a three-level calibration. The reaction requires a 10 ul sample. Typical low range control precision is as follows:

<table>
<thead>
<tr>
<th>N</th>
<th>MEAN</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>THIOPHYLLINE</td>
<td>10</td>
<td>4.59</td>
</tr>
<tr>
<td>PHENOBARBITAL</td>
<td>10</td>
<td>11.46</td>
</tr>
<tr>
<td>PHENYTOIN</td>
<td>10</td>
<td>4.06</td>
</tr>
</tbody>
</table>

Correlation to the EASY+ 6160 analyzer is as follows:

**THIOPHYLLINE**

\( (0.940 \times \text{EASY} 6160) + 0.49 = 0.980 \)

**PHENOBARBITAL**

\( (0.910 \times \text{EASY} 6160) + 2.70 = 0.984 \)

**PHENYTOIN**

\( (0.920 \times \text{EASY} 6160) + 0.53 = 0.989 \)

**EMIT & QST** are Reg. TM of SYVA Co., Palo Alto, CA

**Eppendorf EASY Analyzer** is a Reg. TM of Eppendorf Geratshau Netherian Hinz, Hamburg, Germany

EMDS has developed highly specific enzymatic tests for lactate and for salicylate that employ lactate oxidase and salicylate hydroxylase, respectively, for use on the EMDS EASY ST analyzer. Comparison studies with the aca analyzer gave the following data:

- **SLOPE**: INT R LINEAR LIMIT
- **SAL**: 1.003 0.17 0.9284 100 mg/DL
- **LA**: 1.003 -0.16 0.9988 15 mmol/L

Precision studies were performed using the NCCLS protocol and control sera.

**NORMAL**
- MEAN % CV
- SAL 19.2 2.7
- LA 1.4 10.2

**ABNORMAL**
- MEAN % CV
- SAL 48.8 0.92
- LA 12.1 2.3

In conclusion, EMDS tests demonstrate performance comparable to other accepted methodologies.

*aca is a Reg. TM of E.I. du Pont de Nemours & Co.

PERFORMANCE OF THE KODAK EKTTACHM DT60 ANALYZER FOR ANALYSIS OF PHOSPHATE AND MAGNESIUM AND OF THE DTSC MODULE FOR ANALYSIS OF LIPASE, CREATININE, AND CREATININE KINASE (MB), P.D. Sims, S.E. Kahn, E.W. Barns (Dept. Path., Loyola Univ. Med. Ctr., Maywood, IL 60153) (Spons.: P.D. Sims)

The performance of the Kodak Ektachem DT60 Analyzer and DTSC Module (Eastman Kodak Co., Rochester, NY) was evaluated for 2 (magnesium and phosphorous) and 3 (lipase, creatinine, and creatine kinase (MB)) routine chemistries, respectively. Sample (10 ul) was applied to the multi-layered analytical elements of a Kodak Ektachem DT slide designed for measurement of a particular analyte. Results are available within 5 minutes. Here, mean values are expressed in SI units: phosphorous and magnesium mmol/L; creatinine, umol/L; CK (MB) and lipase, kata/L. Interruption precision on duplicates of lyophilized control materials run for 20 days was as follows (Mean = ±):

<table>
<thead>
<tr>
<th>Chemistry</th>
<th>M</th>
<th>M CV</th>
<th>N</th>
<th>N CV</th>
<th>M</th>
<th>M CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorous</td>
<td>43</td>
<td>3.7</td>
<td>2.2</td>
<td>41</td>
<td>3.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Magnesium</td>
<td>43</td>
<td>5.0</td>
<td>3.7</td>
<td>46</td>
<td>0.8</td>
<td>2.6</td>
</tr>
<tr>
<td>Creatinine</td>
<td>43</td>
<td>10.8</td>
<td>3.1</td>
<td>46</td>
<td>3.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Lipase</td>
<td>40</td>
<td>12.7</td>
<td>3.1</td>
<td>42</td>
<td>2.0</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Each method evaluated appeared to be suitable for expedient and precise analysis on the DT60 Analyzer or DTSC Module.

CLINICAL STUDIES

Animal Studies

- **QUANTITATION OF SERUM PROTEINS IN M. FASCIULARIS (RHEUS MONKEY)**. Susan E. Mitchell and David H. Bing. (CBB Laboratories, Inc. 800 Huntington Avenue, Boston MA 02115.) (Spons.: D. Bing).

Immunochromatographic quantitation of the human serum proteins has proven useful in the diagnosis of infections, hematologic and metabolic human diseases. We have used this method to quantitate serum proteins in M. fascicularis based on the cross reactivity that serum proteins in this primate species exhibit towards monospecific goat polyclonal antiserum to 13 human serum proteins. Analyses were done by the immunometric method performed on the Technicon Auto Analyzer II using Atlantic Antibodies nephelometric grade polyclonal antisera. Quantitation was achieved by comparison to human serum protein standards provided by Atlantic Antibodies. The results of such determinations on 21 adult Rhesus monkeys over a period of 2 months followed total protein 6.96±0.05 g/dl; albumin 3.14±0.31 g/dl; haptoglobin 144.6±8.2 mg/dl; transferrin 238.7±4.9 mg/dl; complement component C3 178±15.2 g/dl; IgA 103±9.4 mg/dl; IgG 103±9.4 mg/dl; IgM 60±8.3 mg/dl; complement factor B 21±7.5 mg/dl and Beta lipoprotein 34±15.1 mg/dl. Complement component C4, alkaline phosphatase and complement component C1 esterase were determined as 31±12.5, 31.6±21.2, and 40.7±16.9% of normal. These results are remarkably similar to values determined for human serum proteins.

While these values may not reflect the absolute level of these serum proteins in this primate species, the ability to detect quantitative changes in levels of such proteins should prove useful in experimental studies on M. fascicularis in which changes in the levels of the serum proteins are used to evaluate the response to infectious, traumatic or drug-induced stimuli.

- **THE ASSOCIATION OF BIOCHEMICAL PARAMETERS TO THE EVOLUTION OF DIABETIC NEPHROPATHY IN THE OBSESS ZUCKER RAT**, Cory R. Ward, J.D. Mahan, P.D. Boll, J.M. Laah, W.M. Sherman (The Ohio State University, Columbus, Ohio) (Spons.: K.M. Ward)

Nephropathy is one of the major long-term complications of diabetes mellitus. The obese Zucker rat is a genetic model of non-insulin dependent diabetes mellitus (NIDDM) (Bray, GA: Edg., 1977). In order to assess the appropriateness of this animal model for the study of diabetic nephropathy we examined fasting blood glucose (FBG), glycated hemoglobin (GHB), insulin (I), 24-hr. urine protein (P), mean arterial pressure (MAP), total cholesterol (TC), triglycerides (TG), and albumin to creatinine ratio (A/C) on a monthly basis for 5 months. The mean doses of insulin used in this study were 0.67 i.u./kg/day. The effects of treatment on the parameters were compared by analysis of variance. The results indicate that insulin significantly lowered FBG, GHB, triglycerides, and the A/C ratio.

- **THE CLINICAL STUDIES**


We are interested in developing an immunocassay for sheep and dog myoglobin (Mb) to assess the effects of mechanical stress on myocardial recovery of the "stunned" myocardium in the dog and sheep animal studies. We wondered whether we could find structural differences between the Mb from heart and skeletal muscles so that the assay specific to heart damage could be developed.

Mb(s) from both heart and skeletal muscles of dog and sheep were isolated following the same procedure described earlier (Clin Chem 1987:33:1037). They were characterized by SDS-polyacrylamide gel electrophoresis, chromatoeocussing and isoelctrofocussing (IEF). Rabbit antisera were also raised against the major subunits (from chromatoeocussing) of Mb from both dog and sheep heart tissues.

We found that Mb isolated from all four muscles are similar in size. While there was no difference found between Mb from heart and skeletal muscles of sheep there was difference between Mb from dog heart and dog skeletal muscles. The major peak of Mb for dog heart was eluted at pH 7.9 after
chromatofocusing whereas the major Mb peak from dog skeletal muscle was eluted at pH 8.1. Dif- ferent IEF patterns were observed between dog Mb (a). Both Mb (a) from sheep heart and sheep skeletal muscles reacted with anti-sheep heart Mb forming intramolecular precipitate patterns, whereas receptors precipitated in human muscle with concentration varying from 0.01 to 0.5 mg/mL, and a line was clearly visible between the samples and 0.01 mg/mL of dog heart Mb. We believe that development of a specific immunoeassay for heart damage will be possible for dog studies in the near future.

210 EFFECTS OF DIFFERENT ANEASTHETICS AND SAMPLING SITES ON SERUM ESTERASES IN THE RABBIT. J. P. B. Clark, R. Wilson, J. Riley (NIESH, RTP, NC 27709) (Spon.: R. Thompson)

Frequently, different anesthetics and sampling sites are selected to collect blood from rodents in toxicology studies. Although various combinations of these may be used at different times within the same study, data often are compared regardless of collection methods. We determined the effects of different combinations of these factors on biochemical analyses in rat serum. Using combinations of 3 sampling sites, retro-orbital sinus (ROS), right cardiac ventricle (RCV), and posterior vena cava (PVC), and 4 anesthetics, carbon dioxide (CO2), methoxyflurane (WF), halothane (AN), and isoflurane (NX) were used, to collect the samples. Serum analyses (ALT, CK, LD, SDH, AP, glucose, creatinine, electrolytes, total protein, phosphorus, and total cholesterol) were measured for each combination of site and anesthetic. Eight male, Fischer 344 rats were bled. Forty-four rats were anesthetized with each anesthetic and bleeding sites were alternated between animals. Analyses were performed using a Centrifichem 500 and an Astra 6. For each assay, significant effects for site and anesthetic were identified using a two-way ANOVA (p < 0.01). Individual comparisons were made using Newman-Keuls test (p < 0.01).

Considering the effects of varying sampling site, the following analyses were significantly different at the listed site when compared to the other 2 sites: K - increased for RCV; LD - increased for ROS; TP, Na, creatinine - decreased for PVC; glucose - decreased for ROS. The following effects occurred for the listed anesthetic: CO2 - increased compared to chloroform, halothane, and isoflurane; total protein - increased with K; SDH - decreased with NF; AP, ALT, K - increased with CO2; LD, CK - increased with PF; total protein, total bilirubin - decreased with PF. Additional data will be presented, but the results of the collection of blood must be established if comparable results are to be obtained from animals in toxicology studies.

211 BILE ACID PROFILES AND SERUM ENZYME ALTERATIONS IN RATS FED THE HEPATO CARCINOGENIC PEROXISOME PROLIFERATOR WT-14,643, Douglas A. Neupauer, Russell G. Cattley, and James A. Pupp (Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709) (Spon.: D. Neupauer)

Traditional indicators of liver injury, AST, ALT, and SDH correlate well with early hepatic toxicity, but are generally not elevated in animals chronically exposed to hepatocarcinogens. However, very little information is available on serum chemistry parameters of animals chronically treated with hepatocarcinogens that induce peroxisome proliferation. Male F-344 rats were fed a potent peroxisome proliferating compound, WT-14,643, (0.1% in the diet) for 22 weeks. Blood samples were drawn from anesthetized animals by cardiac puncture and serum analyzed for SDH and serine acyl transferase activities. Nineteen individual bile acids, including the conjugated forms, were separated by HPLC with post-column derivitization. Bile acid profiles were then run on bile samples from the common bile duct and serum collected from the portal vein. Of the serum enzymes, only SDH and choastic were detected in serum within normal range were significantly elevated in treated rats. Non-conjugated forms of some individual bile acids in the peripheral blood were elevated to 2 to 5 fold by WT-14,643 feeding. Portal blood bile acids were also elevated 2 to 4 fold, consisting mostly of non-conjugated forms of cholic and deoxycholic acid. An abnormal profile of bile acids was noted in the bile of WT-14,643 fed rats. Total cholic and deoxycholic acids were elevated 2 fold in treated rats. These data indicate that serum enzymes are not markedly elevated by peroxisome proliferating hepatocarcinogens. However, significant increases in bile acid profiles were noted and may be important in the carcinogenic process since similar changes have been noted in animals fed deoxycholic acid, a known hepatic tumor promoter.

212 BIOCHEMICAL EFFECTS OF 17-8 ESTRADIOL ON RAT OSTEOLASTIC CELLS IN CULTURE. D. D. Banks, J. N. Kifai, L. M. Silverman (Depts. of Pharm. and Nutr. Lab., Univ. of N.C., Chapel Hill, NC, 27514), and T. K. Gray (Dept. of Med. and Pharmacol., Univ. of N.C., Chapel Hill, NC, 27514) (Sponsor: S. S. Leinbach)

To further elucidate the mechanism of estrogen therapy in osteoporosis we studied the action of 17-8 estradiol (178E) on the metabolic responses of two established rat osteoblast-like, osteostatic receptor-containing cell lines. Both UMR106 (mature osteoblast model) and ROS 17/2.8 (mature osteoblast model) cells were incubated in serum-free DMEM/10% serum (178E and vehicle for 48 h. Cell numbers were quantitated by automatic counting. Enzyme assays of cell lysates (lactate dehydrogenase, LDH, alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT)) were performed on the Roche Cobas-Bio 100.

In UMR106, 178E (at 10-9M to 10-14M) decreased cell growth (p<0.01) and increased the activity (p<0.05) of LDH (1651), ALP (1450), GGT (1500) and AST (1645). In ROS 17/2.8, 178E was not detectable at this cell density (2 x 104 cells/well). Similar responses were observed when UMR106 cells were plated at a higher density (2 x 105 cells/dish), however, cell activity was significantly increased (p<0.01, 10-7M 178E). Intracellular transferase concentration (by immunoturbidimetry) was also significantly increased (61% at 10-7M) by 178E. Intracellular ROS in ROS 17/2.8 cell line was unresponsive to either 178E or 178M. In conclusion, the immature UMR106 cells were responsive to 178E in a specific, dose-dependent manner of the mature ROS 17/2.8 cells were unresponsive despite the presence of specific receptors in both cell lines. These results suggest that 178E may act on osteoblast precursors to enhance bone formation.

213 USE OF A KODAK EKTACHRM DT ANALYZER TO MONITOR CHEMICAL CHANGES IN HAMSTRA HEPATOS OR INJECTED WITH WOODCHUCK HEPATITIS VIRUS (WHV), F. D. Lasky (Clinical Products Division, Eastman Kodak Co., Rochester, NY 14623), and J. B. Snyder (Zoological Soc. of Phila., Phila., PA 19104) (Spon.: F. D. Lasky)

The American woodchuck (Marmota monax) is susceptible to a viral hepatitis infection (Woodchuck Hepatitis Virus, WHV) that is immunologically similar to human hepatitis B. These animals develop hepatic lesions that are histologically similar to those found in some human patients who are chronic hepatitis B carriers. In woodchucks, however, metastasis does not occur beyond the liver. We used a Kodak Ektachrm DT analyzer to study the temporal changes in animals that were administered WHV. In woodchucks, however, metastasis does not occur beyond the liver. We used a Kodak Ektachrm DT analyzer to study the temporal changes in animals that were administered WHV. In woodchucks, however, metastasis does not occur beyond the liver.

We conclude: The Kodak Ektachrm DT analyzer is well suited for small animal testing. AST, GGT, and total bilirubin are elevated in hepatitis positive woodchucks. ALEP, and especially GGT are markedly elevated in animals with confirmed hepatocellular carcinoma.


Monosomoneon urate is a rarely occurring constituent of urinary calculi in humans. It is however occasionally encountered in animals. It is not a component of the urine of the normal animal. Monosomoneon urate can be isolated from simple acid base reaction test.
A drop of 0.3 N HCl is added to a portion of powdered calculi, covered with observed microscopically. In less than a minute monoammonium urate will begin to form distinctive crystals. Oxalic acid is not affected by the acid medium and remains unchanged.

Upon acidification monoammonium urate forms clear or translucent rectangular crystals which will appear white, yellow, or light brown if viewed with polarized light. Some clustering of the crystals may be noted. To date, we have identified 16 monoammonium urate calculi, all of which were confirmed by biopsy. It is noted that this rapid method is of special value to veterinarians who may need this differentiation done without the delay of traditional analysis.


A previous study (JBC 1987;262:13537) demonstrated that isolated adult rat cardiomyocytes treated with inhibitors of oxidative phosphorylation (3 mM/L antimycin and 3 mM/L CCCP) and of anaerobic glycolysis (5 mM/L 2-deoxy-D-glucose) degrade ATP quantitatively, within seconds, to AMP. The AMP is then converted to intracellular adenosine and variable amounts of IMP. The rate of degradation of IMP by adenylate deaminase depends on the metabolic state of the cells just prior to deamination; IMP production in undamaged cells will be limited in ATP stores through anaerobic glycolysis. Here, we demonstrated that this reciprocal relationship between anaerobic glycolysis and myocardial adenylate deaminase activity can be modified by adrenergic stimulation. The data indicate that the inhibition of adenylate deaminase associated with anaerobic glycolysis is abolished by alpha-1 adrenergic stimulation in rat myocytes.

38 min Preincubation Conditions Deaminozylation (pmol/L)

Acrobic
Rotenone (RT)
RT+epinephrine
RT+epinephrine+propranolol
RT+epinephrine+isoproterenol
RT+phenylephrine

(Supported by C.Ohio Heart & USPHS HL 36240 grants.)

216. A COMMERCIAL KIT FOR MEASURING ALDOSTERONE IN NON-EXTRACTED SERUM FROM RATS AND HAMSTERS. W. E. Grizzle, L. J. Smith, J. T. Ingram, Depts. of Surgery, Pathology and Comprehensive Cancer Center, Veterans Administration Medical Center and University of Alabama at Birmingham, Birmingham, AL 35294. (Sponsor: W. E. Grizzle).

We report that a commercially available assay for measuring aldosterone in samples of human serum is valid without modification for determining aldosterone in the serum of small animals including rats and hamsters. The "Cate-A-Count, No Extraction Aldosterone Assay" (Diagnostic Products Corp., Los Angeles, CA) is very useful for determining aldosterone in the serum of small animals since the assay can be performed in duplicate on as little as 0.5 ml of serum and since it does not require extraction of the samples. Reproducible (intra- and inter-assay coefficients of variations of 5% or less) and accurate results were obtained on samples of charcoal-extracted serum (rats and hamsters) which were spiked with aldosterone to produce concentrations of aldosterone ranging from 5 ng/dl to 500 ng/dl. Technical considerations that proved important in obtaining accurate and meaningful results include the importance of adjusting high-level samples with charcoal-extracted serum from the species being studied (e.g., dilute human with human, rat with rat) and of obtaining samples from animals immediately after anesthesia. We also report a slight interaction of this assay with high concentrations of cortisol or of corticosterone. However, this interaction would not be large enough to affect the interpretation of most experimental results.

Supported in part by the UAB Department of Pathology, and by V.A. Medical Research Funds.

217. A SIMPLE DIAGNOSTIC TEST FOR FELINE AND CANINE PANCREATITIS SUITABLE FOR USE IN VETERINARY OFFICE by Deudas and A. G. Casas. (Sponsor: W. E. Grizzle).

While the diagnosis of pancreatitis is of importance in veterinary medicine, rapid, simple tests are generally not available. We have developed a simple colorimetric rate assay for serum pancreatic amylase (EC 3.2.1.1) on the Eslakab-CCS system. The Eslakab-CCS is a table-top filter photometer capable of performing rapid rate assays in the visible and near infrared (NIR) wavelengths. Single reagent assay packaging allows for discrete, low volume use. This format would be applicable to veterinary office testing.

Colorimetric reagents for serum amylase developed for human clinical assays are based upon enzymatic hydrolysis of p-nitrophenyl-maltotetrose (p-NPG) as substrate, which gives rise to p-nitrophenol. The use of this substrate in the veterinary context is inappropriate, since competing endogenous enzyme pathways are likely to interfere. The Eslakab-CCS reagent utilizes a covalently derivatized substrate, which blocks the competitive enzymes. The Eslakab-CCS amylase reagent shows excellent correlation using canine samples compared to a commercial reference method on COBAS-BIO™. The regression equation was:

\[ Y(\text{ESLAMAP}) = 1.01 \times (\text{COBAS}) + 6.7. \]

The reagent is linear over a range of 0 to 2500 U/L and exhibits a within-run precision of approximately 5%.


Sulfhemoglobin was determined in hemolysed blood of monkeys receiving an experimental new drug by measuring that absorbance at 620 nm which does not disappear upon addition of potassium cyanide. (In contrast, any absorbance due to methemoglobin, which at 540 nm is indistinguishable from that of sulfhemoglobin, disappears when KCN is added.) The assay was validated by experimentally inducing formation of both sulfhemoglobin and methemoglobin by addition of WS and Kyp(CN), respectively to normal whole or hemolysed blood, and demonstrating that the sulfhemoglobin band did not disappear upon addition of KCN, but the methemoglobin band did disappear.

The reproducibility of the assay was determined by comparing absorption at 620 nm from 7 aliquots of diluted hemolysed blood from the same animal. The relative standard deviation is 0.3%, which is well within the 2% limit set for the instrument and sampling variability.

Linearity of the assay response was determined by measuring absorption at 620 nm of hemolysed blood from an animal with elevated sulfhemoglobin mixed in various ratios with that from a normal animal. The regression coefficient of the resulting curve (absorption vs percent of elevated sulfhemoglobin blood) was 1.0, with a y intercept of 0.011 absorption units.

The concentration of sulfhemoglobin detected in drug treated animals was dose related, and the effect appeared to be species specific, since a similar study conducted in rats did not result in formation of sulfhemoglobin.


The thyrotrophic response of pentachlorobenzene (PeCB), a chemical used as a flame retardant and as a precursor in the synthesis of a fungicide, pentachlorotribenzene, was assessed by determining serum levels of triiodothyronine (T3), thyroxin (T4), free T3 (FT3) and thyroid stimulating hormone (TSH) and evaluating morphologic changes in the thyroid gland. Ninety-day toxicity studies were conducted in F344 rats and B6C3F1 mice of both sexes using dosed feed. Dose levels were 0, 33, 108, 330, 1000 and 2000 ppm. Blood was collected at 15, 45 and 90 days post-treatment. T3, T4, FT3 and TSH were quantitated using radioimmunoassay procedures.
Both male and female rats exhibited significantly decreased serum T₄, T₃, and FT₄ (p = 0.01–p < 0.0001) levels which were accompanied by significant elevation in TSH levels (p = 0.02–p < 0.0003), particularly in the 1000 and 2000 ppm dose groups at 15, 45 and 90 days post-treatment. Significantly lower values in T₄ and FT₄ were also observed at lower dose levels of 33–330 ppm at all time periods. Male and female mice treated in a similar fashion showed statistically significant decreases in T₄ levels and non-dose related decreases in T₃ and FT₄ levels at all time periods. TSH levels in mice, tested only at 90 days, were unchanged when compared to the control groups. Minimal histopathologic changes were observed at the 2000 ppm dose level in rats which consisted of enlargement of the follicles and the presence of pale-staining colloid. No histologic changes related to treatment with PeCB were discernable in mice.

We can conclude from this investigation that treatment with PeCB is thyrotropic in rats as evidenced by thyroid hormone alterations which are indicative of primary hypothyroidism.

Clinical Studies

**222 EFFECTS OF DIFFERENT EXERCISE TRAINING PROGRAMS ON THE LIPID, LIPROPROTEIN CHOLESTEROL, AND APOLIPOPROTEIN LEVELS OF COLLEGE ATHLETES.** Rifej, N. (Dept. Hosp. Lab., Univ. of NC, Chapel Hill, NC 27514), Gradjean, A. (Swenson Center for Nutrition, Univ. of MS, Jackson, MS 39210), Forsythe, W.A. (Univ. of Southern MS, Hattiesburg, MS 39406) (Spons.: Hader Rifej).

Endurance physical training exerts changes in the lipid lipoprotein cholesterol, and apolipoprotein concentrations that are favorable in lowering coronary heart disease risk. However, the extent of these changes was recently reported to be dependent on the exercise training program. In this study, we compared the lipid, lipoprotein cholesterol, and apolipoprotein levels of the University of Nebraska-Lincoln football players (UWL) with those of UWL basketball players. Thirty-five football players and 14 basketball players were recruited for the study. The two groups had different exercise training protocols. Football players were undergoing a winter conditioning period, whereas basketball players were undergoing a summer training period that consisted of aerobic running, swimming, and skill-drill. None of the participants smoked or consumed excessive amounts of alcohol. No significant differences in body weight and caloric intake were observed between the two groups. Football players had an increase of 23% in LDL cholesterol (p < 0.0005), 40% in triglyceride (p < 0.0005) and 8% in apolipoprotein B (p = 0.0125) and a decrease of 16% in HDL cholesterol (p = 0.025) and 18% in apolipoprotein AI (p = 0.0025) in comparison to basketball players.

In conclusion, the endurance aerobic exercise training program which basketball players were undertaking seems to cause changes in the lipid, lipoprotein cholesterol and apolipoprotein profiles that are favorable to atherosclerosis.

**223 EFFECTS OF ULTRA-LONG-DISTANCE RACES ON LIPID METABOLISM (24 HOUR RACE; 1000 KM RACE).** D. Nagel, D. Seiler, H. Franz and K. Jung (Klinikum Ludwigshafen, W.-Germany). (Spons.: D. Seiler).

In this study we investigated the effects of two ultra-long-distance runs on the serum concentration of total cholesterol, triglycerides and HDL-cholesterol. During a 24 hour race, we examined two different groups of runners (13 non-stop runners and 12 day racers). The 100 km runners were divided into daily distances of approx. 50 km. 55 of the 110 participants finished the race. Blood was taken before, several times during and after the race.

All runners showed a decrease in cholesterol and triglycerides and an increase in HDL-cholesterol.

<table>
<thead>
<tr>
<th>24 hour race</th>
<th>1000 km race</th>
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<tbody>
<tr>
<td>24 hour race</td>
<td>non-stop runners</td>
</tr>
<tr>
<td>Chol</td>
<td>Trig</td>
</tr>
<tr>
<td>Before</td>
<td>214</td>
</tr>
<tr>
<td>After</td>
<td>197</td>
</tr>
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</table>

Mean value of serum concentrations (mg/dl)

During the 24 hour race, the concentrations of cholesterol and triglycerides decreased continuously and HDL-cholesterol increased. Whereas during the 1000 km competition, we found the minimal concentrations of cholesterol and triglycerides and the maximal concentration of HDL-cholesterol on the 8th day and not on the last day as had been expected.

Our results show that changes in concentrations of serum lipids are dependent on the kind of effort. Nevertheless, the positive effects of ultra-long-distance races on the risk factors for cardiovascular diseases is only possible up to a certain extent. After longer periods of extreme running these positive effects are partially reversed but without returning to concentrations found before the race.

**224 SERUM LDH AND LDH ISOENZYMES IN CHRONIC RENAL FAILURE: EFFECT OF HEMODIALYSIS.** D.W. Vastrati, D.S. Miyada, I. Kim, J. Reid, and E.V. Gonzales (Depts. of Nephrology and Pathology, UC Irvine Med. Ctr., Orange, Ca. 92668) (Spons.: D.S. Miyada).
Twenty-five patients with end stage renal disease (ESRD), undergoing hemodialysis had arterial blood samples drawn from the predialysis line shortly before, 30 minutes into and shortly after the conclusion of dialysis. A simultaneous sample of blood leaving the dialyzer was obtained to assess the effect of extraneous bodily fluids. Total serum LDH and LDH isoenzymes were performed on Beckman's IDEAL and Paragon, respectively.

**Effects of Dialysis.** Serum total LDH activity in this group was 155 ± 29 U/L compared to 129 ± 19 U/L for the control group (p<0.001). All LDH isoenzymes were significantly elevated (p<0.01) except LDH-1.

**Effect of single pass.** Serum total LDH increased significantly upon single pass through the dialyzer (p<0.001) and LDH-2, LDH-3, and LDH-4 increased (p<0.03); no significant changes were observed with LDH-1 and LDH-5.

**Effect of hemodialysis.** Serum total LDH rose during hemody-

lalysis (p<0.001 did LDH-1 and LDH-5). No significa-
tant changes were observed with other LDH isoenzymes; thus, there is observed a flip-flop between the effects of single pass and hemodialysis.

With LDH-5, 25 patients showed marked elevations while 9 showed virtually no change.

The results preclude the possibility of 1. hemoconcentration or 2. the kidney as the sole source for the observed changes in total serum LDH and LDH isoenzymes. Rather with the possibility for mixed causes, we feel the attrition of the formed elements of blood, erythrocytes, leukocytes, and platelets to be as likely source for the enzyme changes.

225 **FUNCTIONAL LEVELS OF o-MACROGLUCULIN IN RELATION TO CHRONIC OBSTRUCTIVE LUNG DISEASE AND ASTHMA.** H. Christine Galliard, R. Domanski, T.A. Klirow-Smith (Nat. Ctr. Occ. Hlth., P.O. Box 7478, Jhb, Republic of South Africa) (Spon: L.A. van der Walt)

The amylodicyl activity of plasma o-macrogluculin (o-M) complexed with porcine pancreatic elastase (PPE) was compared in chronic obstructive lung disease (COPD) patients, asthma patients and in healthy subjects. Absolute levels for o-

protease inhibitor (o-PI) were determined in the three groups by an antibody precipitation laser nephelometric method.

The activity of elastase bound to o-M was measured using suc-

cinyl-trialkylamino-nitro-anilide (SAPNA) as substrate. Thirty three COPD patients (26 males and 7 females), and 25 asthma patients (11 males and 14 females) were compared to 54 normal subjects (39 males and 15 females). The age of subjects from all groups ranged from 43 to 79 years. The mean of the acti-

vity of bound elastase in COPD cases was 2.37 ± 0.10 (S.E.M.) elastase units/ml plasma. In chronic asthmatic patients, the mean was 2.51 ± 0.10 (S.E.M.) elastase units/ml plasma. The mean activity of normal subjects was 1.61 ± 0.06 (S.E.M.) elastase units/ml plasma. While results of COPD and asthma cases were not found to differ significantly from each other, there was a highly significant difference when they were compared to the control group (P<0.00005). Results of the levels of o-M paired the COPD group with a mean of 2.67 ± 0.1 mg/ml. The asthma sufferers had a mean of 2.5 ± 0.09 mg/ml. Neither of these results were significantly different from the control group with a mean of 2.4 ± 0.08 mg/ml.

In both groups of patients, the functional level of o-M was significantly raised. This may play an important role in the pathology of lung diseases, and may be useful as a marker.

226 **THE INCIDENCE OF ALCOHOL AND DRUGS AMONG MVA TRAUMA ADULTS 70-100% TRAUMA UNIT, M. Kapur, E. Vingilis, C.B. Liban and B. Mcclellan; (Clinical Laboratory, Addiction Research Foundation, 33 Russell St, Toronto, Dept. of Clin. Bioch, University of Toronto and, Sunnybrook Medical Centre, Toronto, Canada.)** (Spon: Guang H. Kang)

Alcohol has shown to be a major factor in MVA fatalities. In Canada about 50% of all drivers killed in MVA had been drinking. Good comparative data on non-fatal injuries is not available. In Toronto the Addiction Research Foundation and Sunnybrook Metropolitan Truma Unit have embarked on a 3 year study to investigate the role of alcohol and drugs in MVA trauma victims. This paper presents some of the very preliminary findings on the incidence of alcohol and other drugs in the trauma victims.

Method: Blood and urine samples of patients admitted to the trauma unit were analyzed for various drugs. Preliminary analysis for alcohol, urinary cocaine, barbiturates, benzodiazepines and other drugs were done by the Abbott TDXs. Barbiturates and alcohol were confirmed by GLC. Extensive TLC screening was also done to confirm the immunoassay results and to detect other drugs.

**Population characteristics:** Over a period of about 2 months 75 patients were screened. 66 were MVA whereas 11 had sustained non-motor vehicle related trauma. In 8 other cases the cause of injury was not identified.

**Results (MVA only):** 36 (64%) patients had 1 or more drugs in their body fluids. Alcohol (41%) followed by cannabinoids (11%) and benzodiazepines (7%) were the most common drugs. Cocaine (25), barbiturates (5,9), codeine (2,), and diphenhydramine (2.) were among the other drugs. All six patients positive for cannabinoids were male drivers. Their average age was 21 (18-27). Four of these six also had alcohol and one had cocaine in addition to cannabinoids in their body fluids.

227 **ENZYME ABNORMALITIES OF PATIENTS WITH ACQUIRED IMMUNODEFICIENCY SYNDROME.** C.M. Huang, Mark Ruddel and Ronald J. Elin (Clin. Pathol. Dept., N.H, Bethesda, MD 20892) (Spon: N. Papadopoulos)

The acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) and is characterized by a defect in cellular immunity. The progression of the disease is usually based on clinical evaluation and the ratio of helper to sup-

pressor T lymphocytes. We determined the activities of phosphohexose isomerase (PHI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase in serum from normal and AIDS patients. Seropositive individuals confirmed by Western blot and patients with AIDS to assess results in patients from AIDS.

Our findings are the following (mean ± SEM):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>HIV+</th>
<th>AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHI (U/L)</td>
<td>40 ± 3</td>
<td>66 ± 4</td>
<td>113 ± 10</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>17 ± 2</td>
<td>29 ± 3</td>
<td>64 ± 10</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>20 ± 3</td>
<td>33 ± 3</td>
<td>83 ± 10</td>
</tr>
<tr>
<td>LD (U/L)</td>
<td>133 ± 5</td>
<td>176 ± 8</td>
<td>255 ± 17</td>
</tr>
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</table>

* p < 0.05 between HIV+ and normal.
+ p < 0.05 between AIDS and normal HIV+ groups.

There is a significant difference among normal, HIV seropositive and AIDS groups. Four enzymes were tested. The enzyme activities show a progressive increase with the progression of the disease. HIV infection caused liver impairment as evidenced by significant increased enzyme activities related to liver function (AST, ALT, and LD). We conclude that these enzyme measurements may be an additional biochemical marker for the progression of the disease.

228 **THE EFFECT OF INTERLEUKIN 2 (IL-2) ON TESTS OF LIVER FUNCTION.** C.M. Huang, M. Ruddel, C. Elly, Ronald J. Elin, M.T. Lotte, and S.A. Rosenberg (N.H., Bethesda, MD 20892) (Spon: Gloria Ives)

Some patients with metastatic cancer for whom standard therapy has failed have responded to systemic administration of IL-2. The toxicity of IL-2 to the liver has not been fully characterized. We determined the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), and total bilirubin (TBL) for eleven consecutive days (prior to IL-2 therapy, five days with IL-2 therapy and up to five days after IL-2 therapy) in serum from up to 21 patients. Our findings are the following (mean ± SEM, n in parentheses):

<table>
<thead>
<tr>
<th>Test</th>
<th>Peak Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>ALT</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>GGT</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>ALP</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>TBL</td>
<td>5.2 ± 0.2</td>
</tr>
</tbody>
</table>

There was a significant increase in AST for all five analyses occurred close to the end of IL-2 therapy (day 5) and suggested progressive toxicity to the liver during the course of IL-2 therapy. A comparison of the two ratios shows a definite progression towards the baseline value when IL-2 therapy is discontinued. Additional studies are needed beyond five days after cessation of IL-2 therapy. There are obstructive and hepatocellular components to the liver toxicity induced by IL-2. Thus, IL-2 therapy effects toxicity to the liver which subsides when the drug is discontinued.

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988 1199
229 PreSEния of IgG/IgM CARDiOliN PiNties in the Serum of Individuals With AIDS or ARC.

Cardiolipin antibodies (CA) have been reported in a number of conditions, including thrombosis, recurrent spontaneous abortion, thrombocytopenia, lupus, and a number of infectious diseases. The analysis of specificity and sensitivity of CA antibodies is of critical importance in classifying results as elevated or not elevated.

Guidelines have been established to allow standardization of the methodology and calibrators. Recently it was reported that CA are elevated in patients with AIDS (23/28) or ARC (12/14). However, the assay used did not employ the techniques recommended to improve assay specificity and sensitivity.

We report on the presence of CA in patients with AIDS or ARC using an ELISA calibrated against that of the International Workshop. Results:

<table>
<thead>
<tr>
<th>Result</th>
<th>Mean (±2 S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Persons</td>
<td>2.5 (± 1.9)</td>
</tr>
<tr>
<td>AIDS Patients</td>
<td>7.2 (± 2.9)</td>
</tr>
<tr>
<td>ARC Patients</td>
<td>5.7 (± 4.4)</td>
</tr>
</tbody>
</table>

Thirty-seven of 89 AIDS samples and 8 of 28 ARC samples gave elevated results for IgG or IgM Cardiolipin Antibodies, or both. Our data indicate a lower percentage of positive results in AIDS/ARC patients, and demonstrate the need for the standardization of CA assays.


230 MEASUREMENT OF SERUM OSTEOCALCIN IN A GROUP OF WOMEN WITH UNTREATED POSTMENOPAUSAL OSTEOPOROSIS: COMPARISON WITH NORMAL ADULT WOMEN.

Elevated osteocalcin levels have been reported in postmenopausal osteoporotic women (Epstein, S. et al., Lancet 1:307, 1981). However, in other studies osteocalcin concentrations have been indistinguishable between women with osteoporosis and normal adult women (Gundberg, C.M., et al., J Clin Endocrinol Metab. 57:1211, 1983). We compared serum osteocalcin levels of 25 women with postmenopausal osteoporosis with that of 26 normal adult women using a double antibody radioimmunoassay developed in our laboratory.

Bovine osteocalcin standards, "patients" serum, or controls were incubated for 18 hours at 4°C with rabbit anti-bovine osteocalcin antibody and 125I-bovine osteocalcin. The dose response curve was linear between 0.5 and 25 mg/mL and the detection limit was set at 0.5 mg/mL. Inter-run and intra-run imprecision were less than 11.62% C.V. and 9.95% C.V., respectively. Osteocalcin levels of the 25 women with untreated postmenopausal osteoporosis were in the range of 1.2 to 28 mg/mL with a mean ± S.D. of 8.6 ± 5.9 mg/mL, while the osteocalcin levels of the 26 normal adult women were in the range of 1.2 to 10.5 mg/mL with a mean ± S.D. of 3.7 ± 2.4 mg/mL. The reference range was calculated to be 1.7 - 10.9 mg/mL. Ten of the 25 women with untreated postmenopausal osteoporosis had osteocalcin concentrations above the reference range, while the remaining 15 had osteocalcin levels within the reference range. Our reference range is consistent with that reported by Epstein, S., et al., and by Gundberg, C.M., et al. We conclude that serum osteocalcin is elevated in some women with untreated postmenopausal osteoporosis.

231 A METHOD FOR CONFIRMING THE PRESENCE OF ANTIBODIES TO HIV UTILIZING RECOMBINANT PEPTIDES PRODUCED IN E. COlli.

We have developed a convenient method for the confirmation of the presence of antibodies to Human Immunodeficiency Virus (HIV) using purified, recombinant peptides produced by genetic engineering techniques in E. coli. Each peptide represents a portion (or portions) of specific HIV proteins. They include: (1) an 18Kd peptide of envelope protein, Kp41; (2) a 57Kd peptide containing sequences of gp120 (gp precursor), Kp55; (3) a 28Kd peptide of core protein p24 (gp gene product), Kp24; (4) an 85Kd peptide of outer envelope protein, Kp120N; and (6) a 32Kd peptide of outer envelope protein, Kp120OC. The purified peptides are individually coated onto wells of a polystyrene microtiter plate. The wells are incubated with serum, and reactions are blocked with anti-human IgG-alkaline phosphatase conjugate. The wells are then washed and the presence of antibody is demonstrated by incubating with p-nitrophenyl phosphate and reading the absorbance.

Using this assay, a panel of specimens from AIDS and ARC patients seropositive for HIV antigens was compared to a panel of blood donors from random donors in which none reacted. All the AIDS and ARC specimens but none of the seronegative donor specimens reacted to the Kp41 antigen. The majority of AIDS and ARC specimens but very small percentage of seronegative donor specimens reacted to Kp24, Kp55, Kp66/31, and Kp120C.

We conclude that this assay may be a useful method for detecting and confirming the presence of antibodies to specific HIV antigens.

232 SERUM HORMONAL LEVELS IN PATIENTS WITH ROKITANSKI-KUSTER HAUSER SYNDROME.

Rokitanski-Kuster Hauser Syndrome is a congenital absence of vagina and in 95% of cases a rudimentary uterus with normal secondary female characteristics. Serum hormonal level of these patients have been evaluated in different aspects, but the data regarding a complete pituitary, adrenal and ovarian hormones is lacking.

This study was undertaken to investigate the cyclic variations of nine related hormones of two women hospitalized for vaginoplasty surgery. Their blood samples were collected daily over 8 to 9 days consecutively. The sera were kept frozen in aliquots sufficient for each test until the last day of sampling.

Measurements were made by radioimmunoassay method as a separate batch for each patient. In order to correlate the results, a few random samples from each batch were analyzed together with control sera.

The data shows a significant elevation of serum prolactin level as compared to established reference ranges. This elevation is more pronounced in follicular phases. Although serum osteocalcin levels are lower than normal range in one patient, there is no increase in testosterone and dehydroepiandrosterone sulphate concentrations. The variations of cortisol, progesterone and estradiol are in normal ranges. The baseline of pituitary hormones (TSH, FSH and LH) are running in normal patterns with considerable higher midovulatory peaks.

233 CAPTORSIL TEST IN CHILDREN WITH HYPERTENSION.

R.M. Hamed, J.W. Balfe and G. Ellis (Depts of Pediatrics and Biochem, Hosp. for Sick Children, Toronto, Canada, M5G 1X8) (Sponsor: G. Ellis)

Plasma renin activity (PRA) measurement before and 90 minutes after Captorsil (an angiotensin converting enzyme inhibitor) has been proposed as a rapid test for assessment in children. The method was tested in 20 children with hypertension aged 4 months to 19 years (mean age 12.57 years). Blood pressure (BP) was continuously monitored in the supine patient by a Bard Biomedical/A&D monitor A001 monitor. The test was performed on 34 renal patients (mainly outpatients) with hypertension aged 4 months to 19 years (mean age 12.57 years). The BP had stabilized, blood was taken for PRA and Captorsil (0.7 mg/kg) administered orally. BP monitoring was continued and blood taken for PRA at 90 minutes after Captorsil. Sample were sent STA to the lab on ice, centrifuged and stored at -20°C prior to analysis by the Angiotensin I Kit (REA-026) from New England Nuclear, North Billerica, MA 01862. The patients were then classified clinically into three groups on the basis of the etiology of their hypertension. The table shows mean (SD) initial and the mean (SD) changes in diastolic BP and PRA between 0 and 90 minutes in the groups.

<table>
<thead>
<tr>
<th>BP (mm Hg)</th>
<th>PRA (mg/L/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Change</td>
</tr>
<tr>
<td>Renovascular 3</td>
<td>5.8(3.5)</td>
</tr>
<tr>
<td>Paroxysmal 27</td>
<td>86.4(16.7)</td>
</tr>
<tr>
<td>Essential 4</td>
<td>70.3(7.9)</td>
</tr>
</tbody>
</table>

There was a wide variability of response in the renovascular and paroxysmal groups. In the three children with renovascular disease, one showed no PRA response to Captorsil. There was extensive overlap of response between the groups. PRA is higher in normal children than in adults, but age was not a major cause of the variability. We conclude that variability of PRA response in patients with renovascular disease may limit the usefulness of the test in children.

Neurofilaments (NF) constitute a class of intermediate filaments (IF) found primarily in nerve cells. NF consist of three protein components: NF-200, NF-150 and NF-68. Antibodies (ab) to NF have been described in a number of diseases and elevated levels of ab to NF have been found in several neurological conditions. Antibodies to a variety of cell ag (including IF) are found in systemic lupus erythematosus patients (SLE) and, recently, in subjects with the acquired immune deficiency syndrome (AIDS). Because of the common finding of central nervous system (CNS) involvement in SLE and AIDS, we investigated ab to MMW-NF in these subjects.

An enzyme immunoassay (ELISA) was utilized in which a peroxidase-anti-human ab was used to detect anti-NF reacting with MMW-NF covalently bound to microtiter plates (MicroFluor, Inc., Newport, RI). Ortho phenylene diamine/H2O2 was the chromogen and absorbance (OD) at 490 nm was read vs a reagent blank.

A group of healthy subjects (CON) was used to establish the normal range (NR) of 0.00-0.26 (mean ± 2SD). Analysis of variance indicated significance among study groups (p<0.01). SLE group (n=18) was significantly different (0.180±0.19, p<0.05) from CON; however, 4 subjects had OD above NR. AIDS group (n=15) had significant elevation (0.410±0.40, p=0.018) and 4x were above NR with 5 AIDS (33%) >2SD above CON mean.

Anti-NF ab data may be clinically useful in predicting CNS involvement in disease and ELISA may provide sensitive quantitation of these ab.

RELATIONSHIPS BETWEEN PHYSICAL AND BIOCHEMICAL PARAMETERS IN NEONATES, Ann Marie and Raymond Jang, (University of Cincinnati Medical Center, Cincinnati, OH 45267-0714) (Spon:A. Warner).

A group of appropriate weight for age neonates were assigned to premature (<36 wks, n=47) and mature (38-42 wks, n=140) groups on the basis of physical and neurological exams (G Score). Cord blood samples were analyzed for Hgb, Hct, conalbumin, HFA V1, WBC, FEP, AP epalub, pseudo-cholinesterase (CCHO), and creatinine. AFP/WT and HGB F/WT were calculated from birth weight (Rg). Relationships between these biochemical measures and physical measures were determined through six stepwise multiple regressions using the model: *Biochemical measure = f(CCHO, WBC, FEP, etc.)*. Placenta (P), G Score (G). The strongest models obtained were:

**Premature girls**
1) Hgb = 11.611 + 16.924 x 0.110P - 10.163G
R² = 0.965, P<0.01
2) HGB F/WT = 138.670 - 2.516C + 1.073G
R² = 0.930, P<0.001

**Premature boys**
1) HGB F/WT = 48.728 - 0.014P + 0.242G
R² = 0.903, P<0.001
2) Chol = 179.948 + 145.585C
R² = 0.548, P<0.002

**Mature babies**
1) HGB F/WT = 55.931 - 0.005W = -2.66L
R² = 0.512, P<0.05

Mature girls, no models with R²>50, P<0.05 were obtained.

It is concluded that there are strong relationships between biochemical and physical measures particularly in the premature neonate and that these relationships differ by sex.


The incidence of sickle cell trait varies among blacks in North America. Although, the average hemoglobin (Hb)-SS gene frequency is 8 per cent in the U.S.A., a value of 15 ± is recorded in South Carolina. During a two day health-fair (community-sponsored) in Selma,AL a sickle cell gene frequency of 15.96% was observed. In order to confirm the observed high frequency for the sickle cell gene carriers, the screening program was continued for over a period of twelve months. Hemoglobin electrophoresis was performed by two techniques. 1) cellulose acetate, pH 8.6 (Coomasie blue, 0.04) and 2) labeled cellulose acetate. 0.3M HCl, pH 2.2 (Spon: Spon. 36701). MMW CII and SII (Spon: Spon. 36701) were determined simultaneously. Hb A2 and F were quantitatively determined using A2 column and radial immunodiffusion technique respectively. Overall 127 cases were followed up with both techniques. 947 tests in 2 patients were aware of their Hb status prior to the tests provided by the Center. Surprisingly this higher frequency was not detected by the students at the center. The center also serves as a residency training program for the Family Practice Physicians. Thus it became apparent that there was a crucial need for a revision in the curriculum for the physician in training. Through a screening program, we were able to detect sickle cell trait, implement a rigorous training program for the resident physicians in family practice and provide proper counseling to the patients for hemoglobinopathies. We believe that, laboratory screening is a first step towards programs leading to genetic counseling and medical practice by family physicians and general practitioners.

CIRCULATING IMMUNE COMPLEXES AND HYPOCOMPLEMENTEMIA IN HIV-INFECTED PATIENTS, S. Diao (Department of Pathology, Baylor College of Medicine; Veterans Administration Medical Center, Houston, Texas 77030) (Spon.: S. Diao).

Current commercially available assays for Acquired Immune Deficiency Syndrome (AIDS) are capable of detecting various antibody bodies to HTLVIII/HIV as an indicator of prior exposure to the virus, but are neither diagnostic of the infectivity of the tested individual's blood/tissue nor indicative of the clinical course of AIDS or the development of HIV-related complications.

We are attempting to identify prognostic tests for AIDS. In an ongoing study 96 sera from 85 patients over a 3 year period have been studied for hypocomplementemias and circulating immune complexes (CIC) concentrations. CIC's are measured by a microtiter ELISA assay employing monoclonal antibody to CIC binding cig (Gayrtoch, San Diego, CA 1982).

CIC concentrations were increased in 56(58%) of sera and occurred predominantly in late AIDS individuals (36) and in individuals with ARC (20). C4 was decreased in 14(17%) of HIV-infected patients while 27(32%) and low C3. Hypocomplementemias (assessed by IgG, IgM and IgA concentrations) was present in 36(45%) and 35(42%) of AIDS and ARC patients respectively, but was absent in asymptomatic seropositive individuals.

To date there appears to be no statistical correlation between hypergammaglobulinemia and complement concentration. The largest CIC concentrations were present in patients with marked hypergammaglobulinemia.

It appears that CICs are common in symptomatic HIV-infected patients and are not associated with hypocomplementemia. Poly-clonal hypergammaglobulinemia does not account for all of elevated CIC. Studies to determine the relationship between infection and CIC level in AIDS patients are in progress.


The use of DNA probes is a new and an important addition to existing laboratory methods for the detection of carriers of Duchenne muscular dystrophy (MDM). Since the gene for DMD has not yet been cloned, restriction fragment length polymorphisms are presently used within affected families in order to trace the inheritance of the defect in the pedigree.

It has been our experience, in 49 MDM families, that the technique worked particularly well in obligate families (31), in excluding carrier status in isolated cases (36), and in families in which certain members already exhibit creatine kinase (CK). However several potential problem have been encountered: (1) the accuracy of positive carrier detection is limited by the probability that an affected relative represents a new mutation (2) sometimes the affected person or some crucial family member is not available to donate DNA (3) key family members are uninformative for the DNA probes (4) genetic recombination between the polymorphism and the mutation will lead to error in carrier detection (5) approximately 50% of obligate carriers exhibit normal CK levels.

Cases will illustrate the advantages and the problems of the RFLP approach in carrier determination in Duchenne muscular dystrophy.
TOTAL ERROR ANALYSIS OF BEDSIDE GLUCOSE ANALYZERS, Nancy Cossitt; David Mestrich (Children’s Memorial Hospital, Chicago, IL 60614) (Spon: N. Cossitt)

Three levels of assayed Whole Blood Glucose Control, SugarCheck (Streck Laboratories) were used to evaluate whole blood glucose determinations quantified with two reflectance meters, Ames Glucometer 2 and Accuchek II (Boehringer-Mannheim Diagnostics.) The same material was also quantified for glucose on the Beckman Synchron CX-7.

The study was performed over a 25 day period with single determinations of each level of control (low, mid, high) being run by the same operator on each of the three instruments daily.

Mean, S.D. & cv of each level of control material per instrument were calculated. Further calculations were performed to determine bias of mean, imprecision and TEs (total analytical error) to 95% confidence. The TEs 95% ranged from 15-45%.

Instrument: Accuchek II; Ames; Synchron CX-3
Control Low Mid High Low Mid High Mean 58.0 117.5 305.1 76.6 183.8 303.4 62.4 154.8 274.5
S.D. 5.3 11.0 2.6 11.4 5.4 9.8 21.0 1.6 6.5 2.2 3.7
Bias abs 18.6 23.7 10.5 14.2 30.0 28.9 6.9 12.4 154.8 274.5
Bias rel 31.7 20.2 31.7 21.8 34.2 36.6 3.2 4.3 7.3
TEs 95% 45.2 73.7 114.8 37.8 30.1 23.2 5.2 2.8 2.2

In summary, both the Ames Glucometer II and BMD Accuchek II are probably useful for home monitoring. In a hospital setting physicians should not assume that bedside glucose readings are equivalent to laboratory glucose determinations.


The feasibility of measuring lidocaine metabolites in blood as an indicator of liver function was assessed by utilizing a new serum assay from Abbott Laboratories to measure the major metabolite monoethylglycinexylidide (MEGX) by the TDX system. A subtherapeutic 1 mg/kg intravenous bolus of lidocaine was given to test liver function. Eight potential liver donors were evaluated by existing criteria and three livers were used. Five were not used due to elevated liver function test results or inappropriate size. Lidocaine studies indicated that these livers had metabolic capabilities equivalent to the group that was used. Six additional hepatic transplant recipients had 30 lidocaine metabolites performed on them to evaluate stable liver function and function during ischemic injury and rejection as documented by MEGX serum concentrations were compared to biochemical tests (Table). Higher MEGX values were seen in stable patients as compared to those with either ischemic damage or rejection indicating decreased metabolic capabilities. This procedure allows an easy rapid evaluation of liver metabolic function which may enhance selection of appropriate liver donors and better management of liver transplant recipients.

Sex Age Group 50 yr

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age Group</th>
<th>Central 95%</th>
<th>Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>1-8 yr</td>
<td>23 mg/L</td>
<td>6-47 mg/L</td>
</tr>
<tr>
<td>M</td>
<td>9-15 yr</td>
<td>28 mg/L</td>
<td>6-96 mg/L</td>
</tr>
<tr>
<td>F</td>
<td>1-15 yr</td>
<td>12 mg/L</td>
<td>6-81 mg/L</td>
</tr>
</tbody>
</table>


A six-month old boy with glycoprotein storage disease type I was treated with frequent feedings supplemented with glucose infusions. In an attempt to reduce liver size, further treatment consisted of adding glycine to his milk (0.8/day for 8 days). We reasoned that a nitrogen-rich diet would increase urea production which would mean more ATP consumption (4 moles of ATP per mole of urea synthesised) and hence more glycoprotein breakdown. Glycine was chosen because it has the highest N/C ratio of all aminos, and because it is relatively innocuous.

There resulted no change in liver size. However, a 4-fold increase in urea excretion was accompanied by a 10-fold increase in urine lactate. Blood lactate also increased (from 3.1 to 5.2 mmol/L). Lactate was determined by the method of Marbach & Weil (Clin. Chem. 1967, 13; 314-25). Increased lactate production was interpreted as increased glycoprotein breakdown which was balanced by glycogen synthesis, since there was no reduction in liver size. The enhancing effect of elevated plasma glycine on glucagon production, which in turn stimulates glycogen breakdown, may also partly explain the increased lactate production.

Because of the risk of increased lactic acidosis, a nitrogen-rich diet was not recommended in glycoprotein storage disease type I.

PEDICIDICAL REFERENCE INTERVALS BY AUTOMATED RADIOIMMUNOASSAY: CORTISOL, FERRENU, TRICYCLOPYRINE (TB), LUPROIN (LH), PRLACTIN (TSH), THYROID (TH), TRIDODOTHYRONE (TT), AND T4-T3 RATIO, G. Letuneco, S. Canni, E. Parodi and L. Gomard (Laboratories Inst. Gen., Geneva, Switzerland) (Spon: M. H. Dominonak).

For analytes commonly measured by immunosassay, the literature contains relatively little information on expected values in children. Moreover, it is difficult to compare the literature results because of the scoring age classifications used by different groups and frequent inattention to the statistical distribution of the data sets.

We sought to circumvent these limitations by compiling a substantial data base, and by analyzing the results for age-related trends and distribution, to rationalize the choice of age groups and statistical techniques.

LONGITUDINAL MONITORING OF SERUM PROTEINOSIS AND CORTISOLIN IN NON INSULIN DEPENDENT DIABETES (NIDDM), M. H. Dominonak, S. M. MacMillan and K. R. Pender (Dept Path Biochem, Western Infirmary, Glasgow G1 6XH and Dept Med Royal Infirmary, Glasgow OG) (Spons: M. H. Dominonak).

Our objective was to define the interpretation of the fructoseamine concentration in diabetic patients, in relation to hemoglobin A1 (HbA1) levels. Fructoseamine concentrations were monitored for 8-16 weeks in 14 patients with NIDDM (9 males, 5 females, age < SD 57 ± 9 years).

Initial fasting blood glucose (FBG) was 15.3 ± 3 mmol/L, HbA1 13.7 ± 2.2% and fructoseamine 4.2 ± 0.5 mmol/L. At 3 weeks FBG was 11.2 ± 2.9 mmol/L (p0.05), HbA1 11.8 ± 3.6% (p0.01) and fructoseamine 3.9 ± 0.4 mmol/L (p0.01). At 16 weeks they were 11.5 ± 2.6 mmol/L (p0.02), 10.6 ± 1.8% (p0.05) and 3.4 ± 0.6 mmol/L (p0.01) respectively. For the whole group we found significant correlations between HbA1 and fructoseamine at 8 separate visits ranging from r = 0.30 to r = 0.94. When patients were monitored at weekly intervals 62.5% of HbA1 and fructoseamine results showed the same direction of change between visits. When monitored at 2, 4, 6 and 12 week intervals the same direction of change was observed in 62.4%, 31.8%, 52.4% and 90% results respectively (p2 < 0.025).
In conclusion: In NIDDM correlation between HbA1c and fructoseamine may vary between patients and with time in the same group of patients. Trends in glycosyl control are reflected both in fructoseamine and HbA1c levels. However, visits to visit changes show different changes of fructoseamine and HbA1c concentration, particularly during frequent monitoring.


Equivocal results (60 to 80 mmol sodium/L) of sweat tests in the diagnosis of CF have limited the complete informative nature of this test. Recently, DNA probes were linked to the CF locus on chromosome 7 have been used to follow the inheritance of the mutant gene in families with an affected individual. In addition, the reporting of new probes (pM1.19 and pV1.2c, Sambler et al.) in linkage disequilibrium with CF has allowed for haplotype testing. These new probes along with others previously shown to be linked to CF (pMet D, pMet H and p33.111), were used to evaluate an individual with an equivocal sweat test result. DNA was isolated from peripheral blood samples obtained from the individual, an affected sibling, an unaffected sibling and the parents. Restriction fragment length polymorphisms (RFLP) were analyzed using the above probes and the appropriate restriction enzymes. The Met D (BamI RFLP) and Met H (TagI RFLP) probes were fully informative as both parents were heterozygous. The Xv.1c (TagI RFLP) and KM.19 (PstI RFLP) probes were partly informative as only one parent was heterozygous. Using the results from the Met D and Met H probes and assuming no recombinants, it was shown that the individual with the equivocal sweat test did not inherit either of the two chromosomes carrying the CF mutation. The unaffected sibling was shown to be a carrier for the CF haplotype of the affected individual, using results from pM1.19 and pV1.2c, was shown that previously in carriers to have the highest probability of carrying the CF mutation (0.398, Odds = 1 in 5.0, Beaudet). These results indicate that DNA probes linked to the CF locus can be helpful in resolving equivocal sweat test results in individuals with an affected sibling.


We investigated the effects of gestation (completed weeks), maternal weight (pounds), and maternal age on the median of maternal serum AFP from two populations (Utah and Minnesota). Samples submitted to our laboratory during a 19 month period were included in the study if gestation was 20 or more weeks, and if weight and age were known. Patients known to have multiple fetuses or a fetus with structural abnormalities were excluded. The number of samples analyzed was 2494 for Minnesota, and 1980 for Utah, 1992 of 2446. The AFP results (ng/mL) were logarithmically transformed to normalize the distribution.

Means (Standard Deviations) Correlation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard (SD)</th>
<th>Coefficients (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utah</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFP 1.546 (0.213)</td>
<td>1.555 (0.219)</td>
<td>AFP vs. Minn. Utah</td>
</tr>
<tr>
<td>weight 144.3 (26.6)</td>
<td>1.553 (26.8)</td>
<td>weight -0.217</td>
</tr>
<tr>
<td>age 28.2 (4.19)</td>
<td>28.6 (5.20)</td>
<td>age -0.019</td>
</tr>
</tbody>
</table>

*sig. diff. (p < 0.001) | *sig. corr. (p < 0.001)

Stepwise multiple regression for each population of gestation, weight, and age onto log(AFP) demonstrated that age was not useful in predicting AFP. Medians were therefore estimated using:

log(AFP) = A + B * gestation + C * weight

where:

A = 0.346 (0.213) B = -0.019
C = SD of estimate

Minneapolis 0.7045 0.0684 -0.0019 0.183 (F = 1.36)
Utah 0.7182 0.0673 -0.0020 0.186 (F = 1.39)

We conclude that AFP increases by 1% per week gestation and decreases by 0.5% per 10 pound increase in body weight. In addition medians for Minnise were 15% higher than for Utah. Laboratories should consider the effect of geographic location when determining medians for maternal serum AFP screening programs.

246 DIFFERENTIAL EFFECT OF THE ANTISYPHOMATIC DRUG TOISPORINE (BRISTOL-MYERS BNY 13859) ON SERUM PROLACTIN. P.J. Orugula, P.G. Owen, N.M. Kurtz and J.T. Kenney (Mental Health Clinical Research Center, Dept. of Psychiatry, Univ. of Texas Southwestern Medical Center, Dallas TX 75235 and CNS Clinical Research, Bristol-Myers Co., Wallingford, CT) (Spon: J.T. Kenney).

Measurement of circulating prolactin levels is of interest in the study of antipsychotic medications because the blockade of dopamine receptors in the CNS by these drugs is considered to be one of their principal pharmacological effects. All subjects were pretreated with toipronine in 30 patients treated with tiosporine, 33 patients treated with thioridazine, 35 patients treated with haloperidol and 30 patients treated with placebo. All patients were participating in a multicenter, double-blind study and gave informed consent prior to treatment. Serum prolactin levels (ng/mL, X ± S.D) were as follows:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Baseline</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo 9.1 ± 6.6 12.0 ± 13.5 8.3 ± 4.6 8.8 ± 3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thioridazine 11.3 ± 9.0 8.4 ± 4.0 8.3 ± 3.4 8.4 ± 2.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haloperidol 12.4 ± 10.1 35.2 ± 16.9 39.1 ± 20.3 40.0 ± 18.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiosporine 15.7 ± 18.8 15.9 ± 21.1 17.1 ± 17.8 20.2 ± 29.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patients treated with thioridazine or haloperidol showed significant elevations (p < 0.0004) in serum prolactin that were sustained throughout treatment while patients treated with tiosporine showed only a small (N.S.) increase. These findings are consistent with animal studies suggesting that tiosporine differs from other antipsychotics in its effect on the dopaminergic system.


It has been shown that Vitamin E will prevent lipid peroxidation in red cell membranes, and the Vitamin E content of sickle cell plasma has been shown to be low compared to normals. We decided to investigate the levels of tocopherol (T) isomers in the plasma and red blood cells (RBC) of non-SCD patients compared to SCD patients using an improved version of existing HPLC methods.

Comparison of the HPLC results are as follows:

<table>
<thead>
<tr>
<th>Non-SCD</th>
<th>SCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>RBC</td>
</tr>
<tr>
<td>a-T (n = 11)</td>
<td>6.725 ± 1.4 ± 6.725 ± 1.4</td>
</tr>
<tr>
<td>a-T (n = 5)</td>
<td>9.09 ± 4.12 ± 9.09 ± 4.12</td>
</tr>
<tr>
<td>y-T (n = 1)</td>
<td>1.64 ± 0.57 ± 1.64 ± 0.57</td>
</tr>
<tr>
<td>y-T (n = 2)</td>
<td>1.91 ± 0.93 ± 1.91 ± 0.93</td>
</tr>
<tr>
<td>y-T (n = 17)</td>
<td>1.17 ± 0.23 ± 1.17 ± 0.23</td>
</tr>
<tr>
<td>y-T (n = 18)</td>
<td>0.28 ± 0.10 ± 0.28 ± 0.10</td>
</tr>
</tbody>
</table>

a. UV detection, b. Fluorometric detection

The Student's T-Test showed a significant difference between plasma A-T and RBC y-T of SCD patients vs non-SCD patients at the 95% confidence level. The increased amount of y-T in the RBC of SCD patients does not seem to be indicative of a protective effect on the RBC membrane but may suggest a greater transport of the increased plasma y-T to the RBC.


Pancreas transplants (PaT) are a treatment modality for end-stage renal patients who are hypoinsulinemic diabetics. The use of serum glucose levels to monitor rejection of PaT has not been totally successful. We measured serum levels of PP, a possible endocrine hormone, and NT, a product of stimulated macrophage, for urine samples to determine if the levels correlated with the clinical status of the transplanted organ and other biochemical parameters. Serum was drawn at different pharmacologic actions. The use of serum glucose levels to monitor rejection of PaT has not been totally successful. We measured serum levels of PP, a possible endocrine hormone, and NT, a product of stimulated macrophage, for urine samples to determine if the levels correlated with the clinical status of the transplanted organ and other biochemical parameters. Serum was drawn at different pharmacologic actions.
fasting glucose POD 14 amylase (POD 9), and C-peptide (POD 11). Urinary iron levels plated by POD 16. Serum PP levels peaked at 45 U/mL by POD 7 and became NNR by POD 16. Serum NT levels were initially very low (<9 mmol/L) until POD 17 when they rose slightly above normal. At POD 26 the patient was readmitted for a low grade fever, later presumed to be caused by a CMV infection. The patient was not well at this stage and there was no clinical evidence of rejection. Associated with the infection was a rise in blood lymphocytes, peaking 4 days after readmission. This rise in NT and C-peptide by 9 days and PP rose slightly 5 days prior to admission.

Serum PP and NT correlated well with clinical and biochemical indices of rejection. The rise in NT and C-peptide suggested no organ rejection process. The patient was still stable 2 months after surgery, suggesting that serum PP and NT levels may provide useful adjunct data for monitoring patient's status.

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This two-year study was initiated (1986) in order to study the effects of competitive high school swimming on serum iron and serum ferritin levels. Blood specimens were drawn from swimmers after their first high school swim practice (early August) and again at the end of the high school swim season (Late November). In both August and November a test hematologic profile and a complete hematology workup were performed on each swimmer. The findings were dramatic. Even though some of the swimmers were taking supplemental iron and other vitamins, their ion levels dropped significantly during the four months of competition (an average drop of 61 mg/dL). At the end of the 1986 season five of 13 swimmers had serum iron levels of <50 mg/dL and two had serum ferritin levels that were too low to measure. What is the mechanism of iron loss in swimmers? Our data show that swimming does not cause increased iron loss in serum. Swimming does promote increased intravascular hemolysis but normally the iron that results is efficiently recycled. Women who swim 10,000 yards or more per day and maintain a total body fat of 18% or less normally experience amenorrhea. Thus by a process of elimination it appears that the G1. tract is the most likely site of blood (iron) loss. At the beginning of the 1987 swim season swimmers were advised to increase the iron content of their diet. All were made aware of the fact that iron, in excessive amounts, is toxic. Results were very encouraging. Serum iron levels dropped an average of only 25 mg/dL during the 1987 swim season, while there was an increase in the hematocrit, hemoglobin concentration and serum ferritin in 9 of 14 swimmers. In conclusion, swimmers who are associated with a rigorous competitive swimming program must watch the iron content of their diet very carefully if they are to avoid iron deficiency.

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**PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS AND HEPATITIS MARKERS IN PARENTERAL DRUG ABUSERS, Thomas E. UKen, S. Goldin and H. Baker (Worchester Alnsworth Hospital and State Hospital, Worchester, MA 01605). (Spon: Thomas E. UKen)**

Human immunodeficiency virus antibody (HIV-Ab) and serum markers for hepatitis B surface antigen and antibody (HBe-Ag, HBe-Ab), core antibody (HBC-Ab) as well as hepatitis D virus antibody (HDB-Ag) were determined in human volunteers who are known to be parenteral drug abusers (PDA). Blood was randomly collected and the sera analyzed using enzyme immunoassay procedures for all the hepatitis markers and HIV-Ab screening. All HIV-Ab positive samples were confirmed using the Western Blot method.

Of the 65 PDA tested, 25 (38.5%) were HBe-Ag positive and 8 (12.3%) were HIV-Ab positive. The hepatitis antibody profile was variable as follows: 30/65 (46%) were HBe-Ag (+) and 49/65 (76%) were HBe-Ab (+). The prevalence of HBC-Ab in HBe-Ag positive PDA was 36%, an estimate which is in agreement with previously published reports. The frequency of HDB-Ag was significantly lower (23%) in HBe-Ag-positive PDA indicating recovery from HB or HDV virus infection. HIV-Ab presence in PDA in general was 12.3% but significantly higher in HBe-Ag positive PDA (32%). Most of these 32 PDA were positive only for anti-HIV positive PDA, an association which has not been reported previously and may contribute to the understanding of HIV infection.

The results indicate high prevalence of hepatitis B and D infection among PDA and a greater prevalence of HIV among HBe-Ag-positive PDA and among HDV-positive PDA in particular. In addition the data suggest the need to screen for these viral markers in this population to confirm the apparent association of HIV and HDV infection which could have important implications for the pathophysiology of HIV infection.

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**ASSESSMENT OF RENAL FUNCTION BY INULIN CLEARANCE:**

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**COMPARISON WITH CREATININE CLEARANCE DETERMINED BY ENZYMATIC METHODS**, J.F. Agnif. J. P. Halstein, J. Wigley, and M. Bort, NY, Memorial Hospital, Philadelphia, PA 19104. (Spon: J. Wolfson)**

The purpose of this study was to compare creatinine clearance (CrCl) determined by enzymatic and Jaffe methods with glomerular filtration rate measured by standard inulin clearance in patients with varying degrees of renal function. Patients were grouped based on 24-hr CrCl (mL/min) as follows: Group I > 100 (n=6); II 50-100 (n=6); III 25-49 (n=6); IV < 25 (n=6). Timed serum and urine samples were collected before and after inulin infusion. Clearance was calculated by the HPLC, Astra Jaffe, and two enzymatic methods: Kodak Ektachem. Clearance values were collected for body surface area. The results showed the mean SD of all data from all groups combined. The enzymatic tests were not as sensitive and specific as the Jaffe enzymatic assay. Enzymatic assays for serum and urine creatinine measurements should be carefully correlated with Jaffe methods if enzymatic values are to be reliably used to assess renal function.

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**MONITORING PATIENTS WITH PANCREAS TRANSPLANTATION, Peter Woldof, Donald Siegel and Leonard Perloff (Dept. of Pathology & Laboratory Medicine, and Surgery, Hosp. of Univ. of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104).**

Pancreatic transplantation is a well-established procedure with one year allograft survival rates as high as 60% in insulin-dependent diabetics with or without end-stage diabetic nephropathy. The Clinical Transplant Laboratory is a key role in supporting the transplant team effort in the perioperative period and is also quite important for periodic assessment in long term follow-up. A four-day protocol has been developed for evaluation of pancreatic allograft recipients who have exocrine drainage in the bladder. Endocrine function is determined by measurement of hemoglobin A1C, blood glucose, insulin and C-peptide and admission to the hospital for oral administration of glucose. Exocrine function which provides a very early marker of rejection is determined by measurement of urine pH, bicarbonate, amylase, lipase, chymotrypsin and the calculation of excretion rates of amylase, lipase and bicarbonate. The response of the allograft to stimulation in in a Susstall Challenge Test and a Cholecystokin-Secretin Test is also measured. The protocol which provides a convenient method of contrasting patients and for assessing status of individual transplant function has been tested on eight occasions. Data was collected in a database and presented graphically for ease of inspection.
and hip in 109 premenopausal women between the ages of 35 and 50 during the follicular phase of their cycles to determine the relationship between bone density and sex steroids. SHBG and FSH were measured by fluororimmunoassay; 82 and DHEAS.

Neither E2 nor DHEAS correlated significantly with bone density at any site. In women with bone density at three sites in the hip, the femoral neck (r = -0.2822, p = 0.004), Ward’s triangle (r = -0.2777, p = 0.004), and the greater trochanter (r = -0.1346, p = 0.243) were negatively, with SHBG density at the spine (r = -0.2212, p = 0.051), the femoral neck (r = -0.1989, p = 0.04), Ward’s triangle (r = -0.2120, p = 0.04), and the greater trochanter (r = -0.1088, p = 0.04).

Estradiol, which regulates plasma androgen and estrogen availability, and FSH, which is released in response to decreases in plasma estrogens, correlated with bone density. Estradiol may influence bone density in premenopausal women by regulating concentrations of free sex steroids, and FSH may reflect the availability of free estrogen. SHBG and FSH may be predictive of bone density over time.

**254** PROGNOSTIC VALUE OF DIRECT AND TOTAL PLASMA PYRIDOXYL 5'-PHOSPHATE PROTEIN, LIN, M.S., R. L. Naughten, J. J. GuADINOS, K. J. Michael, J. A. K. (Spon: J. J. GuADINOS)

We compared the prognostic significance of direct plasma pyridoxal phosphate (PLP) and total (deproteinized) plasma pyridoxal phosphate (TPLP) in 36 seriously ill surgical patients, using the tyrosine aminotransferase method. But, not TPLP, predicted survival (p < 0.05 versus p = 0.05). This confirmed our previous observations in 222 patients that DPLP was useful in predicting one-month mortality (p < 0.001) (Keniston, Clinical and Physiological Applications of Vitamin B6, Alan R. Liss, New York, in press 1988). The ratio of TPLP to DPLP (R) was also of prognostic significance (p < 0.05). DPLP, but not TPLP, correlated well with the other independent parameters, such as lactate dehydrogenase/albumin ratio, drug use, serum enzyme levels, white blood count, serum electrolyte and calcium levels. The difference between TPLP and DPLP, (DPLP), correlated well with the serum urate aminotransferase level (r = 0.06) and also in serious illness, much of the plasma PLP is tightly bound to enzymes and drug-PLP complexes, leaving less PLP available for uptake by the cells. Based on DPLP levels, we have supplemented almost 26 patients with additional pyridoxine; and we found that increment in DPLP also predicts survival rate. In conclusion, TPLP may not reflect the true vitamin B6 status of patients in serious illness, as DPLP tends to do so.

**255** CLINICAL UTILITY OF CARMAZEPINE METABOLITE IN THE RECOVERT OF CARMAZEPINE OVERDOSE PATIENTS, Jen-Ling Liu, Joe-Fang Deng(Clinical Toxicology Division, Veterans General Hospital, Taipei, Taiwan, ROC) & Stephen S. Yu(San Francisco Public Health Toxicology Lab., San Francisco, CA 94101)(Spon: John Osterich)

The major pathway of biotransformation of carbamazepine (CBZ) consists of epoxidation to CBZ-10,11-epoxide (CBZ-E) which is then converted to trans-10,11-dihydro-10,11-dihydroxy CBZ (CBZ-diol). Biologically inactive CBZ-diol might compete with active CBZ and CBZ-E for binding site and serve as a protector to reduce the toxic effect of CBZ overdose. We studied the CBZ-diol levels in two CBZ overdosed patients.

The analyses of CBZ, CBZ-E, and CBZ-diol were carried out by a C-18 reverse phase HPLC system. Acetonitrile was employed to precipitate serum protein and methanol was used as the internal standard. The mobile phase consists of methanol/water(55/45, v/v) and the flow rate is 1 mL/min. The absorbance of the effluent was monitored at 220 nm. Blood specimens from two suicidal patients were drawn at various time intervals. The results are listed below:

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ingested (mg)</th>
<th>CHZ, mg/L</th>
<th>CBZ-E, mg/L</th>
<th>CBZ-diol, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>50</td>
<td>1.5</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>#2</td>
<td>80</td>
<td>1.2</td>
<td>0.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**256** CLINICAL UTILITY OF URINARY N-ACETYL-α-D-Glutaminidase ASSAY, Stephen S. Yu(Spo: John Osterich)

Urinary N-acetyl-α-D-glucosaminidase(NAG)(EC 3.2.1.30) is a sensitive marker for tubular nephrotoxicity. The elevation of NAG activity in drug-induced renal damage has been well established. In this study, we investigated the NAG activity in drug screening specimens submitted from San Francisco General Hospital.

NAG activity is measured by a modified fluorometric method of Powell et al. (Clin. Chem.,17:175-185, 1981). The urinel specimen was incubated with enzyme substrate, 4-ethyl umbelliferon-N-acetyl-α-D-glucosaminidase(0.5 m/L) in citrate buffer, pH 4.5 for 30 min, at 37°C. The reaction was terminated with 1 M Na2CO3, pH 10.5. The liberation of 4-ethyl umbelliferone from the substrate was measured fluorometrically with excitation wavelength at 350 nm and emission wavelength at 440 nm. The NAG activity is expressed in enzyme unit per gram of creatinine. The sensitivity of the assay is in the range of 0.1 U/G. The intra- and interassay variations are in the range of 3%. The emission fluorescence is linear up to 100 U/G. The enzyme substrate and urine specimens are stable for 3 months or longer at -20°C. The NAG activity of 126 adult urine toxicology specimens and 33 healthy individual specimens is tabulated below:

**257** COMPARISON OF A SIMULTANEOUS RADIOIMMUNOASSAY FOR FREE THYROID STIMULATING HORMONE (TSH) AND THYROTROPIN (TSH) IN ASSESSING THYROID FUNCTION, J. Kalra, K. L. Massey and Y. A. Laxdal (Dept. Pathol., Univ. Hosp., Univ. Saskatchewan, Saskatoon, Sask., Canada)(Spon: K. L. Massey)

We compared and evaluated the diagnostic efficiency of a simultaneous radioimmunoassay for free thyrotropin (FT4) and thyrotrpin (TSH) ("SimulTrac FT4 & TSH (25)"") RIA, Becton Dickinson Immunodiagnostic, Orangeburg, New York) with a sensitive immunometric assay (IRMA) for TSH (TSH MAIACLONE, Serono Diagnostics Inc., Braintree) in assessing thyroid function. The reference interval for euthyroid subjects by both methods were within the reference ranges listed by the manufacturers.

Patients studied (n=71) were divided into different groups on the basis of their clinical findings. In the hyperthyroid group (n=12) all the patients were diagnosed clinically with the IRMA method. The "SimulTrac" method indicated discrepancies in five patients, four having normal FT4 and one having a normal TSH concentration. In the hypothyroid patients, one was correctly diagnosed as overtly hypothyroid by both the methods. The other five patients were shown to be subclinically hypothyroid with a low normal FT4 and low TSH concentration by the "SimulTrac" method. These five patients also had elevated TSH values by the IRMA method. In clinically euthyroid group (n=15) fourteen patients were correctly diagnosed and one patient had an increased TSH value by the IRMA method. The "SimulTrac" method in this group gave discrepancies in five patients; two as having elevated TSH, and three with elevated FT4. All the patients with nonthyroidal illness (n=17) were correctly diagnosed by both methods.

These observations indicate that the results obtained by the "SimulTrac" method provided improved diagnostic efficiency for subclinical hypothyroidism, however the IRMA method was superior in diagnosing hyperthyroid and euthyroid patients.

**CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988**
MODIFICATION OF AN UNCONGENITAL H市の KITT FOR DOW SYNDROME SCREENING IN THE SECOND TRIMESTER OF PREGNANCY. (George J. Kariuki, I.E. Haddad, D.E. Palmeri, and L. Canick) (Department of Biochemistry, Southern Illinois University at Edwardsville, Illinois, and the University of Washington, Seattle. Supported by a grant from the US Public Health Service: Grant No. 5R01-AM16332-15.)

We have recently reported that second trimester (15 to 20 weeks) maternal serum unconjugated estriol levels are significantly lowered in Down syndrome pregnancy (Br. J. Obstet. Gynecol., 1988). These data demonstrate that maternal serum unconjugated estriol levels, when combined with maternal age and the determination of maternal serum alpha-fetoprotein levels, may significantly improve screening for Down syndrome pregnancy.

Measurement of the unconjugated estriol levels found in the second trimester (0.5 to 5 ng/mL) requires modification of commercially available kits, which are designed for use in the third trimester of pregnancy when levels range from 3 to 40 ng/mL. We report here modifications of the Amersham® Estriol (Unconjugated) RIA Kit (Amersham Corp.) for the measurement of second trimester maternal serum unconjugated estriol levels. Modifications include doubling the sample volume to 40 mL, sample standards, controls, and 2) addition of three low standards by serially diluting the lowest kit standard (3 ng/mL) twofold and dropping the two highest kit standards, producing a standard curve ranging from 0.4 to 15 ng/mL (95% BO/ 0.20 ng/mL; 50% BO/ 0.41 ng/mL; 20% BO/ 2.46 ng/mL; 20% BO/ 8.83 ng/mL). Recovery was assessed by spiking in two levels of estriol (1.7 and 25 g/L) to 25 actual patient samples. Average recovery (C/V %) was respectively 105% (8.8%) and 105% (5.6%). Patient samples with high second trimester unconjugated estriol values (4 to 6 mg/mL) were serially diluted down to 0.5 ng/mL with zero standard and showed linearity as expected. Between-assay CV of 11.6% and 5.7% were obtained using control sera containing 1.3 and 5.4 ng/mL, respectively.

Using this modified kit, maternal serum unconjugated estriol values increase by approximately 20% per week in the second trimester. Our validation data suggest that commercially available unconjugated estriol kit can be successfully modified to measure levels found in the second trimester of pregnancy.

WHEN DOES PARATHYROID HORMONE (PTH) START TO INCREASE IN RENAL INSUFFICIENCY? (Lasse Larsen and N. Lundberg. (Dept. of Clinical Chemistry and Nephrology, University of Linkoping, S-581 85 Linkoping, Sweden.)

As patients with hyperparathyroidism can suffer from renal insufficiency we wanted to identify the degree of glomerular filtration impairment at which PTH elimination starts to decrease thus increasing the blood PTH-concentration.

We therefore studied three groups of renal insufficiency (RI patients): 1) slight, (Creatinine clearance (Cr) 40-70 mL/min), 2) moderate (Cr 20-40 mL/min) and 3) severe (Cr<20 mL/min).

Creatine in serum and urine was determined with a conventional Jaffé method, PTH with a micromale radiimmunoassay (Orion Diagnostica, Cambridge, Boston MA) and ionized calcium (Ca²⁺) with an ICA-1 (Radiometer A/S, Copenhagen, Denmark).

Mean ± SD values in the different groups could be found in table 1. (n = number of patients) (p = level of difference significance between group 1/2 and 2/3 respectively).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cr (mL/min)</th>
<th>PTH (mCi/L)</th>
<th>Ca²⁺ (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>70-120</td>
<td>1.1 ± 0.6</td>
<td>1.23 ± 0.03</td>
</tr>
<tr>
<td>Group 2</td>
<td>40-70</td>
<td>3.3 ± 2.3</td>
<td>1.23 ± 0.10</td>
</tr>
<tr>
<td>Group 3</td>
<td>&lt;20</td>
<td>6.0 ± 2.6</td>
<td>2.2 ± 0.6</td>
</tr>
</tbody>
</table>

Peptide levels started to increase slightly when GFR was <50 mL/min, while a two to three fold increase in PTH could be seen when GFR was <20 mL/min. In terms of the PTH-PTH system it would be desirable to increase slightly when >175 mL/min, but did not increase markedly until >350 mL/min.

MOLECULAR ANALYSIS OF S-GLOBIN GENE HAPLOTYPES IN SAUDI SICKLE CELL ANEMIA PATIENTS, M.A.F. El-Hamzi and A.S. Waray (Professor of Medical Biochemistry, College of Medicine and King Khalid University Hospital, P.O. Box 2925, Riyadh 11461, Saudi Arabia) (Spon: Arjumand S. Waray)

The S-globin gene haplotypes in sickle cell anemia patients, with a mild and severe form of the disease, from three different regions of Saudi Arabia, were investigated using restriction endonucleases, Hinc II and Hind III. Significantly different results were obtained in the different regions. In Al-Ula, in the north-western province of Saudi Arabia, where a severe form of sickle cell anemia exists the S-globin gene haplotype in all patients investigated was --. In Ha'il in the south-western province of Saudi Arabia, where the sickle cell anemia exhibits itself generally in severe clinical manifestations, the haplotypes identified in 72 sickle cell anemia patients were --. The predominant haplotype was --. Finally, all 22 patients from the eastern province of Saudi Arabia with a mild form of sickle cell disease carried the haplotype --. The patients were grouped on the basis of their S-globin gene haplotypes, with the hematological data and clinical manifestations were compared. The results showed that the haplotype -- is associated with a mild form of sickle cell disease. The F level in the different haplotypes did not differ significantly.

Comparison of the severity of sickle cell disease and the associated haplotypes in different regions of Saudi Arabia will be presented. The results will be discussed to show that though the different S-globin gene haplotypes in Saudis do not significantly influence the Hb F level but they do influence the severity of sickle cell disease.

TAMOXIFEN BENZYL SULFOTETRIDEOXIDE AND ITS METABOLITES IN BREAST CANCER. L.A van der Walt (Dept. Chem Pathol, S A Institute for Med Research and University of the Witwatersrand, Johannesburg). (Spon: ICR Conte.)

Sex-steroid hormone receptors are accepted predictive indicators of endocrine-responsive breast tumors, are prognostic markers of the clinical course of the disease and may indicate degree of tumor differentiation. When the tumor contained both estrogen receptor (ER) and progesterin receptor (PR), some 80% of breast tumors respond to endocrine manipulation. When biopsies were only ER positive, some 30% of patients would respond positively while less than 10% of patients with ER-PR- respond to hormonal therapy. The most common use of these agents are the periods after surgical resection. In an attempt to improve on the predictive values for classical ERPP, we investigated the binding of radioiodinated tamoxifen to presence of a value for the tamoxifen resistant receptors. We compared the assessment ER and PR by the vertical tube sucrose gradient ultra-centrifugation method in 208 patients with histologically confirmed breast cancer. Tamoxifen appeared to associate with cytosol in a non-specific manner. When tamoxifen was incubated with homogeneate, a membrane-dependent partial hydrolysis could be demonstrated. Shone the active 4- or 5-hydroxyls of the specific protein association while non-specificity was only determined in the 45 fraction. The median specific tamoxifen binding capacity was 45 fmol/mg cytosol protein and appeared to be more closely associated with PR positive rather than with ER positive tumors. Patients with positive tamoxifen receptor have all responded objectively to the drug. Some 4% of patients proved to be PR- and we were able to identify partially that were ER-PR+ with ER+ and those that were ER-PR+ but TR- that did not respond to tamoxifen. Approximately one half of our ER-PR- patients were TR- while only 8 patients were ER-PR-. We contend that specific TR binding is a prerequisite for response to the drug and that this assay may prove superior than classical receptor determination in the prescriptive prediction. Supported in part by a S A Medical Research Council Grant.
263  CALCIUM STATUS AND AGE-RELATED BONE LOSS IN WOMEN,
T.C. Aw, A. Cheang, W.M. Kwok, and S.S. Ratnam (Depts of Clinical
Chemistry and Obstetrics & Gynaecology, National University
Hospital, Singapore 0511) (Sponsor: T.C. Aw)

Despite luminant sunshine osteopetrosis is an important
clinical problem in this country. There is paucity of data
relating calcium status to bone mineral content (BMC) in the
tropics. BMC of the lumbar spine was determined in 132 normal
female Chinese volunteers by dual photon absorptiometry (Nово).
All subjects were free from any medication or disease (amnesia,
diabetes, gastrointestinal, liver, kidney, and thyroid disorders)
that could affect skeletal integrity. Calcium status assessed by
measuring serum calcium (Ca), ionised calcium (Ca++) , alkaline
phosphatase (AP), phosphorus (P), mid-molecule PTH (Pth-M),
Immunonuclear Corp.), and urinary Ca & P excretion. Ca and Ca++
were determined by ion-selective electrodes (Nova 7).

AGE (n) BMC Ca (mg/dL) Ca++ (mg/dL) PTH (pg/mL)
22-39 (61) 0.932 ± 0.096 2.28 ± 0.16 1.19 ± 0.08 86.2 ± 19.0
40-49 (32) 0.833 ± 0.083 2.26 ± 0.11 1.21 ± 0.06 81.6 ± 40.2
50-59 (32) 0.746 ± 0.131 2.26 ± 0.15 1.22 ± 0.07 63.2 ± 21.5
60-70 (27) 0.694 ± 0.124 2.29 ± 0.12 1.23 ± 0.08 83.7 ± 67.0

Women between 30-39 yr were noted to have maximum BMC (0.933 +
0.105 g/mcg). The mean BMC in the immediate premenopausal
period (40-49 yr) was only 0.6 times lower than maximal values,
in contrast to 20-22% for women between 50-59 y and 25.6% for the 60-
70 age group. This underscores the importance of estrogenic
status in the pathogenesis of bone loss in the women studied.
No significant differences were found in renal creatinine, calcium or
phosphorus excretion between the various age groups. The normal serum Ca and Ca++ argue against deficiency of calcium or
vitamin D in the subjects studied. However, it is likely that
elevations of PTH with age probably contributes to the process of
age-related bone loss.

Wednesday Afternoon—July 27

Poster Session 1:00pm–3:00pm

HORMONES

Thyroid Hormones

266  ELISA® HOMOGENEOUS ENZYME IMMUNOASSAY FOR MEASUREMENT
OF SERUM OR PLASMA THYROIDSTIMULATING HORMONE IN THE CONRAS
BIO ASSAY ANALYZER,
Sydney Leeder, H. Sharps, K. Schultze, C. Rekemper, R. Bellet
TSTVA COMPANY, Palo Alto, CA 94304 (Sponsor: Neal Bellet).

We have developed an ELISA assay for the measurement of serum
or plasma TSH (T4) using the Cobas Bio Analyzer. The
ELISA assay, based on a T4-labeled G-6-PDH conjugate system, uses
three reagents. A 40 ul sample is treated with 100 ul of
pretreatment to release protein-bound T4. Then an 18 ul aliquot
of the pretreated sample is mixed with 170 ul of a T4 antibody
working reagent (with enzyme substrates). After a 1-minute
delay, a 50 ul working reagent containing the T4-enzyme
substrate is added. The T4-labeled enzyme competes with sample T4
for antibody binding sites. Unbound conjugate reacts with
HRP, and the reaction is then measured. The concentration of T4 in
the sample is proportional to the concentration of T4 in the sample.

The assay was performed on the Cobas Bio and the following
parameters were established:

Within-run CV's are 4.36 at 3.04 ug/dL, 0.03% at 7.86 ug/dL,
and 3.49% at 16.5 ug/dL. Between-run CV's on samples and controls
are < 6.9% from 2 to 5 ug/dL, < 4.3% from 5 to 12 ug/dL, and < 6.9% from
12 to 20 ug/dL. Results from comparative analysis studies on
patient samples between ELISA and Corting Magic assays were:

The results indicate this assay offers an accurate, precise,
and convenient method to measure serum or plasma T4. Furthermore,
the simplicity of the protocol and the applications of these
reagents to many other general chemistry analyzers.

267  FREE THYROID STIMULATING HORMONE ESTIMATION BY ENHANCED
LUMINESCENCE IMMUNEASSAY,
F.A. Bonini, G. Bandi, M. Portillo, E. Cassar & M. Marone
(Istituto scientifico S. Raffaele, Laboratorio Centrale, Milan,
Italy) (Sponsor: G. Bandi).

The level of free thyroid (FT4), especially associated with
thyrotropin, is considered a good index of the functional status
of thyroid. Radioimmunoassay is almost exclusively the technique
used for FT4 routine measurement in serum. Despite its wide
universally accepted reliability, some analytical problems exist when
"analyzing" assays are used, whereas the "two-step" assays are in-
creasingly time-consuming. We evaluated a new non-isotopic immu-
nochemical technology, based on an enhanced luminescence immuno-
metric assay, developed by Amersham for FT4, and performed with
the AmeRite system. After one-hour incubation of serum, in a micro-
plate with an immobilised monoclonal antibody against FT4,
a horseradish peroxidase-conjugated antibody is added, and the light
emitted from the enzyme-catalysed oxidation of luminol, appropri-
ately enhanced by a special reagent, is read in the AmeRite analy-
zer. The precision of the assay was evaluated on three pools of
routine sera. The within-run and between-run precisions follows:

38.2 ± 0.69 ng/mL (n=10), 3.3 ± 1.04 ng/mL (n=10) and 4.32 ± 5.15
ng/mL (n=10). The between run precision (five replicates for ten
days) presented CV's of 5.95, 4.4 and 8.2 respectively. The system
was linear to 5 ng/mL. The recovery was 95% at 5.3 ng/mL and 93% at
1.5 ng/mL. The minimal detectable dose was 0.19 ng/mL. The Amer-
rite FT4 correlated well with a commercial direct RIA assay (E lect-
on Dickinson):

n=132 r=0.9 slope=0.899 intercept = -0.0024

We tested the Amersite assay to determine FT4 levels in various
patients populations. FT4 levels in young and premenopausal
patients were clearly separated from euthyroid levels, also
in children (n=92) and in third trimester pregnancy (n=15). In
our experience, the FT4 Amersite, due to quite good analytical
performances, the reduced volume of sample and cost and time
saving, can be recommended for the routine use.

ABSTRACT WITHDRAWN

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988
A RAPID, SENSITIVE ASSAY FOR HUMAN THYROID-STIMULATING HORMONE (TSH) ON THE ABBOTT IMMUNOASSAY SYSTEM. Charles H. Kellar, W. Novotny, B. Ramp, J. Hoeschele, and J. Abbott and Abbott Laboratories, Abbott Park, IL 60064 (Spec.: M. L. Safford)

We have developed a sensitive, fully automated microparticle capture enzyme immunoassay (MEIA) for determination of TSH in human serum or plasma. The assay, configured as an automated two step "sandwich" ELISA on the Abbott IMx, utilizes monoclonal antibody-coated microparticles as an antigen capture phase and an alkaline phosphatase-antibody conjugate, each specific for different antigenic determinants on the TSH molecule. Assay time is 30 minutes for a full carousel (24 samples), and less than 20 minutes for 1 sample. Standardization was against WHO 80/558 TSH. Sample volume is 100 ul; dynamic range 0 to 100 UI/mL. The normal range was 0.56 to 5.98 UI/mL, with a mean of 1.85 UI/mL (n=50). Inter assay % coefficients of variation (CVs) were 8.7% ± 1.3% (n=15 runs over 9 days) and 5.9% ± 1.8% (n=15 runs over 17 days), read off a stored standard curve. Recovery on spiking WHO TSH into a normal human serum pool was 96.4 ± 10.4% in the range of 0.1 to 22 UI/mL. Recovery on serial dilution of a 70 UI/mL TSH sample to 2.2 UI/mL was 96.6 ± 10.2%. No cross-reactivity to hCG was observed at 200000 UI/mL. HCG. Sensitivity was < 0.1 UI/mL with a detection limit of 0.2 ng/mL. Normal values were 0.09 to 1.0 ng/mL. The assay incorporates the microparticle capture and fluorescence readout features of the IMx immunoassay system, offers the advantages of significant time and labor reduction.

A FULLY AUTOMATED FPIA 74 ASSAY FOR THE ABBOTT IMX.

Donald R. Balfour, Richard Frank, Richard Bahling, and Priscilla Buchanan (Abbott Laboratories, Abbott Diagnostics Division, Abbott Park, IL 60064) (Spec.: S. Hoyvat)

The new Abbott IMx Analyzer has the capability of performing Microparticle Capture Enzyme Immunoassays (MEIA) as well as the Fast Analyzer Fluorescence Polarization Immunoassays (FPIA) in a manner similar to that of the Abbott IMx Fluorescence Polarization Analyzer. The IMx 74 assay requires no pretreatment and shows excellent agreement with the IMx 74 PLUS assay and EIA assay on patient serum and plasma samples. This study demonstrates the feasibility of the IMx assay on the new IMx Analyzer.

All performance parameters for the IMx 74 Plus were retained in this new IMx 74 assay. Excellent sensitivity was found at 0.9 pg/mL. Reproducibility experiments yielded a mean of 95% with a range of 3% to 5% and recovery was 104% ± 3% with the new assay. Correlation performed with hyper- and hypothyroid specimens versus the IMx assay was excellent with r = 0.96, Y intercept = 0.46, and a slope of 1.00. The assay has been in clinical use for a minimum of two weeks. In summary, the IMx 74 assay performs as well as the IMx 74 Plus assay and will allow the IMx user a broader range of assay capability.

ADAPTATION AND EVALUATION OF CEDIA THYROID ON THE COULTER DASSO CHEMISTRY ANALYZER. F. Lucas, S. Ho (Coulter Diagnostics Division, Hialeah, FL 33014) (Spec.: A. Kansas)

The Closed Enzyme Donor Immunoassay (CEDIA) (CEDIA is a trademark of Microgenics, Inc., Concord, CA) for the quantitative measurement of thyroid utilizes a new concept in immunoassay. By recombinant DNA techniques, the common enzyme B-galactosidase has been split into two totally inactive polypeptide fragments. The two fragments can spontaneously recombine to yield the catalytically active enzyme. The enzyme can then be measured by using an antibody attached to one fragment and when specific thyroid hormone binds, it will inhibit the spontaneous reassembly. Thyroid hormone is measured by the assay of a substrate. Adaptation and evaluation of the CEDIA Thyroid to DASSO Chemistry Analyzer provides STAT serum thyroid hormone assay. Within run precision represents single samples run 31 times.

Performance

Precision

<table>
<thead>
<tr>
<th>n</th>
<th>5.73</th>
<th>0.93</th>
<th>17.24</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>0.59</td>
<td>0.93</td>
<td>4.32</td>
</tr>
<tr>
<td>CV %</td>
<td>10.29</td>
<td>9.59</td>
<td>2.63</td>
</tr>
</tbody>
</table>

In conclusion, the application of CEDIA Thyroid Assay demonstrates results that compare favorably with established methods.

PERFORMANCE OF THE CEDIA THYROID 74 SC ASSAY ON THE HITACHI 740. William A. Cox, Jagdish Sahai, Terry Morres, Larry Lee and Pyare Khana (Microgenics Corporation, Concord, CA 94520) (Spec.: Roger Islam)

The CEDIA Total T4 Assay has been adopted for use in conjunction with the Hitachi 740 clinical analyzer for the determination of T4 levels in serum. The CEDIA 740 method utilizes two inactive fragments of the enzyme B-galactosidase, termed Enzyme Acceptor (EA) and Enzyme Donor (ED), which can spontaneously recombine to form active enzyme. A single T4 molecule is covalently attached to each ED molecule so that binding of anti-T4 antibody inhibits the formation of active B-galactosidase. The CEDIA T4 Assay contains two reagents: an EA Reagent containing anti-T4 antibody and a reagent containing T4 with serum thyroid-binding proteins, and an ED Reagent containing the substrate o-nitrophenyl-8-D-galactopyranosyl (ONPG). Each reagent contains a corresponding co-factor buffer. The T4 SC Assay contains an additional buffer needed to prepare a working solution of ED Reagent for application to the Hitachi 740. The CEDIA 74 SC Assay is performed in a fully-automated manner as follows: A serum specimen (calibrator, control or patient sample) is added to the EA Reagent and incubated for 5 min at 37°C. The ED Reagent is then added, and the amount of active B-galactosidase is measured by the rate of ONPG hydrolysis at 415 nm during the time interval between 4 and 5 min after the addition of ED Reagent. The T4 concentration in the sample is linearly proportional to the amount of B-galactosidase formed, and is determined automatically by comparison with the rates obtained using 0.0 and 20.0 pg/mL T4 Calibrator supplied. Forty-four samples are assayed within 26 min after picking up the first specimen. Performance of the CEDIA 74 SC Assay was evaluated on the Hitachi 740: the following results were obtained:

- Inter assay coefficients of variation (n = 20) were 3.3% at 7.7 pg/mL, 1.5% at 11.2 pg/mL, and 2.3% at 15.0 pg/mL.
- The mean sensitivity (least detectable dose, five determinations) was 0.55 pg/mL. Correlation of patient sample values obtained by single-point measurement with the CEDIA 74 SC Assay to results obtained with a T4 EIA method produced a slope of 1.00, an intercept of -0.06 pg/mL, a correlation coefficient of 0.999 and an SEE value of 1.0 pg/mL, using a least-squares regression analysis.

In conclusion, the preliminary results indicate that the CEDIA 74 SC Assay can be adapted to other clinical analyzers, including the Hitachi 746 and 737. Thus the CEDIA 74 SC Assay is a rapid and effective method for the automated measurement of T4 concentrations in human serum.

EVALUATION OF THE CEDIA THYROID 74 AND T4-PTCA EASEY ASSAYS ON THE LL MONARCH ANALYZER. I. Mally, W. Kohnlein, M. Fialk, Pyare Khana (Microgenics Corporation, Concord, CA 94520) and Carolyn Covington (Instrumentation Laboratory, San Jose, CA 95131) (Spec.: Pyare L. Khana)
We have evaluated the CEDIA™ homogeneous enzyme assays for both T4 and T7 Uptake on the T4 assay, the amount of T4 in the patient sample competes with T4-ED conjugates for a limited number of binding sites. Therefore, the assay T4 concentration is directly proportional to the amount of T7 Uptake assay exhibits linearity from 20% to 80% uptake. The following precision data has been obtained:

<table>
<thead>
<tr>
<th>T4</th>
<th>T-Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ug/ml)</td>
<td>3.64</td>
</tr>
<tr>
<td>S.D. (ug/ml)</td>
<td>0.25</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>8.60</td>
</tr>
<tr>
<td>N</td>
<td>29</td>
</tr>
</tbody>
</table>

The following correlation values were calculated using Clinical Assays T4 and T-Uptake RIA as the reference method:

T4 CEDIA™ = 0.93 (RIA) + 0.76; r = 0.955; Syx = 0.79; n = 122
T-Uptake CEDIA™ = 0.94 (RIA) + 2.1; r = 0.984; Syx = 0.73; n = 130

The above data demonstrates the reliability of the CEDIA™ T4 and T-Uptake assays. In addition, the serum pretreatment and the ease of the operating the CEDIA™ makes these methods convenient as well.

APPLICATION AND EVALUATION OF CEDIA™ T4 AND T-UPTAKE TO THE ROCHE COBAS MBA™, Lori Hollander, Walt Kleinach, Keith Young, Jay Mellick, Mike Scalci, Braden Lynch, (Microgenics Corporation, Concord, California 94518) (Spons: Jeffrey E. Shidell)

CEDIA™ T4 and T-Uptake Assays have been applied to the COBAS MBA™ Chemistry Analyzer (Roch Diagnostics). Both assays are based on recombinant DNA techniques that provide homogeneous assays to test for thyroid hormone levels in the serum. The T4 assay uses digoxin-specific antibodies as the enzyme substrate and the T-Uptake assay uses digoxin antibodies that recognize binding sites in the T4 assay. The T4 assay shows linear correlation in the range of 20% to 80% uptake. In T4, the curve shows linearity to 20 ug/ml. The following intra-assay precision data was obtained:

<table>
<thead>
<tr>
<th>T4</th>
<th>T-Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ug/ml)</td>
<td>5.00</td>
</tr>
<tr>
<td>S.D. (ug/ml)</td>
<td>0.64</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>9.07</td>
</tr>
<tr>
<td>N</td>
<td>25</td>
</tr>
</tbody>
</table>

Correlation of both assays on the CEDIA™ to COBAS BIOC® gave the following results:

T4: CEDIA™ = 0.98 (BIOC) - 0.50; r = 0.94; Syx = 0.75; n = 50
T-U: BIOC™ = 0.97 (BIOC) + 1.26; r = 0.988; Syx = 1.16; n = 77

The ease of operation of the CEDIA™ and the fact that serum pretreatment is necessary for the CEDIA™ T4 and T-Uptake assays, makes these applications both convenient and reliable.

PERFORMANCE OF THE CEDIA™ T4 ASSAY ON THE COBAS BIOC®, Michael A. Zoccoli, Beverly S. McCalla, Stephen B. Friedman, Wayne B Manning and Pyrr Khanna (Microgenics Corporation, Concord, Ca 94520) (Spons: Kim Vallerius)

The CEDIA™ T4 method utilizes two bio-engineered inactive fragments of the enzyme B-galactosidase, a large fragment (EA) and a smaller polypeptide (ED). These two fragments will spontaneously recombine into active enzyme. T4 is covalently attached to the ED without affecting the ability of the fragment to recombine with EA to form active enzyme. However, binding of a specific T4 antibody will inhibit the reassociation of the EA and ED fragments, thereby regulating the amount of enzyme formed.

The CEDIA™ T4 Assay contains two lyophilized reagents: an EA Reagent containing anti-T4 antibody and a releasing agent to dissociate complexes of T4 and serum thyroid-binding proteins, and an ED Reagent containing the substrate, o-nitrophenyl-p-D-galactopyranoside (ONPG). Each reagent has a corresponding reconstitution buffer. The CEDIA™ T4 Assay is performed in a fully-automated manner as follows: Seven microliters of a serum specimen (calibrator, control or patient sample) followed by 18 ug of T4 is mixed with 190 ug or EA Reagent, and incubated for 10 sec at 37°. The ED Reagent (15 ml of 20 ul of diluent) is added. The amount of active B-galactosidase formed by recombination of EA and ED-4T4 complexes is determined by measuring the rate of ONPG hydrolysis at 420 nm during the time interval between 6 and 8 min after the addition of ED Reagent. The T4 concentration in the sample is linearly proportional to the amount of B-galactosidase formed, and is determined automatically by comparison with the rates obtained using the supplied 0.0, 10.0, and 20.0 ug/ml T4 Calibration. Twenty-eight samples can be analyzed within 20 min after pipeting of the first sample. Performance of the CEDIA™ T4 Assay was evaluated on the COBAS BIOC®; the following results were obtained: Intra-assay coefficients of variation (n = 28) were 7.6% at 4.8 ug/ml, 4.6% at 10.4 ug/ml, and 3.0% at 17.2 ug/ml. The mean sensitivity (least detectable dose; three determinations) was 0.93 ug/ml. Correlation of patient sample values obtained by single-point measurement with the CEDIA™ T4 Assay to results obtained with a T4 RIA method produced a slope of 1.04, an intercept of 0.55 ug/ml, a correlation coefficient of 0.98, and a S.E.E. value of 0.98, using a least-squares regression analysis. Thus the CEDIA™ T4 Assay is a rapid and effective method for the automated measurement of T4 concentrations in human serum.

PERFORMANCE OF THE CEDIA™ DIGOXIN SC ASSAY ON THE HITACH™ 704, William A. Coty, Jagdish Saini, Raj Gambir, Patrick Lyn, Lori Briggs, Stanley Friedman and Pyrr Khanna (Microgenics Corporation, Concord, CA 94520) (Spons: James Love)

We have developed a homogeneous Closed Enzyme Donor Immunoassay (CEDIA™) for measurement of digoxin levels in serum which can be used in conjunction with the Hitachi™ 704 automated clinical analyzer. In the CEDIA™ Digoxin SC method, the enzyme B-galactosidase has been split into two inactive fragments, a large fragment (EA) and a smaller polypeptide (ED), which can spontaneously recombine to form active enzyme. A single digoxin molecule is covalently attached to each ED molecule so that binding by anti-digoxin antibodies inhibits the reassociation of EA and ED fragments. The CEDIA™ Digoxin SC assay is performed by first combining a serum specimen with an enzyme reagents in the Hitachi™ 704 and the Digoxin SC (IgG) as detected by a brief (3 min at 37° or 2 min at room temperature) incubation to allow binding of digoxin in the specimen to the antibody. The instrument then adds the sample-antibody mixture and a substrate containing EA and the substrate, chlorophenol red-D-galactopyranoside (CPRG) to the reaction cuvettes, followed by a solution containing EA. The reaction is mixed and incubated at 37° C for the rate of CPRG hydrolysis in present of 570 nm during the time interval of 4 to 5 min after the addition of EA. The digoxin concentration in the sample is linearly proportional to the amount of B-galactosidase formed, and is determined automatically by comparison with 0.0 and 4.0 ng/ml. Digoxin Calibrators supplied. Forty-four samples can be analyzed within 26 min after addition of the first sample-antibody mixture. Performance of three independent lots of reagent was evaluated on the Hitachi™ 704; the following results were obtained: Intra-assay coefficients of variation (n = 20; three determinations per lot) were 7.3% to 14.1% at 0.7 ng/mL, 3.2% to 6.0% at 1.8 ng/mL and 2.7% to 5.5% at 3.0 ng/mL. The sensitivity (least detectable dose; three determinations per lot) was 0.16 ng/ml. Intra-assay coefficients of variation were 0.21 single-point determinations on separate runs for each lot were 10.6% to 13.7% at 0.7 ng/mL, 6.3% to 8.8% at 1.8 ng/mL, and 4.3% to 7.3% at 3.0 ng/mL. Linearity and recovery studies produced results within ±10% or 0.2 mg/mL of the expected concentration.

Correlation of patient sample values obtained by single-point measurement with the CEDIA™ Digoxin SC Assay to results obtained with a digoxin RIA method produced a slope of 1.03 to 1.07, intercepts within the least detectable dose, correlation coefficients of 0.954 to 0.967 and S.E.E. values of 0.22 to 0.25 ng/mL using a least-squares regression analysis. In addition, preliminary results indicate that the CEDIA™ Digoxin SC Assay could be useful for clinical laboratories, including the Hitachi™ 705. Thus the CEDIA™ Digoxin SC Assay is a rapid and effective method for the automated measurement of digoxin concentration in human serum.

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Interfering substances were found among those tested. The Tm
T-Uptake assay performs as well as the TSX T-Uptake assay and,
when used in conjunction with Tm T4, will provide an easy and
accurate estimate of thyroid function.

A FULLY AUTOMATED ASSAY FOR TOTAL T3 UTILIZING
THE ABBOTT IMX ANALYZER, N.R. Groesbeck, S. Hau and L. Sohn
(ABBOTT Laboratories, North Chicago, IL 60064) (Spons.: C. Leskowicz)

A fully automated nonpretreatment assay for total T3
(Triiodothyronine) has been developed for the Abbott IMX
Analyzer. Human serum calibrators (0-8 ng/ml), goat anti-T3
coated microparticles, N-acetyl T3 coupled to alkaline
phosphatase and the substrate methylumbelliferyl phosphate
are utilized in the assay.

In the first step, the incubation of sample, particles and
displacing agent results in the binding of endogenous T3 to
the coated particles. Following transfer of the reaction
mixture to the glass fiber matrix and washing, conjugate is
applied to the matrix. After a 4 minute incubation, the matrix
is washed, methylumbelliferyl phosphate applied and the rate of
formation of methylumbelliflurone measured. Measured rates are
separately related to the T3 concentration in the sample.
Total assay time ranges from 23 minutes for a single sample to
37 minutes for a full carousel (24 samples). All steps and
calculations are carried out automatically by the Analyzer.
Curve storage is valid for at least two weeks.
Sensitivity of the assay is 0.16 ng/ml, average recovery is
105% and crossreactivity with T4 is less than 0.1%. Evaluated
versus Abbott T3 HABRAD, the assay had a slope of 0.90, an
intercept of 0.07, and a correlation coefficient of 0.98.

The assay is simple to perform and can be used for the routine immunoassay of total T4 in serum in clinical laboratories.

A Fluorescence assay for sensitive TSH measurement has been
developed to assess the thyroid function status of a variety of
patients with or without thyroidal conditions. The totally
automated assay is based on a sandwich phase sandwich immunoassay
and pulsed fluorescence technology.

The IMPULSE® reagents for TSH and samples are placed in the
instrument. The TSH assay configuration is selected and the
instrument is initiated. The 200 lL serum sample is pipetted
into a monoclonal antibody coated reaction vessel and incubated.
Unbound components are removed, the vessel is washed, and
fluorescent labeled polyclonal antibody is added. The vessel is
washed again and the bound components are solubilized. Bound
fluorescent labeled TSH is measured for each sample and the
concentration obtained from a calibration curve.

The IMPULSE® reagents are stable for one year, all in liquid
formulations, and the calibration curve can be stored for use for the
life of the kit. The instrument offers complete automation with
calletting, washing, reading, calculating and tabulating.

The quantitation of serum TSH is linear over the concentration
range of 0.1 to 100 mIU/L and the assay sensitivity is
0.04 mIU/L with a 95% confidence of detection from the mean of
the zero standards. The average recovery of spiked TSH from
several serum specimens is 101.5% ± 10.6%.

A HIGHLY SENSITIVE FLUOROMETRIC ENZYME
IMMUNOAASSAY FOR THYROTROPIN (TSH)
ASSAYS, Deborah K. Vicker, A. D. Mansfield, S. Y. T. H. E. P. A.
Dumouline, and C. L. V. O. M. D. W. A. D. J. S. W. D. H. (Spons.: W. K. Miller)

A highly sensitive two-site enzyme immunoassay for thyrotropin
(TSH) has been developed. This immunometric assay employs two
monoclonal antibodies specific for TSH. Du Pont's chromium dioxide
diamagnetic particles provide the solid support for the capture antibody,
which is specific for the intact TSH molecule. The detection antibody,
which is specific to the TSH beta subunit, is conjugated to a high
glycine alkaline phosphatase enzyme.

Serum (25 lL), antibody-enzyme conjugate (200 lL) and antibody-
coupled magnetic particles (35 lL) are sequentially incubated for a total
of 70 minutes. Three sequential washes remove excess conjugate from
the magnetic particles, which are then incubated at 37 °C for 5 min.
with 4-methylumbelliferyl phosphate in diethanolamine buffer. EDTA is

We have developed a new non-isotopic immunoassay for total thyroxine
(T4) in serum. A T4-bovine immunoglobulin conjugate, prepared by the
carboximide method, was used to non-covalently coat white microtiter
wells. In the assay, 10 lL of serum are mixed with 100 lL of a monoclonal
biotinylated antibody and incubated for 2h at 37°C. Serum T4, released
from binding proteins with trypsin treatment, combines with immobilized T4
for binding to the biotinylated antibody. After washing, the degree of antibody
binding which is inversely proportional to the T4 concentration in the sample,
is quantified by adding streptavidin labeled with the europium chelate BCPDA-
(4-7-bis(chlorosulfophenyl)-1,2.9-triazole) in the presence of excess Eu³⁺.
After 30 min, the wells are washed and dried and the fluorescence of the complex:
globulin-T4-antibody-biotin-streptavidin-BCPDA-Eu³⁺ is measured on the solid-phase after
pulsed excitation at 357 nm. In a specially designed time-resolved fluorometer
which also performs the data reduction. The assay characteristics are as
follows: dynamic range 1 - 20 pg/lL; detection limit 0.7 pg/lL; within-run precision
3.5 - 5.0%; day-to-day precision 6.0-8.0%; recovery ranged from
87 to 114% with a standard deviation of ± 2.8%. This assay is entirely
free of a number of thyroxine metabolites and drugs was negligible.
The correlation with an established radioimmunoassay procedure was good:
(Y sliders) =
2.27 + 1.02 x (RIA), r = 0.94, n = 100. This assay is simple to perform
and can be used for the routine immunoassay of total T4 in serum in clinical laboratories.

A NON-ISOTOPIC TOTAL THYROIDINE IMMUNOAASSAY WITH
TIME-RESOLVED FLUORESCENCE AND A MONOCOCLANAL ANTIBODY.
A. Pavlus, M. V. B. M. G. E. D. M. (Spons.: Y. Ten)

A non-isotopic total thyroid hormone immunoassay
with time-resolved fluorescence and a monoclonal antibody.

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used to quench the enzyme reaction. The particles are magnetically separated and the absorbance of the supernatant is measured.

The specificity and high sensitivity of this assay (0.1 μM/mL) effectively distinguishes hyperthyroid and euthyroid populations. The analytical range of the assay is linear from 0.1 to 60 μM/mL, with intra-assay variation within 5% CV. The lower limit of detection was 10 μM, and correlation was observed with the Abbott RIABEAD® assay (r=0.99± 0.14; n=97; r=0.77). The mean analytical recovery for ten serum samples was 98.2% (range 92.8 to 103.7%). Excellent parallelism with the standard curve was demonstrated by diluting each of five serum samples (tHSH = 30 μM/mL) with zero calibrator.

In summary, this non-isotopic assay is rapid, sensitive, and specific for tHSH for thyroid function testing.

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**FLUOROMETRIC ENZYME IMMUNOASSAY FOR THE MEASUREMENT OF TOTAL THYROID (T4) AND THYROID UPTAKE (TU) USING CHROMIUM DIOXIDE MAGNETIC PARTICLES.**


Heterogeneous competitive fluorometric enzyme immunoassays have been developed for the measurement of total thyroxine (T4) and triiodothyronine (T3) in human serum. In the Tu assay, the 

**IMPULSE® reagents for T4, and samples are placed in the instrument. The Tu assay configuration is selected and the instrument is initiated. The 10 μL serum sample and fluorescent reagent are pipetted into the reaction well. The competitive binding reaction takes place between the labeled and unbound components. The unbound components are removed, the vessel washed, and the bound components solubilized. The bound fluorescent labeled Tu is measured for each sample and the concentration obtained from a calibration curve.

The IMPULSE reagents are stable for one year, all in liquid formulations, and the calibration curve can be stored for use for the life of the kit. The instrument offers complete automation with pipetting, washing, reading, calculating, and tabulating.

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**AUTOMATED FLUORESCENCE IMMUNOASSAY FOR SERUM THYROID (T4), THYROXINE (T3), AND FREE THYRROXINE (FT4): TECHNIQUES AND INSTRUMENTATION.**

Heffelfinger, J. D. (Diagnostic Products Corp., Dept. Path. and Lab. Med., Jewish Hospital of St. Louis, St. Louis, MO 63110). (Sponsor: D. Sacks)

TSH assays are promoted as a primary test for the diagnosis of thyroid dysfunction. This study reports the specificity of a sensitive TSH assay (Diagnostik, RIA method) and a non-sensitive RIA method (Diagnostic Products, RIA method). Patients were randomly selected from samples submitted for TSH or TSH measurements. Assessment of thyroid status was based on review of patients' charts and consultation with endocrinologists. A TSH assay was used for the detection of thyroid dysfunction and the RIA technique does not have a defined range for hyperthyroidism.

Clinically euthyroid patients (n=124) showed a broad distribution for both TSH procedures (0.39 to 13 μU/mL; 11 TSH and 19 DP s). Classifying euthyroid patients using a normal FT4 did not alter the TSH distribution.

Clinically hyperthyroid patients (n=11) had TSH values below the reference range greater than values of 0.1 to 11 Status and 0.24 μU/mL DP. TSH results correlated well with the clinical assessment of hyperthyroidism.

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**FREE THYROID CAN BE ESTIMATED FROM AUTOMATED IMMUNOASSAY FUNCTION TESTS.**


We have developed a method for deriving an "estimated free thyroxin" (EFT), from automated fluorescence polarization thyroid (T4) and T-Uptake (TU) assays (T4U, Abbott Labs.). The results of this study were compared to T47/100ml calibration with thyroxin binding globulin (TBG) as measured by radioimmunoassay, 55TU = 0.91 ± 0.16 (TBG/x100mL) + 10.6, r² = 0.94, N = 55. From this relationship the total serum T4 binding capacity and that 55TU represents the binding capacity of TBG as well as other proteins such as thyroxin binding prealbumin (TBP) and thyroxin binding globulin (TBG). According to the mass action equation: T4 + TBP = T4 + TBP, where T4 is T4 binding protein, estimated free T4 (EFT) = [T4] / [TBP] (TBP) / [TBP], where [TBP] is the equilibrium unbound T4 capacity. As approximately

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**AUTOMATED FLUORESCENCE IMMUNOASSAY FOR SERUM THYROID (T4), THYROXINE (T3), AND FREE THYRROXINE (FT4): TECHNIQUES AND INSTRUMENTATION.**

Heffelfinger, J. D. (Diagnostic Products Corp., Dept. Path. and Lab. Med., Jewish Hospital of St. Louis, St. Louis, MO 63110). (Sponsor: D. Sacks)

TSH assays are promoted as a primary test for the diagnosis of thyroid dysfunction. This study reports the specificity of a sensitive TSH assay (Diagnostik, RIA method) and a non-sensitive RIA method (Diagnostic Products, RIA method). Patients were randomly selected from samples submitted for TSH or TSH measurements. Assessment of thyroid status was based on review of patients' charts and consultation with endocrinologists. A TSH assay was used for the detection of thyroid dysfunction and the RIA technique does not have a defined range for hyperthyroidism.

Clinically euthyroid patients (n=124) showed a broad distribution for both TSH procedures (0.39 to 13 μU/mL; 11 TSH and 19 DP s). Classifying euthyroid patients using a normal FT4 did not alter the TSH distribution.

Clinically hyperthyroid patients (n=11) had TSH values below the reference range greater than values of 0.1 to 11 Status and 0.24 μU/mL DP. TSH results correlated well with the clinical assessment of hyperthyroidism.

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**FREE THYROID CAN BE ESTIMATED FROM AUTOMATED IMMUNOASSAY FUNCTION TESTS.**


We have developed a method for deriving an "estimated free thyroxin" (EFT), from automated fluorescence polarization thyroid (T4) and T-Uptake (TU) assays (T4U, Abbott Labs.). The results of this study were compared to T47/100ml calibration with thyroxin binding globulin (TBG) as measured by radioimmunoassay, 55TU = 0.91 ± 0.16 (TBG/x100mL) + 10.6, r² = 0.94, N = 55. From this relationship the total serum T4 binding capacity and that 55TU represents the binding capacity of TBG as well as other proteins such as thyroxin binding prealbumin (TBP) and thyroxin binding globulin (TBG). According to the mass action equation: T4 + TBP = T4 + TBP, where T4 is T4 binding protein, estimated free T4 (EFT) = [T4] / [TBP] (TBP) / [TBP], where [TBP] is the equilibrium unbound T4 capacity. As approximately
Comparison of TDX vs radioactive T4 and T7 levels showed excellent agreement as evidenced by the mean correlation coefficients of 0.917 and 0.924, respectively. This compares favorably with the mean of 0.982 and 0.934 for T4 and T7 when the two radiometric methods were compared to each other. Thus, we conclude that laboratory thyroid status assessments of the 228 patients on the Abbott TDX should agree closely with levels obtained on the two traditional radiometric methods examined.

In the liquid-phase monochromic TMAK kit evaluated (Diagnostic Products Corporation, Los Angeles, CA), the precision involving the concentration of exogenous T3R complexes proceeds with liquid-phase kinetics in a ligand-coated tube. This is followed by the addition of a bridging agent which reacts with the complexes inactivating the ligand-coated on the tube. The assay requires 700 µl of serum sample and can be completed in about four hours.

Precision, accuracy, and parallelism of this kit were evaluated and found to be within acceptable limits. The minimum detection limit was found to be 0.05 µIU/ml. The reference range (95% coverage with 90% confidence, assuming a log normal distribution) determined from 22 healthy subjects was 0.45–5.39 with a mean value of 1.55 ±1/µIU/ml. Comparison of the values obtained with this kit (T) and with a Serono kit (X) gave a regression equation of Y = 0.55X + 0.20 (n = 60, r = 0.993). The serum T3R levels were below the lower limit of the reference range in all twelve patients with hyperthyroidism and in twenty thyroid cancer patients on suppressive T3 therapy. The results of the present study would favor use of the liquid-phase TMAK kit for the analysis of T3R complexes on four patients with thyrotoxicosis and one patient on a suppressive dose of T3 for benign colloid nodules. Their basal T3R levels were all lower than the normal range, which would be expected for these patients with thyrotoxicosis.

The results of this evaluation indicate that this liquid-phase TMAK kit can be a sensitive diagnostic adjunct for thyroid disorders.

**Comparison of TDX vs Radioactive T4 and T7 Levels**

Comparison of TDX vs radioactive T4 and T7 levels showed excellent agreement as evidenced by the mean correlation coefficients of 0.917 and 0.924, respectively. This compares favorably with the mean of 0.982 and 0.934 for T4 and T7 when the two radiometric methods were compared to each other. Thus, we conclude that laboratory thyroid status assessments of the 228 patients on the Abbott TDX should agree closely with levels obtained on the two traditional radiometric methods examined.
To assess the clinical usefulness of sensitive TSH assays for patients undergoing thyroid hormone therapy, we performed TSH on the following groups using an Echolon® TSH assay (BIO-RAD): Healthy controls (CTU), euthyroid patients with no thyroid dysfunction (EU), hyperthyroid patients receiving thyroid hormone replacement therapy designated as euthyroid (RX-E) or hyperthyroid (RX-H) on the basis of their serum TSH (T) concentrations.

GROUP  TSH (mU/L)  T4 (ug/dL)
CTU  12  2.3±1.3 (0.8-3.1)  6.9±1.7 (5.5-12.3)
EU  33  1.8±0.9 (1.0-4.9)  8.4±2.6 (5.5-11.8)
RX+H  8  1±0.3 (0.0-1.0)  10.0±1.8 (7.1-12.3)
RX-  11  0±0.2 (0.0-1.0)  16.2±2.4 (12.8-22.3)

We observed a significant difference in TSH values between Groups EU and HP (p<0.0001), demonstrating the ability of this assay to discriminate among euthyroid states. We observed a moderate increase of TSH values in Group RX-H when compared to Group EU (p<0.005), and a striking decrease of TSH values in Group RX-E when compared to Group EU (p<0.0005). It is noteworthy that although patients in Group RX-E are judged "euthyroid" by conventional T4 testing, their TSH secretion is generally suppressed.

We conclude that compared to the conventional thyroid function tests, sensitive TSH assay may be a more alternative alternative as reference point in the optimization of dose regimens for patients on thyroid hormone replacement therapy.

294 EVALUATION OF HOMOGENOUS ENZYME IMMUNOASSAY FOR T4 UPTAKE ON COBAS-BIO CENTRIFUGAL ANALYZER, Thomas Lohmann, K. Echler (Ochsner Foundation Hospital, New Orleans, LA 70112) (Spon. T. Lohmann, M.D.)

A homogeneous enzyme immunoassay for T4, T-uptake, and calculation of free thyroid index, using a popular automated centrifugal analyzer, the Roche COBAS-BIO, was evaluated. Serum samples of 108 patients were run by RIA (New Organon Teknika) and EIA (Microgenics CEDIA). The correlation coefficient was between samples from inpatient and outpatient services and included hypothyroid, euthyroid, and hyperthyroid patients of both sexes. Fasting prior to venipuncture was not required and samples with mild degrees of hemolysis, icterus, or lipemia were not included.

The CEDIA method utilizes β-galactosidase present as two inactive fragments, an enzyme donor and an enzyme receptor, which can recombine to form active enzyme. Thyroxine is attached to the enzyme-donor, and will bind to anti-thyroxine in the T4 assay or to thyroxine binding proteins in the T4-uptake assay. There is competitive binding between thyroxine in the assay and thyroxine from the serum sample. Resultant enzyme activity is directly proportional to the patient's T4 or inversely proportional to the T-uptake, and is measured by the rate of ONPG hydrolysis at 420nm and 37°C.

For T4 the correlation coefficient was 0.94 with a slope of 1.03 and intercept of -0.28. T-uptake gave a coefficient of 0.98, slope of 0.94 and intercept of -2.92; and FTI correlation coefficient of 0.94, slope 0.95, and intercept of 0.32. Using dilutions of a high patient with 0.004bility, the linearity of the method as stated by the manufacturer was confirmed as 3.5 to 20 mg/dL for T4. Interassay precision (n=10) for T4 is 7.6% CV at 13.0 mg/dL and 11.7% CV at 5.6 mg/dL, and for T-uptake is 2.25% CV at 42.1% uptake and 2.6% CV at 44.3% uptake.

In summary, the CEDIA method for T4, T-uptake, and FTI calculation works well on the Roche COBAS-BIO with good correlation to an RIA assay on a nonselective set of patient samples.


Thyroxine binding of serum proteins was measured using a method described by T. Roberts and N. Holub (Clin Chem 15:367-375, 1969). Serum samples were incubated with [125I]-thyroxine and cold thyroxine (1000 μg/L) to saturate the thyroxine binding globulin (TBG), then electrophoresed in the cold on 5% polyacrylamide gels using a 0.1 M Tris-borate buffer. The rods were sliced and the radioactivity in each disc was measured using a scintillation counter. Three peaks were found in normal serum corresponding to TBG, thyroxine-binding prealbumin (TBP), and thyroxine-binding albumin (TBA). The binding of thyroxine to each protein was calculated by multiplying the fraction of counts in each peak times 1000 μg/L. The CVs of the measurements were 9.3% for TBG, 8.2% for albumin and 1.5% for TBP.

Normal subjects (n=100) had thyroxine bindings of 176 ± 7 μg/mL (mean ± SD) to TBG, 225 ± 11 μg/mL to TBP, and 396 ± 108 μg/mL to TBA. Twenty euthyroid patients (11 males and 9 females) with elevated thyroxine concentrations of 12±29 μg/mL had increased binding to TBP and correspondingly less binding to TBP (thyroxine bindings of 180 ± 39 μg/mL to TBG, 469 ± 83 μg/mL to albumin and 350 ± 75 μg/mL to TBA.) The total albumin concentrations were determined in these patients and the 14 patients having triliodothyronine measurements all had values within the normal range. Two other euthyroid patients with elevated thyroxine concentrations showed differential binding to TBP and TBA.

We describe a radial partition immunoassay for serum triiodothyronine (T3) saturating dose for use on the Stratus system. Antibody is preimmobilized onto glass-fiber paper tabs. An alkaline phosphatase T3 conjugate is used as label. The conjugate was prepared by covalently linking T3 to a neoplastic alkaline phosphatase via an active ester T3 intermediate. The assay is based on the competitive protein binding principle. Interference by the thyroxine binding proteins is eliminated by addition of 8-methyl-1-naphthalene sulfonic acid (8-MNSA), which free hormone binds to the antibody binding sites on the coated tab. After a 2-minute incubation, enzyme-labeled T3 is applied to the sample antibody reaction area. The enzyme labeled T3 binds to the unconjugated antibody binding sites. After a 3-minute incubation, unbound enzyme labeled T3 is washed away from the field of view by the application of a wash solution to the center of the reaction zone. A substrate for the enzyme label is incorporated into the wash solution so that the enzyme reaction is initiated simultaneously with the wash step. The reaction rate of the bound fraction is measured by front surface fluorometry with the observed rates being inversely proportional to the T3 concentration. A set of serum controls containing T3 at three different levels gave coefficients of variation of 8.5%, 6.4% and 3.8% for the hypothyroid, normal and hyper controls, respectively. A comparison of values obtained using the assay samples using the Stratus system and a commercially available RIA procedure yielded the following least squares equation: T3 = -0.2 + 0.99 x, r = 0.98, T3 values were spiked at four different levels. Recoveries range from 90% to 103% with an average recovery of 98%. The optimum sensitivity of the assay is in the range of 0.7 to 8 μg/mL T3.

297 CHELATIONMENT IMMUNOASSAY FOR FREE-THYROID WITH ACIDIDINUM-ESTER-LABELED T4 IMMUNOLOBULIN CONJUGATE. M. Boumaud, J.T. Boumaud, P. Regn (Laboratoire de Biophysique Cellulaire, Service de Medicine Nuclaire, Hopital Jean Bernard, BP 577, 69061 Poitiers Cedex, France).

A new chelationmetric assay of free thyroxine (FT4) involves a T4-immunoglobulin conjugate labeled with acididinum ester ("Magic Lite System," Giroux Corning Diagnostics Corp.). The assay is rapid, with one incubation of 1 hour, requires two standards per run, takes 10 s per sample for the quantification step.

Intra-assay precision at three different FT4 concentrations (6, 30, and 30 pmol/L) yield results of 4.1, 7.6 and 3.5% respectively. Inter-assay precision for the three control levels were 12.5, 8.5 and 2.5 respectively. Hemolysis, bilirubin and lipemia had no significant effect. Addition of either 5% cholesterol to plasma sample, to simulate hepatic injury, showed significant FT4 increasing from 1 pmol/L. Results obtained with the "Magic Lite" system (x) were compared with values obtained with the Scivio assay (y), x = 1.25 + 8.625. The correlation coefficient was 0.96 and n = 43.

The FT4 distribution was log-normal in the reference euthyroid population (n=40). 95% of the values were between 12.5 and 25 μg/L (mean±SD). 125 patients were treated with thyroid hormone and were compared to 125 control patients with normal FT4 values. The FT4 was performed with data from the reference population, and 81 hyperthyroid and 46 hypothyroid subjects without treatment, showed a significant cut-off points at 12.5 pmol/L and 25 pmol/L. The
method was also tested with euthyroid subjects who had biological parameters (hypercalcemia, hyper T3G or receiving therapeutic substances (heparin, amiodarone) which can modify the FT4 diagnostic value; results well agree with those obtained by Sclavo.

We conclude that the "Magic Lite System" is efficient and reliable for use in routine measurement of FT4.

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ABSTRACT WITHDRAWN

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ABSTRACT WITHDRAWN

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ABSTRACT WITHDRAWN

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301 COMPARISON OF FREE THYROID MEASUREMENT BY CHELOLUMINESCENCE AND EQUILIBRIUM DIALYSIS, S. Thomas Bigos, J. Maclean, and B. Butler (Div. Endocrinology, Maine Medical Center, Portland, Me. 04102 (Spon.: K. Provoost)

It is desirable in many clinical laboratories to minimize use of radioactive reagents. To address this issue with respect to free thyroid (FT4) assays a preliminary study compared FT4 measurements by equilibrium dialysis (E.D.) with a cheloluminescence immunoassay (C.I.) using acridinium ester-labeled T4 conjugate (Magic Lite FT4 Immunoassay; Ciba-Corning Immunodiagnostics). C.I. is a competitive protein binding system requiring 25 ul sample site simultaneously incubated with 100 ul labelled T4 and 500 ul of solution containing T4-antibody coupled to paramagnetic particles incubated 60 min. at room temperature; bound labelled T4 is separated by a magnetic separator, samples are washed and precipitated, decanted and quantitatively assessed for photon emission. Estimated assay sensitivity was 0.06 ng/dL. 239 sera were grouped and analyzed as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>FT4 (ng/dL)</th>
<th>C.I. (ng/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Hyperthy</td>
<td>4.1</td>
<td>2.7-9.9</td>
</tr>
<tr>
<td>Hypothy</td>
<td>29</td>
<td>0.4-2.0</td>
</tr>
<tr>
<td>Pregnant</td>
<td>10</td>
<td>1.7-2.4</td>
</tr>
<tr>
<td>Estrogen Rx</td>
<td>13</td>
<td>1.8-3.0</td>
</tr>
</tbody>
</table>

Summary: In this study measurement of FT4 by C.I. and E.D. appear to give comparable results. E.D. is more obviously affected by circulating heparin than C.I.

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AN AUTOMATED NON-ISOTOPIC IMMUNOASSAY FOR THE DETERMINATION OF TOTAL THYROID UPTAKE USING THE AFFINITY(TM). D.N. Spencer, P.B. Simenson, G.H. Krauth, T.R. Witty. (Becton Dickinson, Towson, Maryland 21204) (Sponsor Thomas R. Witty).

The AFFINITY(TM) is a benchtop, totally automated, random access immunochemistry analyzer utilizing coated tube technology and utilized reagent packaging (Immunit(TM)). The T Thyroid Uptake assay requires that 50 ul of serum be placed in the Immunit(TM). The AFFINITY(TM) dispenses the serum into the antibody coated tube along with a dilution of horseradish peroxidase coupled to a thyroid analog. The 1st incubation is 15 minutes at 37C. The tube is washed, substrates added and the solution incubated an additional 10 minutes. The reaction is stopped and the amount of color generated is measured photometrically. The AFFINITY(TM) then calculates the % Uptake value compared to the stored (for at least two weeks) reference value.

The % Thyroid Uptake assay is not impacted by physiological or therapeutic levels of Phenotoin, Salicylic acid, Intravenous medication observed over a range of total 20-40% T4 uptake was 3X (N=20). Inter-assay precision monitored over a two week period utilizing a single calibration at time zero was 4X over the same range (N=15). The AFFINITY(TM) free thyroid index, calculated using a commercial thyroid assay, correlated well with an established procedure: slope = 1.1, intercept = -1.85, and correlation coefficient = 0.95 on 201 clinical samples.

Thus, the AFFINITY(TM) % Thyroid Uptake assay provides a rapid, precise and accurate assessment of thyroid status.

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AN AUTOMATED, NON-ISOTOPIC ENZYME IMMUNOASSAY FOR THE DETERMINATION OF TOTAL SERUM THYROXINE, M.C. Elliott, G.H. Krauth. (Becton Dickinson, Towson, Maryland 21204) (Spon: N. Schneider).

A competitive coated tube enzyme immunoassay for the determination of serum thyroxine has been adapted to Becton Dickinson's AFFINITY(TM) System. This system is a benchtop, totally automated device for the performance of in vitro diagnostic testing. Each assay is contained in bar code specific packaging, ready-to-use on the system. The thyroxine assay requires that a minimum of 30microliters of sample be dispensed into the specific package (Immunit(TM), patent 4608331) which also contains a specific anti-thyroxine antibody adsorbed to a 10mm polystyrene tube. The AFFINITY(TM) precisely dispenses sample and thyroxine coupled to horseradish peroxidase to the
coated tube to initiate the competition. After the primary incubation, the mixture is aspirated, the tube washed and reagents added for color development. Color development is stopped and then quantitated directly in the coated tube by the AFFINITY(TM) Total processing time is 30 minutes.

Intra-assay precision observed over a range of 3.0-20 ug/dl was 7.3% (N=10). Inter-assay precision monitored over a 3 week period utilizing a single calibration curve at time zero to 8X over the same range of values (N=7). The sensitivity of the assay is 0.25 ug/dl and shows less than 10% cross reactivity with triiodothyronine (T3) and reverse triiodothyronine (rT3).

Many pituitary hormones (e.g. GH, ACTH) are known to show diurnal rhythms, however there have been contradictory reports about the 24-hour pattern of serum TSH in normal subjects. We have studied the daily variation in serum TSH, triiodothyronine (T3), and thyroxine (T4) and their relationship with the daily variation in cortisol concentration.

The normal subjects studied (n = 11; age range 19 to 46 years) were clinically euthyroid. Blood samples were collected four-hourly for a period of 24 hours. Serum TSH was measured by a sensitive immunoradiometric method and a radioimmunooassay was employed for the measurement of T4, T3 and cortisol. The mean serum levels decreased from 0800 hours to 1600 hours, being at lowest level at 1600 hours. There was a progressive rise of mean TSH concentration that began at 2000 hours and reached a maximum value at 0200 hours. The mean TSH concentration at 1600 hours and 0200 hours were significantly different. While comparing the changes in TSH concentrations with that of cortisol levels, the mean cortisol levels were lowest at 2400 hours, with a progressive rise at 0200 hours, peaking at 0800 hours. The rise in serum TSH preceded the rise in cortisol. Reversal of the normal serum TSH and cortisol secretion pattern was observed in one normal male subject who had been working the night shift for 3 weeks prior to blood sampling. There were no significant changes in the mean serum T4, T3 values over the 24-hour period.

Our observations indicate that there is an existence of a circadian rhythm for serum TSH in normal subjects. These results seem to strengthen the hypothesis that the thyroid hormones, at least T4 and T3, do not play an important role in this regulation.
Most cortisol immunoassays generate similar results and show good correlation with respect to serum cortisol measurement. However, they tend to show large variations in estimating urinary free cortisol (UFC) levels. To assess the validity of UFC measurement by fluorescence polarization immunoassay (FPIA), we measured UFC by FPIA (Abbott Diagnostics), RIA (Micromedic Systems, Inc.) and HPLC (Clin. Chem. 28:2418-2420, 1982) in a panel of 60 urine specimens, which included samples selected from carefully selected normal individuals. The samples (100 μl aliquot for UFC and RIA and 2 ml aliquot for HPLC) were extracted with methanol, derivatized with fluorescamine and analyzed by the above techniques. The interaction assays for two specimens were in the range of 8 and 9 with FPIA and IOX with RIA and 1.8 and 3.4 with HPLC. The mean normal values obtained with two procedures were 30 ± 2 (S.D. 5.0) μg/hr for RIA and 33 (S.D.) μg/hr for HPLC. UFC correlated almost linearly with RIA and correlated closely to FPIA (r = 0.56, slope 1.06). The correlation coefficient (r) for HPLC and TDX was 0.94 (slope 0.97) and for HPLC and RIA was 0.91 (slope 2.4). Although RIA gave values almost twice as high as FPIA for most specimens, the two procedures showed significant correlation (r = 0.88, slope 2.25). Our data suggest that RIA over estimates UFC possibly due to cross reactive substances and that such interference seems to be minimal in FPIA. Also a high degree of correlation between FPIA and HPLC confirms the validity of UFC measurement by the FPIA.


We have developed a fluorometric enzyme immunoassay for cortisol, using a monoclonal antibody-alkaline phosphatase conjugate (75 μl). After a 30 minute incubation at 37 °C, the particles are magnetically separated and washed three times. A substrate (450 μl of 4-methylumbelliferyl phosphate in diethanolamine buffer) is added and incubated at 37 °C for 8 minutes; then the reaction is quenched with EDTA. The supernatant is measured fluorometrically.

The assay's analytical range is from 0.4 to 60 μg/dL with the sensitivity determined using a non-paired Student's t-test (95% confidence) comparing fluorescent intensity of 0 vs. 0.4 μg/dL calibrators. Within-run precision (n = 20) is 8.2% CV at 7.5 μg/dL and 6.7% CV at 22.5 μg/dL.

In summary, this non-isotopic cortisol assay is rapid and simple to run, and exhibits excellent performance comparable to commercial assays.

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We have developed a new immunnoassay of cortisol using time-resolved fluorescence and the immobilized antigen approach. Thyroglobulin-cortisol conjugate, prepared with the mixed anhydride method, was immobilized by adsorption on white opaque microtiter wells. In the assay, immobilized cortisol competes with cortisol in the sample (20 μL) for binding to a biotinylated monoclonal antibody (100 μL), for 1 h at 37 °C. After washing out the unbound species, the amount of bound antibody, which is inversely proportional to the cortisol concentration in the sample is quantified by adding streptavidin labeled with the europium chelate 4,7-bis-(6-aminohexyl)-1,10-phenanthroline-2,9-dihydroxy acid (BCPDPA-Eu3+) in the presence of excess Eu3+. After incubation for 30 min at 37 °C, the wells are washed and dried and the fluorescence of the solid phase consisting of thyroglobulin-cortisol-antibody-biotin-streptavidin-BCPDPA-Eu3+ is measured by time-resolved fluorescence on a specially designed...
analyser. The method is simple to perform and rapid (~100 samples in 100 min). The performance characteristics were as follows: Dynamic range 1 - 50 µg/dl. Detection limit ≤ 1 µg/dl; within-run precision of 4 - 6%; day-to-day precision of 7 - 9%. Recovery ranged from 78 to 119% (mean 99.3 ± 11.9%). Major corticosteroids (53%), prednisolone (40%), prednisone (30%), corticosterone (30%) and 11-deoxy cortisol (12%). The comparison with two established radioimmunoassay procedures gave the following equations: y(TR-FIA) = 0.203 + 0.976 X (DPC, RIA); r = 0.96; n = 9 and y(TR-FIA) = 0.318 + 1.000 X (Dade, RIA); r = 0.95; n = 65. We propose that the method is suitable for routine use in the clinical laboratory.

313 MEASUREMENT OF CORTISOL WITH A NON-RADIOACTIVE RADIOIMMUNOASSAY BASED ON ENHANCED LUMINESCENCE, J. K. Martin, G. Brett, W. Thompson, M. Ramaker, T. Macareese-Tamison (Amersham International plc, Amersham, Bucks, UK) (Spon.: D. Enright)

The measurement of cortisol is an established aid in determining the integrity of the hypothalamic-pituitary-adrenal (HPA) axis. The Amersham cortisol assay has been developed for the in vitro quantitative measurement of total cortisol using a competitive assay technique with an end-point detection based on enhanced luminescence. 50% of serum, plasma or urine samples were incubated with 11H labelled cortisol and 11H labelled cortisol-RSA conjugate and 10H% of sheep anti-cortisol antisera. The wells are incubated for 30 minutes at 37°C with shaking on the Amersham Shaker/incubator. After washing the wells, signal reagent is added and the luminescent signal is measured in the Amersham™ Analyzer.

The assay range is 0 - 1700nmol/l with a typical sensitivity of 30nmol/l. The coefficient of variation for serum samples across the range 44 - 800nmol/l is ≤ 7% within assay (n=12) and 9% between assay (n=10). The corresponding figures for urine samples are ≤ 9% (within assay) and ≤ 12% (between assay) across the range 45 - 1700nmol/l. Cross-reactivity to 11-deoxy cortisol and cortisone is ≤ 3%. Excellent correlation is obtained with GC-MS (aloe = 1.005, r = 0.975) and discrimination between clinical states is supported with the use of dynamic tests. Mean serum cortisol in normal subjects (n=20) was 450±190nmol/l at 9am and 190±30nmol/l at 6pm. Free cortisol ranged from 34 to 229nmol/l in normals (n=17).

In conclusion, the Amersham cortisol assay is fast, reliable and convenient. The sensitivity, precision, specificity and close agreement to GC/MS of the assay make it suitable for routine laboratory use.


We have developed a fully automated HPLC system using a bovine serum albumin-coated ODS short column (BSA-ODS column, TSK) that excludes serum proteins but absorbs small molecular hydrophobic substances, an ODS column as an analytical column and a column switching technique for determination of serum cortisol.

Serum (300 µl) was directly injected onto a precolumn (BSA-ODS, 20 µm, TSK: θ ≈ 4.6 x 10 mm) by an autosampler (AS-8000, TSK). Samples trapped on a precolumn with 0.01% trifluoroacetic acid were removed with 40% methanol in pH 7.4 phosphate buffer with a step-wise elution program (PT-8000, TSK), and transferred to an analytical column (ODS-50 Tm, 5 mm, TSK: θ ≈ 7.5 x 50 x 0.75 x 50 mm) with 60% methanol in pH 7.4 phosphate buffer with a column-switching technique (PT-8000, TSK). Cortisol was monitored at 245 nm (UV-8000, TSK) and its concentration was calculated by an integrator (CP-8000, TSK).

The retention time of cortisol was 8.95 min. Good separation of cortisol from other glucocorticoids except for prednisolone was obtained. The standard curve, which was plotted from the cortisol spiked control serum, showed linearity between 20 - 200 µg/dl. In the precise test, intra-day and inter-day reproducibility was 3.4 and 3.4%, respectively (n = 10). Recovery was about 100 %.

In summary, this fully automated HPLC system for serum cortisol using a BSA-ODS precolumn allows direct injection of serum, rapid analysis of cortisol under 10 min per specimen and produces reliable data.

Catecholamines

315 INTERLABORATORY COMPARISON OF THE DETERMINATION OF VMA AND HVA IN URINE: HPLC/ECD VS. COMBINED GAS CHROMATOGRAPHY MASS FRAGMENTOGRAPHY

N. Hengen, R. Wörndl (Recipe Pharma, 8000 Munich); K. Jacob, B. Kempfer, M. Kneidel (Inst. Klinische Chemie, Klinikum Grosshadern, Universität, 8000 Munich, FRG). (Spon.: N. Hengen)

Vanillylmandelic acid (VMA) and homovanillic acid (HVA) are measured for the clinical diagnosis of pheochromocytoma and neuroblastoma. To demonstrate the accuracy and specificity of a new reversed-phase liquid chromatographic method (HPLC) with electrochemical detection (ECD) (Proceed. Königstein Chromat. Tage 9, 155 (1987)) two laboratories performed determinations of VMA and HVA on 20 urine samples of patients. The methods used were: an isotope-dilution gas chromatographic mass fragmentographic (GC/MS) assay in one laboratory and HPLC applying the Recipe chemicals kit in the other one. The latter method uses simultaneous solid phase extraction, sample preparation and uniform HPLC conditions for both substances. The measured range was 1 - 300 mg/l (5 - 1500 µmol/l) and 1 - 1200 nmol/l, respectively. The correlation coefficients of the GC/ MS versus the HPLC method were 0.994 (r = 0.999x + 1.07) and 0.995 (y = 1.003x - 0.04) for VMA and HVA, respectively.

In conclusion this study shows excellent agreement of the GC/MS vs. HPLC/ECD method and a good quality control in both laboratories.


The need for and the optimal temperature and/or preservation procedures for 24-hour urine collections for catecholamines (CAT) are not clearly documented. In order to compare these procedures, a split sample (cold vs room temperature) multi-armed (GN HCl (0), EDTA-Na2HPO4 (O), post-collection HCl (P), Al preservative) study was performed. The optimal protocol was performed on urine collected from 11 surgical/anaesthesia patients during the acute phase of illness. Using electrochemical detection methods, we performed CAT analysis on these samples within one week of collection using both cold- and room-temperature collection (CC) methods. Repeatability was obtained: *meant as % of collection (N=11)

<table>
<thead>
<tr>
<th>DOPAMINE</th>
<th>NEUROPEPTIDE</th>
<th>NOREPINEPHRINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>RF</td>
<td>CE</td>
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<tr>
<td>IECD E</td>
<td>429±348</td>
<td>432±372</td>
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<tr>
<td>H</td>
<td>423±348</td>
<td>433±342</td>
</tr>
<tr>
<td>PH</td>
<td>448±466</td>
<td>447±475</td>
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<tr>
<td>NP</td>
<td>448±455</td>
<td>446±461</td>
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<tr>
<td>ROOM E</td>
<td>422±369</td>
<td>402±330</td>
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<tr>
<td>TEMP E</td>
<td>420±167</td>
<td>419±328</td>
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<tr>
<td>PH</td>
<td>439±465</td>
<td>437±471</td>
</tr>
<tr>
<td>NP</td>
<td>442±460</td>
<td>449±466</td>
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<tr>
<td>P value (treatment)</td>
<td>0.87</td>
<td>0.838</td>
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<tr>
<td>P value (analytical)</td>
<td>0.995</td>
<td>0.339</td>
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Using factorial ANOVA, no statistically significant differences were found between CATs in urine collected on ice or room temperature, nor between the analytical techniques (RF vs CE). Among the four arms studied *only in epinephrine by RF, were results identical (10%) which may not be sufficient to establish a relevant clinical difference. Results do not show combination of methods in this study using CE would be applicable for collection of 24-hour urine samples for CAT analysis.

317 NEW BUFFER FOR EXTRACTION OF URINARY CATECHOLAMINES WITH CATION-EXCHANGE RESIN, Mary Bibb E McEachern and Steven R. Binder (Clinical HPLC Group, Bio-Rad Labs., Hercules, CA 94547) (Spon.: Mary McEachern)

The measurement of urinary catecholamines in clinical laboratories is useful in the differential diagnosis of pheochromocytoma. New protocols use Bio-Rad's Bio-Rex 7x8 column for extraction of noradrenaline and epinephrine from acid-diffused urine, followed by elution with formic acid and analysis of the TMA derivatives. Initial dilution of the 5 ml sample with 15 ml 0.1ς sodium EDTA and

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988 1217
Due have conditions drugs throughout 3.5 switch VMA/HVA
94547)
dl), 3 acetic methylsilyltrimfluoracetamide
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ANP ARE NOT INVOLVED IN THE NATRIURETIC AND DIURETIC EFFECT OF DOPAMINE IN HUMANS


The administration of Dopamine (DA) to experimental animals and human subjects determines a potent natriuretic and diuretic effect. It is believed that DA determines its renal effects by increasing the renal plasma flow and the glomerular filtration rate, and by inhibiting the tubular transport of sodium. DA exerts a chronotropic effect on the heart enhancing norepinephrine release from cardiac sympathetic nerve terminals. Mammalian atrial cardies contains a group of peptides called, Atrial Natriuretic Peptides (ANP), with potent diuretic and natriuretic activities. The release of ANP seems to be related to blood volume expansion, arterial stretch and tachycardia. It is postulated that cardiac sympathetic nerves may be involved in the release of ANP. Aim of this study was to evaluate whether in human or animal-injected natriuresis is related, at least in part, to an increase of ANP secretion. Eight normal men, aging from 23 to 28 years, were chosen for this study. The subjects, in the fasting state, received an infusion of DA (hydrochloride REVIVAN®, Sime, Italy - 2 μg/Kg bw/min) dissolved in 100 ml of 5% dextrose aqueous solution in 1h. Blood samples were drawn at -60, 0, 30, 60 and 90 min. To ensure adequate urine flow, all subjects received 200 ml of distilled water every 60 min. ANP plasma levels were measured by radioimmunoassay (after a Sep-Pak extraction). Urine samples were collected at -60, 0, 60 and 120 min. DA infusion did not change systolic and diastolic blood pressures, but significantly increased heart rate (+15%, p<0.05). DA infusion caused a marked natriuresis and diuresis, and significantly reduced the ANP plasma levels at time 30, 60 and 90 min. (p<0.05). Our data indicate that diuresis and natriuresis due to pharmacological doses of DA are not mediated by ANP. To clarify the significance of plasma ANP reduction after DA administration further investigations are required.

EVALUATION OF A COST EFFECTIVE CATION EXCHANGE HPLC METHOD FOR CLINICAL URINARY CATECHOLAMINE ANALYSIS


One of the major problems of using urinary catecholamines in clinical practice has been the relatively high cost of the assay. This cost is due in part to the multi-step labor intensive alumina extraction procedure which is necessary prior to separation by HPLC on C18 reverse-phase (RP) columns. Recently, disposable cathodic columns, prepacked with Bio-Rex70, have been released for preparation of samples prior to analysis on silica based weak cation exchange (CE) columns (BIO-RAD, Richmond, CA). Using electrochemical detection methods for both analyses, we compared this new device/column concept to the C18 RP column. The following results were obtained for dopamine (DOP), epinephrine (EP), and norepinephrine (NORE) from human 24-hour urine collections.

<table>
<thead>
<tr>
<th>Samples (μl)</th>
<th>DOP</th>
<th>EP</th>
<th>NOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1.86</td>
<td>1.88</td>
<td>0.98</td>
</tr>
<tr>
<td>Coefficient of Corr. (r)</td>
<td>0.994</td>
<td>0.992</td>
<td>0.98</td>
</tr>
<tr>
<td>Significance (p)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</table>

Recovery range is typically 85-90%. We believe the accuracy and precision of CE to be comparable or better than the RP method and based upon its lower cost per analysis: the dollars savings for 284 samples was $2,384 it could be used in the clinical field as a cost efficient analysis for urinary catecholamines.

hCG

A microparticle capture immun assay for the measurement of human chorionic gonadotropin (hCG) in serum has been developed for the Abbott IMx analyzer. The assay consists of a monocalonal anti-hCG coated latex microparticle and a polyclonal anti-hCG alkaline phosphatase conjugate in a sequential one-step format. The product of the alkaline phosphatase catalysis, 4-methylumbelliferone, is detected and quantitated with the IMx analyzer. Each assay requires 125 μl of sample and specifically detects intact hCG with a dynamic range of 2 - 1000 mIU/ml (1st IFP). Within-run and between-run precision was 5-8%, with sensitivity of less than 2 mIU/ml. Crossreactivity with LH, FSH, and hTSH was 2.0%, 0.1%, and 1.1%, respectively. hCG concentrations of 0 - 9.3 mIU/ml were determined for post-menopausal sera (n=25). LH level, 1.2 - 8.5 mIU/ml for hyperthyroid samples (n=17). hTSH level, 32-353 uIU/ml. Recovery of hCG spiked into a human serum containing 50.2 mIU/ml hCG ranged from 94.1% to 105.6%. Triglycerides (1000 mg/dl), hemoglobin (750 mg/dl), and bilirubin (50 mg/dl) did not interfere in the quantitation of hCG. Patient samples (n=17) with hCG concentrations ranging from 2.7 - 998 mIU/ml exhibited the following correlation with a commercial enzyme immunoassay (r =0.984; slope = 0.94; intercept = 14.1). The IMx analyzer can process 24 specimens per batch with the ability to repel the hCG concentration of 5 specimens in less than 15 minutes.


A highly sensitive two-site enzyme immunoassay for hCG has been developed. This immunoassay employs two monoclonal antibodies specific for hCG. Du Pont's chromium dioxide magnetic particles provide the solid support for the capture antibody, which is specific for the alpha subunit of hCG. The detection antibody, which is specific to the hCG beta subunit, is conjugated to colloidal alkaline phosphatase enzyme.

Serum (100μl), antibody-enzyme conjugate (50 μl) and antibody-coated magnetic particles (50 μl) are simultaneously incubated at 37 °C for 30 min. Three sequential washings removes excess conjugate from the magnetic particles, which are then incubated at 37 °C for 5 min. with 1,4- dianisylbenzenesulfonic acid disodium bisulphate buffer. EDTA is used to quench the enzyme reaction. The particles are magnetically separated and the fluorescence of the supernatant is measured.

The analytical range of the assay is linear from 1 to 200 μIU/ml, with intra-assay precision of <2% CV between 5 and 200 μIU/ml. Excellent parallelism with the standard curve was demonstrated by diluting each of five serum samples (hCG >100 μIU/ml) with zero calibrator.

In summary, this non-isotopic assay is rapid and very sensitive to intact hCG, making it suitable for pregnancy screening or ectopic pregnancy testing.

USE OF MONOCOLONAL-POLYCfree (WOLLYTH) sandwich enzyme immunoassay in microtiter wells for quantitating hCG levels in serum samples, Brenda R. Iw, J. Fu, F.-H. Chang, and M. Wu (United Biotech Inc. 3000 Spacepark Way, Mountain View, CA 94043) (Spon.: Dynetech Corp., Redwood City)

To study the feasibility of using monoclonal-polyclonal sandwich enzyme immunoassay in microtiter wells to determine hCG levels in serum, we studied the performance characteristics and correlations between a monoclonal-monoclonal pair and a monoclonal-polyclonal pair. Both pairings use same solid phase monoclonal antibody which was immobilized on Dynatech Immunol I removawell strips. Enzyme conjugates were prepared by conjugating purified IgG with 111m Ag oxidized horseradish peroxidase. The assay procedure involves (1) incubation of hCG standard (hCG5/537) or samples and enzyme conjugate in coated wells for 30 min. at 24 °C, (2) wash. (3) color development with hydrogen peroxide and tetramethylbenzidine for 10 min. (4) stop reaction with ZnCl2 and read O.D absorption.

Some performance characteristics were compared and listed as below: (Abbrev. M, for monoclonal; P, for polyclonal)

<table>
<thead>
<tr>
<th>Recovery (%)</th>
<th>M-NONO</th>
<th>M-POLY (WOLLYTH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraassay C.V.</td>
<td>7%</td>
<td>8%</td>
</tr>
<tr>
<td>Intraassay C.V.</td>
<td>7%</td>
<td>8%</td>
</tr>
<tr>
<td>Recovery</td>
<td>3 mIU/mL</td>
<td>3 mIU/mL</td>
</tr>
<tr>
<td>Cross-reactivity</td>
<td>mIU/mL</td>
<td></td>
</tr>
<tr>
<td>hLH</td>
<td>0.7%</td>
<td></td>
</tr>
<tr>
<td>FSH, mIU/mL</td>
<td>1.3%</td>
<td></td>
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</tbody>
</table>
| HCG correlation (r) | 0.986

C119

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988
In conclusion, DEAE-purified IgG fraction of the given polyclonal anti-HCG serum can be used to pair with the immobilized monoclonal antibody in microtiter wells to determine serum HCG levels.

We describe a new "sandwich" type non-isotopic immunoassay for the determination of HCG in serum. In the assay, HCG is captured by a beta-subunit specific monoclonal antibody which is immobilized in a microtiter plate well. A three-site sandwich is then created by the addition of two biotinylated monoclonal antibodies specific for different epitopes on the HCG molecule. The degree of binding of the biotinylated antibodies, which is proportional to the amount of HCG present in the sample, is determined by a bridge reaction with a streptavidin-based universal detection reagent. This reagent contains (a) streptavidin covalently linked to 350 ng/ml aBCDPA labeled thyroglobulin and (b) excess Eu(ll). BCPDA [4, 7-dioxabicyclo[2.2.1]hept-2-ene-2-carboxylic acid, perchloroethyl] is a Eu(ll) chelator. The fluorescence of the final complex formed (monoclonal-antibody-HCG-monomoclonal antibodies-biotin-streptavidin-thyroglobulin-BCPDA-Eu(ll)) is measured on the dried solid surface by excitation at 337 nm with a nitrogen laser, and monitoring the emission at 615 nm in a specially designed gated fluorometer working in a time-resolved mode. The assay procedure requires a total incubation time of 90 minutes. Semi-quantitative results can be obtained by using a more simplified and rapid protocol (20 min incubation time). The assay characteristics are as follows: dynamic range 1-5000 units/L; detection limit 1.0 units/L; within-run precision 4.3-6.1%; day-to-day precision 5.2-9.7%; recovery range from 92-105%; and cross-reactivity with no other hormones was negligible.

Results were in close agreement with those obtained with a commercially available immunoradiometric assay (r = 0.99, n = 64), and a time-resolved immunofluorometric procedure (r = 0.99, n = 74).

Comparative immunoassay of human chorionic gonadotropin (HCG) by time resolved fluorescence spectrophotometry, with a new europium chelator.

The assay features two protocols: a) 60 minute incubation for serum and plasma and b) 15 minute incubation for serum, plasma and urine. Preincubation labeling of the monoclonal antibody against HCG (100 ng/ml) was added to microtiter wells which were coated with a sheep polyclonal to HCG. 50μl of sample or standard is then added. The wells are incubated for 60 minutes (or 15 minutes) at 37°C with shaking using the Merlin(TM) Shaker/Incubator. After washing the wells, signal reagent is added and the luminescent signal is measured in the Merlin(TM) Analyzer.

The assay range is 0 to 1000 mIU/ml (1st IRP 75/573) with a typical sensitivity of < 1.0 mIU/ml (observed using either protocol). Cross-reaction to LHRH, FSH and HCG is < 1%. The coefficients of variation (using both protocols) for within assay precision (n=12), are 8% and 5% for the ranges 15 - 40 mIU/ml and 40 - 300 mIU/ml respectively. The coefficient of variation (using both protocols) for between assay precision (n=12) across the range 15 - 300 mIU/ml is < 10%. A comparison with an established RIA assay (Torontolab HCG) gave a correlation coefficient of 0.973 and a slope of 0.974 (1st IRP 75/573).

In conclusion, we have developed a rapid, sensitive and convenient solid phase immunoassay for the quantitative measurement of HCG in serum, plasma or urine. The assay has been found to be specific for HCG and is not cross-reactive with the other human glycoprotein hormones (LH, FSH and TSH). The method has a wide dynamic range (0-1000 mIU/ml) and is rapid and simple to perform.

Chorionic Gonadotrophin (HCG) that includes positive and negative controls. The colorimetric reagent consists of two monoclonal antibodies to different epitopes of HCG covalently attached to dyed sub-microcrystal particles. The assay procedure is succinct. First, the colorimetric reagent is added to urine in an immunoassay vessel. After approximately ten minutes, the urine-reagent mixture is drained through a membrane by means of the disposable device. This is followed by an agreed washing step (a positive reaction), then the color is observed. If HCG is not present (a negative reaction), then the colorimetric reagent passes through the membrane and no color is observed. Positive and negative controls are incorporated as references. The absence of color in the positive well indicates an invalid result. Color in the negative control indicates a contaminating substance. The assay is useful over the range of 50 to 300,000 mIU/ml. In a study of sixty-four patient samples in which 48.4% were from pregnant females, the assay had 100% sensitivity and 100% specificity. The pH of the urine ranged from 4.7 to B.O.
Rapid Urease Assay for Visual Detection of Human Pregnancy

G. J. McClune, N. S. Norkus, and H. C. Warren (Life Sciences Research Laboratories, Eastman Kodak Company, Rochester, NY 14650, Xianning Shih (Cetus Corporation, Emeryville, CA 94608) (Spons.: F. F. Mellis)

A rapid (less than 4 minutes), simple, enzyme immunoassay for urine human chorionic gonadotropin (hCG) was developed using two monoclonal antibodies to form a solution immunocomplex and an avidin-biotin capturing system. The addition of a unique dye reagent causes a colorless-to-red reaction to occur, catalyzed by the presence of a peroxidase enzyme in the immunocomplex. The test features built-in quality control verification of the reagents and procedure simultaneously with each sample being tested. In addition, considerable latitude exists in the reagent addition and the timing of each step. The test involves four simple reagent additions and two one-minute reagent incubations. The assay range is from 30 IU/L to 300,000 IU/L, and all components are room temperature stable for at least three months.

Patient specimen comparative data on 1056 urine samples, pregnancy tested in a multi-center evaluation including physician offices and clinical laboratory settings, demonstrated a sensitivity of 100% and a specificity of 100% (482 positive and 574 negative samples). To confirm specificity, 30 healthy males and 30 post-menopausal females were tested and demonstrated to be hCG negative. No major interferences including hTSH, hLH and hPRL were identified. To ascertain the limits of sensitivity, 20 normal, nonpregnant female urine samples were spiked with 10-70 mIU/mL. The test indicated that 30% of the 10 IU/L, 70% of the 20 IU/L, and 100% of the 30-70 IU/L were positive for hCG.

Miscellaneous Other Hormones

Radial Partition Immunoassay: Quantifying hFSH Levels in Human Serum or Plasma

Kathleen Leung, Samuel Dawson, James Rugg (Baxter Dade Division, P.O. Box 520672, Miami, FL 33152) (Sponsor: C. F.iss)

We describe a radial partition immunoassay for the determination of hFSH in serum or plasma. The clinical significance of hFSH measurement has been primarily the diagnosis of disorders of the hypothalamic-pituitary-gonadal axis. The assay is described for the automated Stratus® Fluorometric Enzyme Immunoassay System.

The assay is a "sandwich" type employing two monoclonal antibodies: one specific for the intact hFSH molecule and the other for the alpha subunit. The solid phase consists of immobilized antibody on glass fiber filter paper. An unlabeled sample of serum or plasma is first applied to the central "reaction zone" of the test allowing hFSH to bind. Then a buffered solution containing enzyme-labeled Fab' fragments of the second monoclonal antibody is applied, completing the "sandwich" formation. Finally, a wash buffer containing a fluorogenic substrate is applied, eluting unbound conjugate to the top periphery. Bound enzyme conjugate is quantified by measuring the rate of increase in fluorescence and varies directly with the hFSH concentration. Rates are compared to clinical units by comparison with a stored calibration curve.

The Stratus hFSH assay is sensitive over the range of 0.3 μIU/mL to 150 μIU/mL. Total coefficient of variation single measurements are 10% or less down to 1.5 μIU/mL. Recovered concentrations of high hFSH sera diluted with the calibrator base gave linear correlation coefficients of 0.99. No significant interference is seen by hLH up to 600 μIU/mL, hTSH up to 20,000 μIU/mL, and hCG up to 250 μIU/mL. Comparison of patient sample recoveries with those obtained with the Bio-Rad MAIA/1 method resulted in the following linear regression data:

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<tr>
<th>Method</th>
<th>Slope</th>
<th>Intercept</th>
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<tbody>
<tr>
<td>Bio-Rad</td>
<td>1.09</td>
<td>0.97</td>
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<tr>
<td>Stratus</td>
<td>1.00</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*Registered Trademark

A Rapid Automated Radial Partition Immunoassay for the Measurement of Human Lutropin

Kathleen Leung, Samuel R. Dawson, and James A. Rugg (Baxter Healthcare Corporation, Dade Division, P.O. Box 520672, Miami, FL 33152) (Sponsor: K. Leung)

Human Lutropin (hLH) is a glycoprotein hormone comprised of two polypeptide "subunits" alpha and beta. The beta subunit is noncovalently intermolecular forces. Measurement of hLH levels is important in assessing the integrity of the hypothalamic-anterior pituitary-gonadal axis. The assay was developed as a rapid, highly specific radial partition immunoassay for determination of hLH for use in the Stratus® Fluorometric Enzyme Immunoassay System. The assay employs monoclonal antibodies directed against different antigenic sites: one antibody recognizes the beta subunit; both samples are pipetted onto the center portion of a glass fiber paper where it reacts with immobilized monoclonal anti-hLH antibody and a two minute incubation, enzyme-labeled Fab' fragments of the second monoclonal anti-hLH antibody are applied, forming an antibody/antigen-labeled antibody sandwich. The unbound labeled antibody is radially eluted by applying a fluorogenic substrate wash solution to the center of the reaction zone. Initiation of enzyme activity occurs simultaneously with the elution of the bound fraction directly with the concentration of hLH present in the sample, is measured by front surface fluorometry.

The assay calibrators are standardized to the WHO 1st International Reference Preparation 68/40. The assay is calibrated to 200 mIU/mL and is sensitive to 0.3 mIU/mL. Inter- and intra-assay coefficients of correlation are below 10%. Recovery of hLH added to samples at levels between 4-160 mIU/mL range from 96-98%. The assay is specific for hLH and shows no significant cross-reactivity with hCG, hFSH, or human pregnancy and HCG, on 212 clinical samples comparing Stratus® and the Sarono MAIAClone® IRIA produced a correlation coefficient of 0.97 and a regression equation of y (Stratus) = 0.94 (MAIAClone) + 0.09.

*Registered Trademark

Identification of Progestrone and Cortisol as Immunoactive Plasma Digitalis Like Factors in Pregnancy

Linda Lopate, D. Brent, E. Johnson, and J. Johnson (Research Department, Dade Division, P.O. Box 520672, Miami, FL 33152) (Sponsor: C. Flass)

Increased levels of digitalis like factors (DLF) in pregnancy have been reported in association with hypertension, toxemia and premature labour. To further evaluate the nature of DLF in pregnancy, we studied 3 women late in the third trimester. DLF was measured in plasma to pit a comparison with that in non-pregnant women. DLF extraction on disposable C18 columns followed by radioimmunoassay using New England Nuclear reagents. Plasma values for immunoreactive DLF were 0.13, 0.13 and 0.40 ng digoxin equivalents/ml. Progesterone accounted for 34%, 35% and 1% of the total DLF in the three women respectively, and cortisol 41%, 18% and 7% based on HPLC analysis. Plasma dehydroepiandrosterone sulfate was also studied by HPLC and RIA and accounted for 5%, 17% and 9% of the total DLF. Both progesterone and cortisol displaced [125I]-digoxin from antibody and also [125I]-digoxin from hCG in the radioimmunoassay dependence on hormones. Progesterone, at 1000 nmol/L, gave a digoxin RIA value of 0.29 ng digoxin equivalents/ml and Na-K-ATPase inhibition of 1.0 ng digoxin equivalents/ml. Cortisol, at 6200 nmol/L, gave 0.14 and 1.1 ng digoxin equivalents/ml, respectively. Only progesterone in the plasma concentrations found in these patients (up to 0.5 ng/ml) and cortisol (up to 25 ng/ml) caused a significant inhibition of Na-K-ATPase. HPLC separation of the plasma DLF showed several peaks. Two of these peaks were identified as progestrone and cortisol by retention time, immunoassay and GC mass spectrometry.

Time-Resolved Immunofluorimetry of Lutropin

A Highly Sensitive and Rapid Procedure, M. J. Khosravi, and E. P. Diamandis (Cyberfluor Inc., 179 John Street, Toronto, Ontario, Canada M5T 1X4) (Spons.: Alan Pollard)

Immunoassays based on time-resolved fluorescence incorporate the positive features of radioimmunoassays with the speed and sensitivity of fluorescence detection but eliminate the well known disadvantages of radiolabeled tracers. We describe here a new immunfluorometric method for the quantitation of lutropin (LH) in serum. The Stratus microtiter plate was used. A sandwich principle with time-resolved fluorescence measurements, using the europium chelate 4,7-bis(chlorosulfonyl)-1,10 phenoanthroline-2,9-dicarboxylic acid (BCPDA) as label. In the antibody reaction, the alpha-subunit of lutropin is adsorbed to the walls of a white opaque microtiter well to form the solid-phase-capture antibody. A biotin-labeled soluble monoclonal antibody is used for detection. Interfacing the biotinylated antibody with streptavidin which has been linked to the bound europium chelate with multiple BCPDA residues, completes the detection system. In the presence of excess europium, the fluorescence of the final complex attached to captured lutropin molecules is measured on the dried solid phase with an
337  FLUORROMETERIC ENZYME IMMUNOASSAY FOR HUMAN FOLLITROPIN (HUMAN FOLLICLE STIMULATING HORMONE) IN SERUM. Charles W. Banner and Deborah K. Vickers. (E.I. du Pont de Nemours & Co., Inc., Medical Products Department, Wilmington, DE 19898) (Sponsor: D. P. Lehane)

A sensitive and rapid two-site enzyme immunometric assay has been developed for the measurement of FSH in serum. The method utilizes chromium dioxide magnetic particles as the solid support.

Reagents for this assay include monoclonal antibody specific for the alpha-subunit of follicitropin coupled to chromium dioxide particles and an sex-specific anti-FSH conjugate to chromium dioxide particles for the beta-subunit of FSH. The assay is standardized against the WHO 2nd IRM-HPO.

The assay is accomplished by combining serum (25 µL), magnetic particle conjugate (25 µL) and enzyme antibody conjugate (25 µL). The reaction is incubated for 40 minutes at 37 °C. The particles and enzyme antibody conjugate separate readily. The fluorescent signal is generated by incubating the washed particles with 4-methylumbelliferyl phosphate in diethanolamine buffer for 5 minutes.

Excellent correlation with the Diagnostic Products Corporation FSH RIA kit was observed (Y = 0.86X + 2.9; r = 0.99; n = 23) utilizing an analytical range of 0 to 100 mIU/mL. Within-assay CV's of 2.6% and 9.2% at 26.4 and 8.5 mIU/mL, respectively, were observed on serum control panels analyzed by traditional clinical methods. However, this assay was able to measure concentrations of less than 0.004% (range 100-106.7 %), determined by adding 10 mIU/mL of FSH to random sera. Excellent parallelism with the standard curve was demonstrated by diluting each of five sera having FSH concentrations greater that 45 mIU/mL with zero calibrator.

In summary, a rapid enzyme immunoassay has been developed for follicitropin and it is suitable for manual or automated analyses.


We measured hGH in serum samples obtained every 20 min for 24 hours from ten prepubertal subjects with short stature using IRMA assays from Hybritech Inc. (H) (San Diego, CA) and Nichols Diagnostics (ND) (San Capistrano, CA). Threefold higher results were obtained with the former when compared to the latter (r = 0.94; p < 0.0001). We therefore compared the IRMA assays with the standardized hGH RIA from Nichols (Human Growth Hormone Program) (N) using the following genetically engineered hGH preparations from Genentech Inc. (South San Francisco, CA): methionated 22K hGH, 22K hGH, and methionated 20K hGH. We also assayed human pituitary hGH (NIH, lot # APF-478B). Each of the hGH preparations were diluted in 3 buffer systems: bovine serum obtained from both H and ND and human serum. The N-RIA and H-IRMA gave superimposable standard curves for all hGH preparations in each dilution. The methionated 20K hGH was not detected in the Hybritech assay. Human serum in the ND-IRMA caused a left shift in the standard curves for all hGH preparations in each dilution. Results showed similar results in diluted horse serum. Control quality controls from Ciba Geigy (maximal and minimal results in ND vs the NRIA) were not results in H, and yet human serum pools containing hGH gave similar results in the N assay, compared to ND, and higher values in ND. While hGH was used in the assay as an immunogen, the IRMA assays gave similar results to the N assay for human serum samples. HGB reference ranges should be determined for each analytical laboratory to prevent patient maldagnosis.

340  A SIMPLIFIED SINGLE COLUMN EXTRACTION OF 1,25 DICYCLOXYDROXY VITAMIN D FOR RADIORECEPTOR ASSAY MEASUREMENT. Eva Guthrie, D. Garcia, R. Zahradnik, *E. Natoli, and *M. Holick (Nicholas Institute Diagnostics, San Juan Capistrano, CA 92675; & Boston University School of Medicine, Boston, MA 02128) (Sponsor: Diana Garcia)

A single column extraction of 1,25 dihydroxy vitamin D was developed for use in our ovine thymus radioassay assay kit with modification of a published procedure (Clin. Chem. 32: 2060, 1986). The extracted serum supernatant was applied to a monomeric C8-hexyl Bond Elut reservoir column (Analytichem Int’l, Harbor City, CA) which was preswashed with additional solvent (5 mL). The hazardous methylene chloride wash was eliminated and the 15 isopropanol (IPA)/Hexane wash was increased to 8 mL. The 1,25 dihydroxy vitamin D was completely eluted in a single peak with 6 mL of 67 IPA/water.

The average recovery of the metabolite by this single column purification was 78.47 ± 7.67 (n = 57). Lot to lot variation of 6 different batches of columns averaged 79.4 ± 2.1; while our current lot shows column to column recovery for 16 columns of 95 ± 26.3. Use of a quality control sample and a receptor binding assay shows the intra-assay coefficient of variation (CV) to be 6 % for a sample with a mean value of 33.3 pg/mL 25.5 (n=23). The inter-assay CV for the same sample was 8.5% with a mean of 35.0 ± 3.0 pg/mL of 1,25 dihydroxy vitamin D (n=16).

This extraction procedure represents an improved method over current commercially available multiple column extraction protocols for 1,25 dihydroxy vitamin D.
**AN ANTIBODY-COATED TUBE.** \(^{131}I\) RADIOIMMUNOASSAY FOR 17alpha-HYDROXYPROGESTERONE IN NEONATAL BLOOD SPOT SAMPLES: ANALYTICAL AND CLINICAL STUDIES, A.S. El Shami and K. Mansbich (ShеПа, Amman, Jordan and Ankara, Turkey).

Measurement of circulating 17alpha-hydroxyprogesterone (17α-OHP) plays an important role in the diagnosis and management of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency. Recent advances in the diagnosis and management of CAH have stirred interest in the precise quantitation of 17α-OHP levels ranging from 1.25 to 65 pg/mL (1 to 75 ng/mL of serum). No preliminary extraction is required. 18B-inch disks punched from blood spot samples are incubated overnight with 17α-OHP in antibody-coated tubes, which are then decanted and counted. Serum samples and controls—diluted 1 in 20 and supplemented with zero blood spot disks—can also be assayed by this method.

17α-Hydroxyprogesterone crossreacts at 3.5%, 11-deoxycortisol at 0.8%, 17α-hydroxyprogesterone at 0.29%, deoxycortisol at 0.1%, corticosterone at 0.0%. Cortisol (tested at 4.000 ng/mL), corticotropin (100,000 pg/mL), and other relevant steroids all yield negative cross-reacting results. Intra- and inter-assay CVs measured 7.1-6.2% and 7.1-13.7% over a concentration range of 4 to 35 pg/mL, for blood spot samples processed in duplicate. The standard curve is essentially linear in a log-log-log representation. As a test of linearity/interval calculation, disks punched from the same blood spot were either cut in half and assayed, or assayed intact; the ratio of results (summed halves, divided by intact) averaged 100%. As a test of accuracy, eighty-five serum samples with 17α-OHP levels ranging from 1.8 to 355 pg/mL were assayed by the present method (NEW) and by two antibody-coated tube 17α-OHP radioimmunoassay methods (DPC and IC) designed and validated strictly for use on serum (Baxter, Los Angeles; Ciba). Linear regression yielded the following relationships and correlation coefficients: (NEW) = 1.00 DPC - 0.01; (NEW) = 0.98 (DPC - 0.01); (NEW) = 0.99. 17α-OHP results for eighty-seven blood spot samples from normal infants ranged from 0.05 to 68 pg/mL, with a mean of 4.2 ± 0.3 pg/mL. 90% had results below 7 pg/mL, while 93% had results of at most 9.3 pg/mL. Two blood spot samples obtained before treatment from infants with confirmed CAH had results ranging from 17 to 33 pg/mL. In the first of these cases the CAH was due to aldosterone deficiency; in the second case there was evidence that the CAH was due to 17α-hydroxylase deficiency. In the absence of such a screening program, diagnosis of CAH in infants may be delayed by a year or more (Lebovitz et al., Amer J Dis Child 1984; 138:571). Moreover, in its salt-wasting form, CAH can be life-threatening.

In neonatal screening programs, collection of heel-stick blood into heparinized capillaries is generally recommended. This, however, is believed to be an acceptable alternative to direct transfer of a drop of blood from heel to filter paper (Nasser document LAA4-A). According to the authors, it is easy to rule out the possibility that heparin might interfere with assays intended for neonatal screening, or that differences in incubation conditions might affect the accuracy of results. The authors therefore conclude that, as we studied a direct, phase radioimmunoassay (El Shami and Durham, Clin Chem 1988;34, this issue) designed for measuring 17α-OHP in blood spots. The standard curve was generated with 17α-OHP standards ranging from 0.01 to 1,000 ng/mL. The standard curve was generated with 17α-OHP standards ranging from 0.01 to 1,000 ng/mL. We have developed a sensitive phase radioimmunoassay (IRA) for measuring 17α-hydroxyprogesterone (17α-OHP) in human plasma. The assay, which uses 17α-OHP bound to a monoclonal mouse anti-17α OHP antibody, is specific for the intact 17α-OHP. A difference of 0.1 ng/mL is considered to be significant for serum samples analyzed at room temperature on a horizontal rotator for two hours. After aspirating the tubes are counted in a gamma counter. Radiolabeled 17α-OHP demonstrated a linear relationship to the fraction and the quantity of LA added.

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A fully automated avidin-biotin immunoenzymometric assay for serum prolactin has been developed and applied to the kone specific antibody analyzer. The assay is based on a double antibody binding and two monoclonal antibodies. The first antibody immobilized on the solid-phase reactions sequentially with sample and biotin-labeled second antibody. This is followed by avidin-enzyme conjugate and enzyme substrate.

In the fully automated assay the KONE specific dilutes standards and samples into the coated cuvettes, incubates the sample removes unbound material and adds substrate and performs incubations. The instrument then measures the color intensity of each well and calculates the results using the spline curve. The total time of the assay cycle is about 1 hour.

The range of the prolactin assay is 0-100 ng/ml (1st IRP 75/504) and the sensitivity is better than 1 ng/ml. The intra-assay CVs were over the entire range between 3 - 8%. The assay correlated well (r = 0.95, n = 35) with the corresponding manual one which, in turn, showed a good correlation (r = 0.95, n = 44) with a commercial RIA (Farsmo Diagnostics).

In conclusion, the fully automated assay provides a sensitive, labor, and time-saving method for the determination of prolactin in serum.

A rapid and nonisotopic solid-phase immunassay for serum prolactin has been developed and applied to the KONE specific antibody analyzer. The assay is based on a double antibody binding and two monoclonal antibodies. The first antibody immobilized on the solid-phase reactions sequentially with sample and biotin-labeled second antibody. This is followed by avidin-enzyme conjugate and enzyme substrate.

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In conclusion, the fully automated assay provides a sensitive, labor, and time-saving method for the determination of prolactin in serum.
Based on the characteristics of association with progesterin binding sites in breast and endometrial cancer and the higher specific radioactivity of [3H]estradiol-17β, we highly recommend that this steroid be used as a ligand for PR in clinical assays. Combined with [3H]estradiol-17β, it may be used in a double isotope mode when small tissue biopsies are encountered. Supported in part by PH Beta Psi Sorority.

**354**
ATRIAL NATRIURETIC FACTOR IN HUMAN EDTA PLASMA BY RADIOIMMUNOASSAY. Donald Wong and Wellington L. Paul, Research Department, SmithKline BioScience Laboratories, Yan Nuy, CA. 91605. (Spon.: Donald Wong).
We have developed a radioimmunoassay (RIA) for the measurement of atrial natriuretic factor (ANF) in human EDTA plasma. Synthetic ANF and antibody specific for the C-terminal end of ANF (Peninsula Co.) are used in the assay. ANF is extracted from plasma using solid phase C18 cartridges. The RIA assay has a sensitivity of 15 pg/mL. ANF is stable in plasma at 37°C up to 3 hours without protease inhibitor, in whole blood at room temperature for at least sixty minutes, and at -20°C for at least 60 days.

The accuracy of the assay was demonstrated by: 1) the low cross-reactivity for 5 ANF fragments shown to have negligible biological activity, 2) the high recovery for ANF spiked specimens with a mean of 102 ± 2%, and 3) a good parallelism, elevated ANF samples were diluted and all of the resultant curves paralleled the standard curve.

The plasma concentrations of ANF (mean ± SD) were: 1) 25.9 ± 8.9 pg/mL in 40 healthy volunteers, 2) 418 ± 126 pg/mL in 9 samples of patients with class 3 and 4 congestive heart failure.

**355**
Determinations of estrogen (ER) and progesterin receptors (PR) are primarily by a radioligand titration assay. Virtually all clinical correlations have been performed using this procedure without sodium molydate (MoO₄²⁻) (Wittliff, Cancer 53:630, 1984). Many studies indicate 10 μM MoO₄²⁻ acts as a stabilizer of ER and PR. Influence of MoO₄²⁻ on specific binding capacities and affinities of ER and PR and the effect on classification was examined in 304 tumor biopsies from women (61 ± 14 yr) as compared with assays on 268 biopsies, mean age 61 ± 14 yr.

**Table 1.**

<table>
<thead>
<tr>
<th>ESTROGEN RECEPTORS</th>
<th>PROGESTIN RECEPTORS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>MoO₄²⁻</td>
<td>206</td>
</tr>
</tbody>
</table>

We evaluated the effect on classification using the arbitrary cut-off value of 10 fmol/mg with the following results:

**Table 2.**

<table>
<thead>
<tr>
<th>NO MOLYDATE</th>
<th>MOLYDATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER/PR</td>
<td>PERCENT (NUMB)</td>
</tr>
<tr>
<td>NEGANEG</td>
<td>24.5</td>
</tr>
<tr>
<td>NEG/POS</td>
<td>10.9</td>
</tr>
<tr>
<td>POS/NEG</td>
<td>12.5</td>
</tr>
<tr>
<td>POS/POS</td>
<td>54.5</td>
</tr>
</tbody>
</table>

These data indicate that, although MoO₄²⁻ increased [PKR] in variously all tumors, there was little effect on classification of either receptor. These data suggest that MoO₄²⁻ may be used as a ligand in clinical assays of ER and PR does not alter receptor status. Supported in part by USPHS grants CA-32102, CA-31946 and CA-25224 from the NCI.

**356**
In order to establish ranges and frequency distributions of steroid hormone receptors in uterine cancer quantitative analyses of estrogen (ER) and progesterin receptors (PR) were performed on biopsies from 64 patients at various stages of endometrial carcinoma. ER was estimated using [3H]estradiol + diethylstilbestrol; PR was determined with [3H]MoO₄²⁻ + R5020 using ligand titration procedures. Information on steroid hormone receptor status assessed were monitored.

ER values (range 0 - 203 fmol/mg cytosol protein) were within those observed in nonmalignant uteri, while PR levels (0 - 3647 fmol/mcpg) appeared to be lower. As in breast cancer, [ER] and [PR] were higher in well and moderately differentiated tumors. An inverse correlation was found between ER and PR distribution with clinical stage or histopathologic category. The frequency distribution of receptors was similar to that reported in breast cancer with 56% of the
tumors exhibiting both ER and PR, whereas 8% contained only ER and 6% contained only PR. The addition of progestin to ER- and PR- 6% of endometrial tumors were PR+ER- similar to breast cancer. We examined this observation using tamoxifen, an anti-estrogen, to induce [PR] in uterine cancer to increase response to progestin therapy.

We report on a new immunofluorometric assay for prolactin (PRL). This is a two-site sandwich-type time resolved immunofluorometric assay based on the use of two monoclonal antibodies. PRL binds to an antibody immobilized in a microtiter well and to a soluble biotinylated antibody. The addition of streptavidin labeled with Eu(III) chelate 4,7,10-bis(cholesterolamido)phenanthroline-2,9-dicarboxylic acid (BCPDA) and excess Eu(III) results in the formation of the fluorescent complex. Fluorescence is quantitated on the dry surface in a gated - fluorometer. Serum samples can be assayed in one incubation step of 1.5 h with the biotinylated antibody followed by a 30 min incubation with streptavidin. The dynamic range of the assay is 0 - 200 ng/mL. The sensitivity was found to be 0.25 ng/mL. The intra-assay CV for PRL concentrations of 7.5, 17.0 and 39.8 ng/mL was 5.9, 6.3 and 7.0% respectively. Inter-assay CV's for PRL levels of 17.1, 25.4 and 55.3 ng/mL were 7.0, 9.1 and 3.7%, respectively. The recovery of PRL added to serum samples ranged from 86% to 110% with a mean of 100 ± 9.8%. Correlation with an RIA kit and one time-resolved fluoroimmunassay kit (LKB, Deltafia) was good. The regression equations were y(TR-FIA) = 0.8291 x + 0.981 x (RIA), r = 0.98, n = 94 and y(TR-FIA) = 0.107 + 0.6614 x (Deltafia), r = 0.94, n = 65. No significant cross reactivity or interferences were found. The assay has the advantages of a non-radioactive label/step and it does not suffer from problems of exogenous Eu(III) contamination which constitutes the main limitation of current time-resolved immunoassays which use Eu(III) as label. This is due to the different design of the assay and the use of BCPDA as label, in the presence of excess Eu(III).

**LIPIDS**

**Lipids and Lipoproteins**

**358**


We studied the effect of lyophilization on the determination of cholesterol with four different analytical systems: SMAC (Technicon), RA-1000 (Technicon), aca (Du Pont), and TDX (Abbott). Lyophilization of serum is related to the standardization of cholesterol, because current materials for justifying accuracy of SMAC and aca experiments are lyophilized as well as many calibrators.

We made low and high serum pools, which we divided into the groups, freezing two at -20°C and lyophilizing the other. We determined cholesterol at intervals over the next 94 days. The concentration of cholesterol decreased a maximum of 1.35 at the end of 94 days for the pools kept at -70°C and 2.25 for the pools kept at -20°C. The value decreased more than 8% for the aca and TDX soon after lyophilization and maintained that value over the 94 days. The results for the lyophilized pools compared with fresh sera are shown here:

<table>
<thead>
<tr>
<th></th>
<th>aca</th>
<th>TDX</th>
<th>SMAC</th>
<th>RA-1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Pool</td>
<td>1 change</td>
<td>-16.1</td>
<td>-8.7</td>
<td>-15.9</td>
</tr>
<tr>
<td>(1.8 g/L)</td>
<td>SIC</td>
<td>-7.1</td>
<td>-1.2</td>
<td>-1.2</td>
</tr>
<tr>
<td>High Pool</td>
<td>1 change</td>
<td>-16.3</td>
<td>-11.9</td>
<td>-3.2</td>
</tr>
<tr>
<td>(2.1 g/L)</td>
<td>SIC</td>
<td>-9.0</td>
<td>-4.3</td>
<td>-1.2</td>
</tr>
</tbody>
</table>

**359**

**LILY, 4, 5, AND 6 MAY BE THE MOST HETEROGEOUS LDL SPECIES.** Judith R. McMahon, John S. Miller, Erling Billlevens, and Ernest J. Schaefer (Tufts University, Boston, MA 02111) (Spon.: J.R. McMahon)

Low density lipoproteins (LDL) are the major cholesterol-carrying particles in plasma and can vary considerably in size and molecular weight. Most individuals have one major LDL type; however, at least seven different LDL species (LDL I-7) can be observed in plasma using 2-16 gradient gel electrophoresis. In our analysis system LDL 1 are the largest particles and LDL 7 are the smallest.

We used a microtiter plate immunoassay to investigate the composition of LDL in the plasma of normal and atherogenic individuals. We determined the precise composition of different LDL sizes by isolating the LDL (d = 1.019-1.063 g/mL) of 22 subjects who had only a single major LDL type (10% of total LDL). Free cholesterol and phospholipid content decreased as particle size decreased from LDL 1 to LDL 7 (10% to 4%, and 25% to 20%, respectively). Conversely, triglyceride and protein content increased with decreasing LDL size (5% to 17% and 20% to 28%). Cholesterol ester (CE) content increased from LDL 1 to LDL 2 to a peak of 41% in LDL 5, and then decreased to 36% for LDL 7. The percent of LDL-C as CE increased steadily from 74% in LDL 2 to 87% in LDL 7. When LDL-C concentrations were calculated from total LDL-C levels for each type, the highest concentrations of plasma LDL-C were found in LDL 4 to 6. Moreover, in a study of normal male and female subjects and 115 cases with severe coronary artery disease (CAD), the mean LDL type was 2.64 in women, 3.63 in men, and 4.52 in CAD patients. These data are consistent with the concept that LDL 4 to 6 may be the most atherogenic LDL particles because they are associated with the highest LDL cholesterol values as well as the greatest LDL cholesterol ester concentration.

**360**


The goal of this study was to apply GLC to profile total serum fatty acids (derived from triglycerides, phospholipids and sterol esters) in disease and health. Several groups of hyperlipemics, rural dialysis patients and healthy donors were studied.

The serum (3-5 ml) was lyophilized, and the dry powder was hydrolyzed with 3.5 ml of 34% BF3 in MeOH (Sigma Chemical Co) at 90°C for 20 minutes. Then 4 ml of '70 b was added and fatty acid methyl esters were extracted with hexane (15 ml x 2) and purified by passing through a small silicic acid column using hexane/ether (80/20 by vol) and analyzed by GLC. Fatty acid profiles were calculated according to Djerassi et al. (Lipids 6:561-563). The hypertensive group was found to have lower saturated/unsaturated fatty acid ratio (0.57) compared to normal (0.72) and dialysis patients (0.71). Saturated fatty acids were significantly different in hypertensive patients than normals (p<0.005) but not between normals and dialysis patients.

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=9</td>
<td>n=10</td>
<td>n=10</td>
</tr>
<tr>
<td>Saturated</td>
<td>36.1 ± 1.5</td>
<td>36.5 ± 1.0</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>57.5 ± 4.9</td>
<td>63.5 ± 1.6</td>
</tr>
</tbody>
</table>

However, unsaturated fatty acids are present in higher amount in hypertensive patients (0.92) and smaller amount in dialysis patients (0.81) compared to normals (0.84). Linoleic and linolenic acids, the precursors of arachidonic, were increased in plasma in abnormal amounts in both dialysis and hypertensive patients (p<0.02). We conclude that fatty acid profiles rather than their saturated/unsaturated ratio are more informative about on the presence of abnormalities in dialysis and hypertensive patients. We recommend such a profile as a useful adjunct in assessing fatty acid status of patients.
The method was evaluated at 80% and 100% on the "Chol" system and compared to results from the Abaland method.

The assay is based on the separation of the HDL (High Density Lipoprotein) fraction from other lipoprotein fractions. The HDL cholesterol is precipitated by addition of Dextran Sulfate-Mg++. The results were compared against those obtained using an established enzymatic total cholesterol method. The method follows the guidelines of the AACC Standard Method.

Calibration stability is at least 80 days and on system reagent stability was thirty days.

The system's Analytical Range was 0 to 100 mg/dL and Sensitivity was 0.0544 ABA per mg/dL HDL.

Within-run and total imprecision data was generated from patient samples duplicate and multiple replicates of controls.

**Results**

The method's performance on the Chol 1 HDL method was equivalent to the AACC Standard Method.

**Measurement of Serum APOproteins, ApoA-I, ApoB and Triglycerides**

Sera from 43 normal females (F) (age: 21-91) and 24 males (M) (age: 25-75) were analyzed for apoA-I (apoA-I) and apoB using an immunochromatography system analyzer from Beckman Instruments, Inc., CA. Total cholesterol (TC) and high density lipoprotein (HDL) cholesterol (C) were measured after precipitation of sera in a Kodak Ektachem 700 unit. Low density lipoprotein (LDL) cholesterol (LDLC) was determined using an enzymatic assay system analyzer from Beckman Instruments, Inc., CA. Total cholesterol (TC) and high density lipoprotein (HDL) cholesterol (C) were measured after precipitation of sera in a Kodak Ektachem 700 unit. Low density lipoprotein (LDLC), HDL/LDL/HDL/TC, and apoA-I and apoB were calculated. Serum concentration (mg/dl) or ratio (unit) were expressed as mean ± S.D. and results were as follows:

**TC** HDL - LDL HDL/TC

F: 254 ± 9 176 ± 16 54 ± 2 171 ± 18 3.3 ± 0.2 0.026 ± 0.013 M: 252 ± 16 198 ± 12 63 ± 2 152 ± 12 3.5 ± 0.2 0.024 ± 0.018

-ApoA-I, apoB, and apo A/B were 162 ± 4.7, 102 ± 4.6, and 1.77 ± 0.12 for F and 150 ± 8, 85 ± 5, and 1.95 ± 0.19 for M, respectively. The following significance for all women) are (0.001) for A/B as compared to men. No differences between the values of men and women were found possibly because of inclusion of 19 post-menopausal females or elevated apoA-1 and apoB values.

**Evaluation of the Beckman Clinical System 700 for Cholesterol Measurement**

The Beckman Clinical System 700 was evaluated for total cholesterol (TC) measurement. Precision, accuracy, and linearity were examined.

The System 700 is a fully automated analyzer which determines serum or plasma TC enzymatically. The assay method is as follows: 600 μL of reagent is added to 6 μL of serum, incubated at 25°C for about 20 min after which the sample is read at 500 nm. The precision (within-day and day-to-day) was measured at three concentrations (x ± 1 S.D., mg/dL).
Numerous biochemical parameters are used in the clinical laboratory to assess potential risk for coronary heart disease (CHD). The reported increased sensitivity and specificity of apolipoproteins AI (Apa), B (ApoB), and their ratio as CHD risk indicators prompted us to include these assays within our lipid profile. We investigated the correlation of ApoA, ApoB, and their ratio to the time consuming HDL-cholesterol (HDL) measurement and the Friedewald calculated LDL-cholesterol (LDL) result.

We performed triglyceride (TG), total cholesterol (TC), HDL, ApoA, and ApoB on 665 patients undergoing routine physical examination. In addition LDL, TC/HDL, and LDL/HDL were measured. Samples with TG > 300 mg/dL were excluded from further study because of the known errors associated with HDL precipitation procedures and subsequent LDL calculations.

The correlation analysis by least squares regression (LSR) and the method of Deming were performed on appropriate combinations of TC, lipoproteins, apolipoproteins, and ratios. Results are:

<table>
<thead>
<tr>
<th>LSR</th>
<th>x</th>
<th>y</th>
<th>Slope Int. r</th>
<th>y</th>
<th>x</th>
<th>Slope Int. r</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC/HDL</td>
<td>LDL/HDL</td>
<td>0.78</td>
<td>-0.34</td>
<td>0.96</td>
<td>0.81</td>
<td>-0.67</td>
</tr>
<tr>
<td>TC</td>
<td>LDL</td>
<td>0.85</td>
<td>-44</td>
<td>0.33</td>
<td>0.31</td>
<td>-0.58</td>
</tr>
<tr>
<td>HDL</td>
<td>LDL</td>
<td>0.56</td>
<td>8.0</td>
<td>0.89</td>
<td>0.59</td>
<td>2.3</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>LDL</td>
<td>0.37</td>
<td>53</td>
<td>0.83</td>
<td>1.30</td>
<td>31</td>
</tr>
<tr>
<td>LDL/TG</td>
<td>LDL</td>
<td>6.92</td>
<td>-0.15</td>
<td>0.91</td>
<td>8.34</td>
<td>-0.48</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>LDL</td>
<td>0.19</td>
<td>0.18</td>
<td>0.89</td>
<td>0.19</td>
<td>0.17</td>
</tr>
</tbody>
</table>

We conclude that ApoA and ApoB performed by nephelometry on the Beckman RXA™ Protein System are highly correlates to HDL and LDL respectively. These parameters are direct serum assays and avoid the need for ultracentrifugation and precipitation techniques which can lead to imprecision.

A simple and sensitive colorimetric method was developed for quantitative assay of lipid peroxides (LOP) in biological materials and foods. It is based on the reaction of LOP with a leuko- methylene blue derivative; (3,5-dimethylcarbonyl-3,7-dimethoxy-10H-phenothiazine (NCDP), in the presence of hemoglobin.

The amount of methylene blue formed by incubation of the reaction mixture at 30°C for 15 min., was measured by the absorbance at 675 nm and the amount of LPO was calculated using cuine hydroperoxide as standard.

There was clear relationship between the amount of conjugated diene and that of LPO measured by this method. A linear relationship between the LPO concentration and the change in absorbance was confirmed in the range less than 30 mol/L. For LPO assay in serum, interferences with ascorbic acid and transferrin were eliminated by adding ascorbate oxidase and cholesterol oxidizing agents. Other significant interferences were detected in serum. Cumene hydroperoxide was added to normal human serum and the mean of each recovery was found to be 102.6%. We checked the within run precision by manual assay using fresh and pooled human sera. The mean LPO concentrations were 0.8 and 4.9 mol/L and the CVs were 11.3 and 2.25, respectively. As a result of preliminary studies with fresh human sera from 39 healthy adult blood donors, the range of LPO was obtained to be 0 to 1.4 mol/L.
were 1.08 and 1.05 respectively. Isobal HDLs (Y) were compared to a deuterium sulfate (X) precipitation method \( Y = 0.92 X + 4.4; r = 0.89; n = 29 \). Isobal LDLs (Y) were compared to a calculated (X) LDL value using triglyceride levels from the Ektachem 400 (Y = 1.33X - 18.0; r = 0.89; n = 29).

Samples with significantly elevated triglyceride or of a chylous nature yielded results which are not suitable for isolated HDL or LDL determinations. Commercially purified control materials do not yield usable values for isobal HDL or LDL.

We conclude that the Isobal LDL-Direct system is potentially an accurate, simple analysis easily adaptable to automation. The unavailability of commercial quality control material must be resolved before this assay is suitable for clinical laboratory use.

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A 'CAPTURE' ELISA IMMUNOASSAY FOR HUMAN LP(a) LIPOPROTEIN. Karl Doetzch, Paul S. Roehmer, and James J. Thompson (Departments of Pathology, Physiology, and Microbiology; L.S.U. Medical Center, New Orleans, LA 70112) (Spons. Karl Doetzch)

Lp(a) is a genetically controlled independent risk factor for heart disease. Elevated blood levels in subjects less than 60 years of age are associated with a 2.5-fold increase in the occurrence of premature atherosclerosis.

We have developed a sensitive, specific ELISA immunnoassay for quantification of the antigen in unfractionated plasma or serum. An enriched IgG fraction derived by Sepharose Protein A chromatography from rabbit polyclonal anti-human Lp(a) is the reagent that 'catches' the Lp(a) protein. The bound Lp(a) then is 'recognized' by a specific mouse monoclonal anti-human Lp(a) (HLH-1). An alkaline phosphatase-labeled IgG substrate and pararosanilin phosphate substrate 'detect' and 'indicate' the amount of bound monoclonal antibody. We investigated the reaction kinetics, slope/bound/free ratio reagents at each stage of the assay as well as the precision and accuracy of this solid-phase microtiter plate ELISA method. Plate-to-plate variation in Lp(a) is less than 10%.

Plasminogen/plasmin has partial structural homology with Lp(a) and therefore could cross-react in immunnoassays for Lp(a). By our technique using the monoclonal antibody, crossreactivity is less than 1%.

**372**

THE CHARACTERIZATION AND USE OF A MONOCLONAL ANTIBODY TO QUANTIFICATE HUMAN APOLPOLIPROTEIN A-I BY AN ENZYME IMMUNOASSAY. T.C. Rothwell, T.M. Scott, L. Battaglia, Thomas Felley, Beth Cooper, Angie Lackey (Hycor Biomedical, Inc., Fountain Valley, CA 92708) and Charlotte R. Elliott, Mfti L. Kashyap (Univ. Calif., Irvine/V.A. Med. Ctr., Long Beach, CA 90822) (Spons: T. C. Rothwell)

Apolipoprotein (Apo) A-I, major protein of high density lipoprotein (HDL), is a very important marker for coronary disease. The production of a monoclonal antibody and the development of an accurate, specific and precise immunochemical technique for Apo A-I quantitation will be useful in meeting the demand for a rapid, high-throughput and standardized method. We have produced a mouse hybridoma high-affinity IgG1 monoclonal antibody, HB-22, against HDL. Its binding increased upon treatment of EDTA plasma with Tween 20. Excess HB-22 bound at least 90% of available Apo A-I in human plasma. A competitive enzyme-linked immunosorbent assay (ELISA) was developed by coating polystyrene microtiter wells with HDL, blocking uncovered sites, and incubating diluted EDTA plasma samples or purified Apo A-I and HB-22 conjugated to horse-radish peroxidase. Bound enzyme was quantitated by oxidation of o-phenylenediamine and spectrophotometric measurement at 492 nm. The dynamic range of the primary standard curve was 4-128 μg/ml. Displacement curves for Apo A-I, HDL, HDL2, HDL3, delipidated HDL, delipidated chylomicrons, and a plasma pool were parallel. Apo A-II, LDL, VLDL, IDL, albumin, and globulin did not cross-react. Apo A-I values from seventeen normal volunteers ranged from 71 to 138 mg/dl. A significant correlation between ELISA and electroimmunoassay using a commercial polyclonal antisera was found. The correlation between Apo A-I and HDL cholesterol was 0.80. The within-assay coefficient of variation (CV) was 5.7%.

Conclusion: The data indicates that HB-22 may be a useful monoclonal anti-Apo A-I, and therefore automated ELISA for an accurate determination of Apo A-I in human plasma.

This was supported by SBIR Grant #SSS-4(A)SR489L33043-03.

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PERFORMANCE EVALUATION OF THE SERALYSER REFLECTANCE PHOTOIMETER. Judy Hundley, John Savory, (University of Va. Medical Center, Charlottesville, VA 22908) (Spons. C. Glenn Attek (Diagnostics Div., Miles, Inc., Elkhart, IN 46515))

We evaluated the performance of reformulated reagent strips on the Seralyser Reflectance Photometer. A recent report (Clin Chem 33, 2262, 1987) describes a similar evaluation using the previously formulated strip. Features of the reformulated strips are improved accuracy and longer stability at room temperature.

Accuracy of the Seralyser Cholesterol Assay was evaluated by comparison to an enzymatic cholesterol assay (BMD Auto Cholesterol High Performance Reagent) adapted to an automated analyzer (Technicon RA-1000). The comparative method uses a measurement at equilibrium and a separate serum blank correction. Comparison data is as follows:

<table>
<thead>
<tr>
<th>METHOD</th>
<th>X</th>
<th>Y</th>
<th>n</th>
<th>slope intercept r</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD Enz. Chol.(RA-1000)</td>
<td>194</td>
<td>0.949</td>
<td>0.047</td>
<td>0.997</td>
</tr>
<tr>
<td>Strip I</td>
<td>96</td>
<td>0.980</td>
<td>0.036</td>
<td>0.991</td>
</tr>
<tr>
<td>Strip IIA</td>
<td>100</td>
<td>0.923</td>
<td>0.035</td>
<td>0.972</td>
</tr>
<tr>
<td>Strip IID</td>
<td>196</td>
<td>0.946</td>
<td>0.066</td>
<td>0.966</td>
</tr>
</tbody>
</table>

In addition we have performed cholesterol measurements on two serum pools which have been analyzed by the reference Abell-Kendall method at the Atlanta Center for Disease Control. Results are as follows:

<table>
<thead>
<tr>
<th>METHOD</th>
<th>X</th>
<th>Y</th>
<th>n</th>
<th>slope intercept r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abell-Kendall Strip I</td>
<td>1</td>
<td>1</td>
<td>20</td>
<td>0.80</td>
</tr>
<tr>
<td>Abell-Kendall Strip IIA</td>
<td>2</td>
<td>2</td>
<td>20</td>
<td>0.80</td>
</tr>
<tr>
<td>Abell-Kendall Strip IID</td>
<td>2</td>
<td>2</td>
<td>20</td>
<td>0.80</td>
</tr>
</tbody>
</table>

**374**

EVALUATION OF THE ACCURACY OF A GC/MS REFERENCE METHOD FOR PROSTAGLANDIN E2 AND 6-KETO-PROSTAGLANDIN F1α. Michael Kinter, Judy Hundley, David A. Herold, Michael R. Wills and John Savory (Dept. of Path., Univ. of Va., Charlottesville, VA 22908) (Spons: Richard Ross)

We have evaluated the accuracy of our isotope dilution mass spectrometry assay for prostaglandin E2 (PGE2) and 6-keto-prostaglandin F1α (6-keto-PGF1α) in a culture of Mycobacteria tuberculosis. Such a system is attractive because it provides a good model of the matrices encountered in biological systems and because these microorganisms have 6-oxygenation activity which converts prostanooids to their dioxygen metabolites, a significant potential interference particularly in immunochromatographic assays. In this validation scheme, the results of the isotope dilution assay are compared to the results obtained using a 3-point standard addition assay. The values provided by the isotope dilution experiment should agree with the values predicted by the standard addition experiment within the limits of the experiments and the slope of the standard addition line should equal 1.0. This criteria would indicate that the two methods are providing comparable results and that the amount of analysis which is added to the sample is not a significant factor.

In this experiment, approximately 250 ng each of PGE2 and 6-keto-PF were added to an approximately 25 ml culture. Aliquots were removed at t=0, 3, 7 and 14 days and analyzed by isotope dilution mass spectrometry and standard addition. Results showed the expected decrease in PGE2 and 6-keto-PF concentration due to 6-oxygenation. The concentrations determined by the two methods were never significantly different for the four determinations of each compound; a slight negative bias of approximately 5% for each compound was indicated. In addition, the slopes of the standard addition curves were always within experimental error of 1.00. In conclusion, these results indicate excellent accuracy for the isotope dilution assay of PGE2 and 6-keto-PF.

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TOTAL CHOLESTEROL AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL MEASUREMENTS: RELIABILITY OF TESTING ON SEPARATE DAYS WITHOUT AND WITHOUT DUPLICATE ANALYSIS. F. R. Tschettert, T. E. Watson, J. M. Hla (Duke Univ. Med., Ctr. and Health Services Research Field Program, VA Med. Ctr., Durham, NC) (Spons: B. Oxford) Total cholesterol (TC) and high density lipoprotein (HDL) and the TC/HDL ratio are subject to both analytical and biological variation. Because of these variations, the National Heart, Lung, Blood Institute and other recommend repetitive measurements of TC and HDL, rather than a single determination, for assessment of cardiovascular risk and intervention monitoring. To expand on these recommendations, we compared the reliability of testing TC,
HDL and TC/HDL ratio, one week apart, with and without duplicate analysis in a group of 14 male outpatients ranging in age from 50 to 65 years.

Four TC and HDL measurements, i.e. duplicate determinations on day 1 and day 6, were used to generate the following 4 combinations: 1) single test, single measurement on day 1; 2) duplicate tests (Dup), average of two determinations on day 1; 3) test-retest, average of single measurements on day 1 and day 6; 4) test-retest + Dup, average of four measurements, two on day 1 and two on day 8. For these combinations, intraclass reliability coefficients (R) were calculated from within and between-day variance components generated from a nested analysis of variance.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Days</th>
<th>Reliability (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>HDL</td>
<td>HDL/TC/HDL</td>
</tr>
<tr>
<td>Single Test</td>
<td>1</td>
<td>0.72</td>
</tr>
<tr>
<td>Test-retest</td>
<td>1&amp;2</td>
<td>0.89</td>
</tr>
<tr>
<td>Test-retest+Dup</td>
<td>1&amp;2</td>
<td>0.95</td>
</tr>
</tbody>
</table>

We conclude that: 1) single test measurements of HDL and TC/HDL ratio are of questionable reliability (R=0.79 and 0.68 respectively); 2) duplicate test both single-day and test-retest, minimally improve reliability; 3) test-retest, that is averaging 2 repeated measurements from separate days, substantially improves reliability and supports NIH/WHO recommendations.

Experiences in monitoring cholesterol analyses by means of various Lipid Research Clinics protocols, and methodology comparisons for lipoprotein subclass analysis, were reported by Luepker et al. (Lipid Research Clinics Program Working Group, 1985). The Lipid Research Clinics (LRC) in 1978 and 1980, respectively, recommend the use of methods for total cholesterol and triglyceride determination that are at least 0.1 mmol/L and 0.2 mmol/L, respectively, and within-clinical pathology laboratories procedures for direct measurement of HDL and LDL cholesterol, respectively.

The National Reference System for the Cholesterol Assay (NRS-Chol), established by the National Heart, Lung and Blood Institute (NHLBI), is a major component of the National Cholesterol Education Program (NCEP), which was established in 1980. The NRS-Chol was designed to provide a standard method for the determination of total cholesterol and LDL cholesterol in serum or plasma. The system is based on the following criteria:

1. The method must be a standardized laboratory procedure.
2. The method must be sensitive, specific, and accurate.
3. The method must be reproducible and reliable.
4. The method must be controlled and monitored.

The NRS-Chol is a collaborative program that involves laboratories from throughout the United States. The program is coordinated by the NHLBI and is funded by the National Institutes of Health (NIH). The program's objectives are to:

- Develop and standardize a laboratory method for the determination of total cholesterol and LDL cholesterol.
- Coordinate the use of this method in clinical laboratories.
- Ensure the accuracy and precision of laboratory results.
- Provide quality control feedback to laboratories.

The NRS-Chol has been successful in achieving these objectives and is widely used by clinical laboratories throughout the United States. It has also contributed to the development of other national reference systems for other laboratory parameters, such as hemoglobin and creatinine.

ABSTRACT WITHDRAWN

LOW AND INDEPENDENT DENSITY LIPOPROTEIN SUBCLASSES IN PATIENTS WITH MILD AND SEVERE HYPERTENSION
Jan J. Ojpt and Euan S. Holberg
Cleveland Metro. Gen. Hospital, Cleveland State University
Cleveland, Ohio 44109

An analytical micro-ultracentrifugation method was developed for differentiation of subclasses of atherogenic lipoprotein classes LDL and HDL. The method was based on an equation developed by H. Fujita (1956). Our contribution is based on recognition that the concentration gradient formed by a floating homogeneous lipoprotein is Gaussian in form. The concentration gradient curves of LDL and HDL subclasses, observed in analytical ultracentrifugation at density 1.05, were correlated well with the calculated theoretical curves for F, 20 to 70, constructed by applying our modified equation.

The correct number of subclasses with specific flotation coefficient range was controlled by the simultaneous use of experimental absorbance scans with calculated curves and by summation of these separational techniques.

For demonstration of practical use of the above experimental and theoretical methods, we present results from 8 patients with mild primary hypertension and from 17 patients with a severe form.

SEVERE FORM HYPERTENSION

<table>
<thead>
<tr>
<th>SUBCLASSES:</th>
<th>LDL1</th>
<th>LDL2</th>
<th>LDL3</th>
<th>LDL4</th>
<th>LDL5</th>
<th>LDL6</th>
<th>LDL7</th>
</tr>
</thead>
<tbody>
<tr>
<td>MILD PRIMARY HYPERTENSION</td>
<td>20.5</td>
<td>34.7</td>
<td>0.0</td>
<td>44.8</td>
<td>16.4</td>
<td>76.5</td>
<td>13.1</td>
</tr>
</tbody>
</table>

SENSITIVE ENZYMIC METHODS FOR HDL AND HDL SUBCLASS CHOLESTEROL MEASUREMENT, Joseph Bateson, Joseph D. Artiss, and Bennie Sak (Pathology Dept., Wayne State Univ., Detroit, MI 48201) (Spon: Joseph Bateson)

We have developed sensitive enzymic assays for HDL-C and HDL subclass cholesterol quantitation. Lipoprotein separation was achieved by a modified double precipitation method reported by Talamas et al (Clin. Chem. 22:1227, 1976). Enhanced sensitivity was obtained by substituting 2-hydroxy-3,5-dichlorobenzoate (HDCBS) for phenol in the indicator reaction. This facilitated a greater than 4-fold increase in sensitivity thus allowing us to decrease the sample-to-reagent ratio from 1:20 with phenol to 1:100 with HDCBS.

Precision studies (n=25) demonstrated both within- and between-run reproducibility of <4.2% at cholesterol levels of 0.54 and 1.33 mmol/L. The OS-HDL subclass specific precipitation method was compared with another precipitation technique (Gidez-Eder, J. Lipid Res. 22:1206-1982) (n=22). Results were highly correlated for HDL-C (r=0.985), HDL-C (r=0.966) and HDL-C (r=0.786). The present method gave a linear response between 0.2 mmol/L and 7.5 mmol/L cholesterol.

INTEREFERENCE was not noted from lipemic (TG<4 g/L), bilirubin (300 mg/dL), hemoglobin (5 g/L), nor with the precipitants dextran sulfate (MW 50,000) and magnesium acetate at the theoretical maximum concentrations. We conclude that the proposed method is precise, accurate, and well suited for routine use in the clinical laboratory.
Cholesterol values from patient specimens, on the other hand, gave regression statistics that fall well within the NHLBI-CDC guidelines; in comparison to a CDC-certified Abell-Kendall reference method, analysis of 81 patient samples gave a slope = 1.00, intercept = -1.7 mg/dL, Sy.x = 5.3 mg/dL, and a correlation coefficient of 0.998.

We conclude (1) that the cholesterol method on the Ektachem 70.700/year is adequate for measurement of patient specimens and (2) that current CDC and CAP matrix materials do not emulate human patient serum on the cholesterol slide and are not adequate to demonstrate cholesterol standardization and competency of laboratories using the Ektachem analyzer.

PROCEEDURES FOR APO AI AND APO B DETERMINATIONS IN UTAH: BECKMAN NEPHELOMETRIC VS. VENTREX RADIOMUNOASSAY (RIA). Lily L. Wu (Dept. Path, Univ Utah Sch Med, Salt Lake City, UT 84132), R.R. Williams, D.C. Hunt, B. Streeter (Cardiovascular Genetics, Univ UT Sch Med), J.M. Lateau (Howard Hughes Med Inst, Univ UT Med Ctr), Lydia Dodan-Lehrer (Beckman Instr. Brea, CA 92821). (Spon. Lily L. Wu).

We evaluated the time-consuming RIA and the rapid nephelometric procedure using Beckman reagents for apolipoprotein determinations. Lipid studies were performed on specimens from 131 hypertensive patients with normal to highly abnormal lipid profiles. Determination of HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) were performed on an Encore II Autoanalyzer using baker reagents after isolation. Multiple determinations were made for each specimen analyzed by both methods.

Lab Precision Of Replicate Assays on the Same Samples

For apo AI by RIA 0.981 (n = 10) and for apo B by nephelometric method 0.998 (n = 9) with r = 0.99. Apolipoproteins correlated well with their respective lipids for both methods in 131 patients. (apo AI vs HDL-C r = 0.6, r = 0.7 neph, apo B vs LDL-C r = 0.7 for both). Conclusion: Both methods are precise and highly correlated with each other but different means suggest the need to adjust to a common method for comparisons.

ABSTRACT WITHDRAWN


Accuracy and precision of cholesterol determination have been emphasized recently due to the National Cholesterol Education Program's recommended guidelines. Their current goals are 5% precision and 5% bias vs. the nationally recognized Abell-Kendall (A-K) reference method. By 1992, the panel recommends reaching 3% performance in both precision and accuracy. Available data from interlaboratory survey programs show significant differences between instruments. Several methods exceed 3% bias when compared to results by A-K on these materials, suggesting an accuracy problem. What is difficult to assess is whether the bias is due to fundamental analytical flaws or to matrix effects caused by the lyophilized materials.

Fresh human serum must be used to determine the accuracy of a cholesterol method by comparison with A-K. In a collaborative study, patient specimens were analyzed by several commercially available methods in one laboratory. In a second laboratory (a CDC-certified cholesterol reference lab), all samples were analyzed by A-K. For the DuPont aca and the Kodak Ektachem, the instruments were operated (including calibration) according to the manufacturer's standard protocol. The Cobas FARA and Hitachi systems (both BMD reagents) used modified calibrations to gain better agreement within-lab. Regression analysis (versus A-K) provides the following data:

Analysis for all-methods gives the following:

N X Slope Y-int Sy.x B SE BD mg/dL
aca 107 229 0.99 0.20 3.99 0.999 2.24
Ektachem 111 195 1.01 -0.22 6.80 0.997 1.14
FARA 106 227 0.98 -0.15 5.56 0.999 4.95
HDL chld 107 229 1.00 3.07 7.50 0.998 3.07

Precision data for each method give CV's ranging from 1.0 to 2.7%. These four commercially-available cholesterol methods exhibit a negligible bias vs. A-K.


A simple liquid reagent for HDL cholesterol determination was developed for the IQ™ IMMUNOCHEMISTRY SYSTEM. The IQ™ SYSTEM, a compact fluorometer-colorimeter, is designed for thyroid function testing and therapeutic drug monitoring in satellite labs and physician offices. In addition, the system provides the capability to measure HDL cholesterol and other clinically important analytes.

In the HDL cholesterol assay, 200 μL of precipitating reagent (phosphotungstic acid, 1.39 mmole/L), was introduced to 200 μL of serum and a control mixture. Precipitated lower density lipoproteins were collected by centrifugation at ambient temperature for 5 minutes on a physician's compact office centrifuge. The precipitated superantigen was carried out on the IQ™ SYSTEM using a liquid cholesterol esterase/oxidase reagent.

When this method was compared with a manual HDL cholesterol assay (SCLAVO) using magnesium and dextran sulfate as precipitating agent in samples of 37 post partum sera, the following regression statistics were obtained:

correlation coefficient (r) = 0.969
slope (m) = 1.02
y-intercept (b) = -0.7

The within-assay CV of the IQ™ method was determined to be 2.7% throughout the useful range of the assay.

We conclude that the HDL cholesterol assay for the IQ™ IMMUNOCHEMISTRY SYSTEM is precise and convenient, suitable for the SPOT laboratory HDL cholesterol determinations.
UTILITY OF LIPID MEASUREMENTS IN PREDICTING CORONARY ARTERYATHEROSCLEROSIS, James C. Boyd and T. Horizon (Univ. of Virginia Med. Ctr., Div. of Clin. Pathol. and Cardiology, Charlottesville, VA 22908) (Spon.: James C. Boyd)

A group of 304 consecutive patients who underwent coronary catheterization at the University of Virginia had blood collected at the time of catheterization for measurement of apolipoproteins A-I and B-I00, total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides. Coronary angiograms were rated for the number of vessels having clinically significant (≥50%) occlusion.

Stepwise logistic regression analysis was used to identify which of these measurements were important laboratory risk factors for the presence of significant coronary arterial occlusion. The most important risk factor in the multivariate prediction model was the ratio of apolipoproteins A-I to B-I00 (P value 0.09) followed by total cholesterol (P value 5%). Apolipoprotein A-I was the next most important risk factor (P value 2.80) but was not statistically significant.

With use of the ratio of lipoproteins A-I to B-I00 and the total cholesterol concentration in a logistic function, the sensitivity of the function in predicting clinically significant coronary artery occlusion at the optimal discrimination value was 67% and the specificity was 88%. Based on the low classification accuracy achieved by this model, factors other than lipids influence the development of coronary artery disease, and lipid analyses alone should not be used to predict patient prognosis or risk of cardiovascular disease.

HDL CHOLESTEROL ASSAY FOR THE ABBOTT VISION™ SYSTEM, W. McGowan, D. Bodziony, and T. Eber (Abbott Laboratories, North Chicago, IL 60064) (Spon.: W. McGowan)

An HDL cholesterol assay has been developed for the Abbott VISION system using a pretreatment of samples with magnesium/dextran sulfate 500 ppm to precipitate the non-HDL lipoproteins (LDL and VLDL), followed by centrifugation to pellet the precipitated lipoprotein. The supernatant is then assayed for HDL cholesterol in the VISION analyzer.

The pretreatment reagent is packaged as a unit-dose tablet in a microcentrifuge tube. The assay requires 0.5 ml of sample to be added to the tube, which is then shaken for 10 seconds, incubated at room temperature for 5 minutes, and centrifuged for 5 minutes. HDL Cholesterol can be measured in serum or plasma over the range of 10 to 120 mg/dl. Between-day coefficients of variation were 1.4 and 1.6% at HDL cholesterol concentrations of 37 and 69 mg/dl, respectively. Comparison to other HDL Cholesterol assays yielded the following results:

<table>
<thead>
<tr>
<th>VISION vs.</th>
<th>A-agent</th>
<th>RAT1000</th>
<th>Hitachi 705</th>
<th>TDX</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>250</td>
<td>161</td>
<td>147</td>
<td>106</td>
</tr>
<tr>
<td>Slope</td>
<td>1.02</td>
<td>1.04</td>
<td>0.98</td>
<td>0.97</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.4</td>
<td>1.77</td>
<td>2.54</td>
<td>3.98</td>
</tr>
<tr>
<td>r</td>
<td>0.981</td>
<td>0.966</td>
<td>0.977</td>
<td>0.987</td>
</tr>
</tbody>
</table>

We conclude that the VISION™ assay is a precise, accurate, and simple system for the determination of HDL Cholesterol.

ACCURACY OF THE DRY-REAGENT CHOLESTEROL TEST ON THE AMES SERVOTHER, James L. Miller, John A. Lott (Dep't. Path. The Ohio State U., Columbus, OH 43210); Lawrence E. Crowley (Ames Division, Miles Labs, Elkhart, IN 46515) (Sponser: Lawrence E. Crowley)

Amaes has a reformulated cholesterol reagent strip for use in the Beckman Bichrom Refractance Photometer. The strip was manufactured to permit storage at RT. We compared the results on patients' specimens using the original formulation strip (2), two new formulations (II and IIT) and the Kodak Ektachem 400. Comparison Data for 100 patients are:

<table>
<thead>
<tr>
<th>Str.</th>
<th>Slope</th>
<th>Intercept</th>
<th>RMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>EK40</td>
<td>0.815</td>
<td>11.2</td>
<td>15.4</td>
</tr>
<tr>
<td>EK40 Str.IIA</td>
<td>0.968</td>
<td>3.8</td>
<td>14.2</td>
</tr>
<tr>
<td>EK40 Str.IID</td>
<td>0.967</td>
<td>6.2</td>
<td>13.7</td>
</tr>
<tr>
<td>EK40 Str.IIA</td>
<td>0.986</td>
<td>19.3</td>
<td>13.6</td>
</tr>
</tbody>
</table>

We conclude that the reformulated SeraLyzer cholesterol strips have satisfactory precision & accuracy.

PLASMA LIPIDS AND APOLIPPROPROTEINS IN PATIENTS UNDERGOING CORONARY ANGIOPLASTY, Demetrios Sioutos, Margaret Hanson, Gary Rozanski, Steven J. McGowan, James M. Spencer, (Dep't. Path. and Lab. Med. and the Andreas Cruessztag Cardiovascular Center, Emory Univ. Sch. Med., Atlanta, GA 30322) (Spon.: D. B. Sioutos)

The aim of this study was to assess the potential use of lipoproteins and apolipoproteins as specific and sensitive markers of coronary artery disease (CAD). Plasma lipids and apolipoproteins were assessed in 56 patients undergoing coronary angioplasty (GA). The patients were classified on the basis of previous coronary angiography as having a one, two or three vessel disease (groups I, II, III, respectively). Twenty six healthy control subjects were included in the study.

The 56 patients had total cholesterol (TC), low-density lipoprotein(LDL) cholesterol and apolipoprotein B(apoB) significantly (p<0.01) increased; high-density lipoprotein(HDL), cholesterol, apolipoprotein Al(apoAl) and the apoAl/apoB ratio (p<0.01) significantly decreased compared to 12 controls. The LDL/HDL cholesterol ratio was significantly (p<0.01) increased in the patients. A one way analysis of variance suggested that apoAl was a better discriminator than apoB to HDL cholesterol and TC, in that order. The apoAl/apoB ratio was the best discriminator. A one way analysis of variance showned also, significant differences among the means of the four groups. Next, the groups were grouped pairwise using Duncan's multiple range test. For variables like TC, LDL and HDL cholesterol, groups II and III were significantly (p<0.05) different only from controls not from group I. For apoAl, apoB, LDL/HDL cholesterol and apoAl/apoB ratio, controls and group I were grouped together and they were significantly (p<0.05) different from group II and III. A stepwise discriminant analysis suggested that HDL and LDL cholesterol were good markers and that apoAl, apoB and their ratio added significant discriminatory value to the information obtained by measuring LDL and HDL cholesterol alone. The results suggest that lipoproteins and especially apolipoproteins can be used as markers of CAD, although the diseases presents gray zones not defined by critical values of lipids or apolipoproteins.

A REINVESTIGATION OF THE HDL-CHOLESTEROL DETERMINATION AFTER PRECIPITATION OF B-LIPOPROTEINS

R. Schols, R. Vorbroek and R. Nelger (Diagnostic Res. Depts. E. Merck, D 6100 Darmstadt, FRG) (Spon.: U. Wurzburg)

Several precipitation reagents for the determination of HDL-cholesterol (CHOD-PAP-method containing phosphomolybdenic acid (PTA) and magnesium chloride (MgCl2), in varying amounts were investigated for their specificity and completeness in b- lipoprotein precipitation. We obtained two sets of precipitation curves, which showed that HDL-cholesterol values were constant over the range of 0.75 to 2.0 mmol/l PTA and 0.5 to 35 mmol/l MgCl2 in the assay.

A reagent was developed with a PTA/MgCl2 ratio of 1 : 6.14 adjusted to pH 3.65. The final concentration of PTA in the assay may vary from 0.9 to 1.1 mmol/l, without any change in the result. This allows for a wide range of sample/reagent ratios useful for the determination in turbid sera. The result is independent from variations of pH (3.0 - 7.0) and ionic strength (up to 400 mmol/l).

The recovery of HDL-Chol. up to 5.2 mmol/l was 100 %. Added LDL-Chol. (up to 6.5 mmol/l) and VLDL-Triglycerides (up to 16 mmol/l) did not interfere. Supersaturation reexamined by protophorultracentrifugation showed only onal sharp band at a density of d = 1.063 mg/ml.

The precision within run was 3.1 % at 0.93 mmol/l
(= 20), the precision from day to day was 3.2 %. The precipitate is very compact and does not resuspend within 16 hours.

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The first obtained results will be described. The procedure was a single intraperitoneal injection of Streptozotocin (60mg/kg B.W.). The second consisted of evaluation in a 2a e 1DL (directly measured value) with cholesterol concentration of the same duration. A simple clinical-chromatographic assay to detect LP is the thiazooarobic acid test (TIA) which hydrolyzes LP to malicindiolaldehyde, followed by fluorescence product.

In the animal study, the baseline mean and standard error for LP following an overnight fast was 0.410±0.24 μmol/L (n = 24). The value changed after injection of the diabetic condition with Streptozotocin, the median concentration of cholesterol was 17.8 ± 3.3 μmol/L (normal=6-6) and the LP value was elevated sevenfold to 2.905±0.92 μmol/L (n = 21). At 23 days, the upward trend was increased five times above normal to 6.192±0.28 μmol/L (n = 18). The concentration of LP in the treated rats was significant at p <0.002 and p <0.001 respectively.

LP values for normal rats and human obtained in this pilot study were within the range of other published reports. The elevated levels observed in diabetes suggests that lipid peroxidation may represent important factor of the disease not previously appreciated and deserves further systematic evaluation. Furthermore, the present results indicate that lipoperoxide measurements appear to correlate with duration of disease. The TIA assay is inexpensive, easy to perform and should be considered as a valuable addition to existing methodologies.

SEMPLIFIED ENZYMIC DETERMINATION OF TOTAL CHOLESTEROL IN GALL STONES
J. S. Wei, H. M. Huang, R. W. Kuo, and C. S. Wu
1. Department of Biochemistry, Chang Gung Medical College
2. Gastroenterology Unit, Department of Internal Medicine, Chang Gung Memorial Hospital at Lin Kou, Taiwan, R.O.C.
(Spon.: IRC Cote.)

Human gall stones generally fall into two categories: pigment stone and cholesterol stone. The classification into the type of stone was done on the basis of their cholesterol content determined. Gall stones containing more than 70-75% of dry weight as cholesterol were named as cholesterol stones, and those having less than 15-20% of dry weight as cholesterol were called pigment stones. The remainder were mixed stones.

The present report presents a simplified method for rapid enzymatic determination of total cholesterol contents in human gall stones. Gall stone powder was dissolved in N,N-dimethylformamide/dimethyl sulfoxide (80/20 by vol.) solution, and was reacted with a mixture of two aqueous enzymatic reagents, without further treatment. The enzymatic test was performed on 10 ml samples that are added to 1 ml of the reagent mixture (Sigma Diagnostics, Sigma Chemical Company, St. Louis, MO). The reagent mixture has the following composition: 4-aminoantipyrine, p-hydroxybenzene-sulfonate, cholesterol oxidase, esterase, and peroxidase. The presence of organic solvents do not interfere with enzymatic activities. The method is reproducible with CVs ranged from 3.7 to 6.0% (n=15 and up). The recoveries of the pure cholesterol standard in two gall stone samples were determined as 99.7% and 102.0%, respectively. The r values of the linearity test for three cholesterol stones revealed excellent, ranging from 0.997 to 0.999. Furthermore, the results obtained from the present method correlate well with those by FTR approach.

CURVATATION OF THE ISOLAB-DIRECT PLUS KIT, A. Malekpour, and S. Radad. (Central Reference Lab., Anaheim, CA 92806.) (Spon.: S. Radad)

The Isolab (Drawer 4350, Akron Ohio 44321) LDL Direct Plus Kit chromatographically separates serum lipids into Alpha (High Density) and Beta (Low and Very Low Density) fractions.

The aim of this present study was to evaluate the contents of these two fractions for Cholesterol and Apoproteins.

To determine HDL and Total Cholesterol we used the Abbott (Abbott Park, IL 60064) and the Boehringer Mannheim Diagnostic (305 W Hege Rd., Indianapolis, IN 46268) reagents. The results were measured with immunoturbidimetric procedures. A group of 43 normal to hyperlipemic persons were assessed. The results of the assay were as follows:

<table>
<thead>
<tr>
<th>TEST</th>
<th>SAMPLE</th>
<th>MEAN</th>
<th>SD</th>
<th>SLOPE</th>
<th>INTERCEPT</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Serum</td>
<td>230</td>
<td>76</td>
<td>1.033</td>
<td>-6.0</td>
<td>0.994</td>
</tr>
<tr>
<td>Chol.</td>
<td>Alpha</td>
<td></td>
<td></td>
<td>228</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beta</td>
<td></td>
<td></td>
<td>8.3</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>Supematant</td>
<td>15</td>
<td></td>
<td>0.8</td>
<td>31</td>
<td>0.84</td>
</tr>
<tr>
<td>Chol.</td>
<td>HDL Chol.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alpha</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fraction</td>
<td>19</td>
<td></td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APO-A1</td>
<td>Supematant</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL Chol.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APO-B</td>
<td>Serum</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We conclude that whereas the Isolab LDL-Direct Plus Kit has proved itself reliable in the measurement of Total Cholesterol and HDL-Cholesterol, it needs to be further tested for Apoproteins.

Monitoring Fetal Lung Maturity

EVALUATION OF THE ABBOTT TDX FLM FOR PREDICTING FETAL LUNG MATURITY, C. Andrew Robinson and Catherine N. Ketchum (Clinical Pathology, UAB, Birmingham, AL 35294) and Carly Cooper, John Russell (Abbott Lab, Abbott Park, IL 60064) (Sponsor: C. Andrew Robinson).

The L/S ratio and PG by thin-layer chromatography are the most frequently used laboratory methods for assessing fetal lung maturity in amniotic fluid. Fluorescence polarization methods of assessing fetal lung maturity have been attempted in the past but have several drawbacks: the lack of suitable standardization and expensive equipment requirements. We have evaluated a new fluorescence polarization method (FLM) developed by Abbott for use on the Abbott TDX. In this study, amniotic fluids from 104 patients (120 specimens) were compared with respect to FL0, L/S ratio, and PG. Approximately one-half of the patients were considered to be high risk patients with complications including diabetes, hypertension, and Rh incompatibility.

Utilizing L/S<2.0, PG absent, and FLM<50 mg/g to indicate immaturity, all three tests compared favorably. In our study we had 6 cases of respiratory distress syndrome: all studies indicated immaturity. FLM<70 mg/g corresponded well with L/S ratios<2.5 and PG present to indicate maturity.

Specimen preparation was also evaluated: filtration vs centrifugation at 500G and fresh vs frozen. Sample preparation by filtration was found to be rapid and simple and would allow for most standardized results avoiding the wide range of centrifugation conditions associated with L/S ratio procedures. Freezing the filtered specimen was found not to significantly affect the FLM value.

The Abbott TDX FLm is simple and rapid (total time approx. 30 min.) and offers a reliable alternative to thin-layer chromatography.

ASSESSMENT OF FETAL LUNG MATURITY BY FLUORESCENCE POLARIZATION, Ann E. Reed and David A. Herold (Path. Dept., Univ. of Virginia, Charlottesville, VA 22908) (Spons.: D. Herold)

Respiratory distress syndrome is a serious disease that occurs in premature neonates whose lungs are immature and fail to provide adequate ventilation. As the fetal lungs mature, they begin to secrete pulmonary surfactant containing phospholipids rich in phosphatidylcholine (lecithin) and, with maturity, phosphatidylglycerol. Since the fetal tracheobronchial tree is in communication with the amniotic fluid, the fetal surfactant phospholipids [lecithin (L) and sphingomyelin(S)] found in amniotic fluid has been used to utilized to determine maturity of the fetal lung. Fluorescence polarization (FP) is a procedure which uses added fluorescent dye to determine the ratio of pulmonary surfactant aggregates to albumin, providing a direct measurable property of the sample. In the presence of low amounts of surfactant aggregates, most of the fluorescent dye is bound to albumin and measured polarization is high; when surfactant is plentiful it binds most of the added dye and measurable polarization is reduced. Precise studies were performed at 3 levels and the results are reported in my surfactant per gram of albumin (mean ± SD):
Amniotic fluid samples (n=45) unconfirmed by blood or meconium were evaluated by FP and the L/S ratio as determined by thin-layer chromatography. Results show a linear relationship:

<table>
<thead>
<tr>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>run</td>
<td>14.9±0.4</td>
<td>47.1±1.4</td>
</tr>
<tr>
<td>run-to-run</td>
<td>16.7±2.4</td>
<td>48.7±2.1</td>
</tr>
</tbody>
</table>

This FP method has the advantages of good precision, ease of performance and speed of analysis. There is reasonable correlation between the L/S ratio and FP methodology. This FP assay should be clinically evaluated as an efficient alternative to L/S ratios in assessing fetal lung maturity.

**RAPID ASSESSMENT OF FETAL LUNG MATURITY BY NUMERICALLY REPORTED "FOAM TEST" RESULTS, S. N. Milliner, E. Katz, and N. Buchanan (Dept. Path., Univ. Illinois, Chicago, IL 60680) (Spon.: S. N. Milliner)**

Amniotic fluid (AF) lecithin/sphingomyelin ratio (L/S) and qualitative phosphatidylglycerol (PG) assays remain the standard tests to evaluate fetal lung maturity. An L/S >2.0, determined by thin-layer chromatography of acetone-preincubated HXJ3x extracts, and the presence of PG indicate a sufficient level of alveolar surfactant. Considerable time and skill are required to generate valid results. Other tests are faster and easier, but many sailors are more confident when such results are confirmed by L/S+PG.

Clements introduced the surfactant titer (ST) ("foam" or "shake" test) to measure surfactant. This is a qualitative visual assessment of the ring of bubbles remaining at the fluid surface 15 min. after vigorous shaking of 3 tubes containing AF serially diluted 1:1, 1:2, 1:4, 1:8, 1:16, 1:32. The number of bubbles within the middle tube is scored: 0 (none), +; 1, a half ring; and ++ if the amount of surfactant is insufficient to stabilize more than a few bubbles. The index is used to record and compare results and it is difficult to compare results with those of other tests. To make ST more useful, a numerical score was devised here, in which 0.0 corresponds to +++, 0.5, to +, and 0, to -. Thus, a dilution series which would be recorded as +++, +++, +++, +++, +++) is 5.0. +.00.0.0.0. = 1.0. +, etc. This system has proved effective and unambiguous in testing several thousand AF.

To ascertain if ST is available several hours before L/S, could be used to predict L/S and allow prompt clinical action or to avoid a lengthy L/S, results of the clinical decision 300 analyzed by AF compared. AF was grouped by ST: 22-76 AF in each of the 7ST from 0 to 5.0. 97% of AF with ST>0 were immature by L/S, 95% by PG. AF with ST>2.0 were mature by L/S in 3 and 2.9% by PG in 95% of cases, and transitional in the remainder. While immature by L/S+PG increased with ST from 0.5 to 1.5, predictions in individual cases would be unreliable. ST of 0 or ST>2.0 show good correlation with lung maturity as predicted by the other test criteria.


The amniotic fluid L/S ratio, with additional detection of PG, is considered the standard laboratory test for directly assessing fetal lung maturity (FLM), and predicting hyaline membrane disease (HMD). The methodology is subject to numerous preanalytic and analytic sources of variation, making interlaboratory comparison difficult. Intraobserver evaluation is necessary to improve clinical reliability of the test. An L/S ratio of 2.0 or greater has been widely accepted as indicative of FLM. This figure was included in a range of values to create clinical decision values at Vanderbilt. L/S ratios obtained at this institution in the past 39 months were reviewed. Of the cases, 72% delivered within 72 hours after the test, and were included in a retrospective clinical evaluation. The diagnosis of HMD was based on rigorous clinical criteria. Selected levels of L/S ratios were categorized according to PG status (present or absent), and compared for determining probability of HMD.

**TABLE 1**

<table>
<thead>
<tr>
<th>PL RESULTS</th>
<th>PROBABILITY OF HMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>2.0-3.4</td>
</tr>
<tr>
<td></td>
<td>3.1%</td>
</tr>
<tr>
<td>+</td>
<td>2.0-3.4</td>
</tr>
<tr>
<td></td>
<td>33.3%</td>
</tr>
<tr>
<td>+</td>
<td>2.0-3.4</td>
</tr>
<tr>
<td></td>
<td>3.4%</td>
</tr>
</tbody>
</table>

The results indicated a high risk of HMD with L/S values <2.0, which sharply decreased as the L/S ratio increased from 2.0 to 3.4, with near elimination of risk seen with values >3.5. These ranges allowed grouping of the L/S values into categories of immature (<2.0), borderline (2.0-3.4), and mature (>3.5) fetal lung states for clinical decision making in obstetrical practice.

**A COMPARISON OF FLUORESCENT POLARIZATION, L/S RATIO, SATURATED PHOSPHATIDYLCHOLINE AND UNSATURATED PHOSPHATIDYLCHOLINE IN AMNIOTIC FLUID IN THE EVALUATION OF FETAL LUNG MATURITY, Katherine M. Cooper, Michael Y. Tsai (Laboratory Medicine and Pathology, University of Minnesota Hospital and Clinic, Minneapolis, MN 55455) (Spon: Michael Y. Tsai)**

Fluorescent polarization (P) of amniotic fluid has been studied as a means of predicting fetal lung maturity. A recent study utilizing synthetic test mixtures, demonstrated that P is affected by the presence of unsaturated phospholipids and albumin (Clin. Chem. 33:1195, 1987). In the current study, we correlated fluorescent polarization with the L/S ratio, phosphatidylglycerol (PG), saturated phosphatidylcholine (PC) and unsaturated PC in amniotic fluid. Net fluorescent polarization was measured by an assay developed for use with an Abbott ABS-9000. The fluorescent probe NBD-PC. The L/S ratio, saturated PC and unsaturated PC were measured by thin layer chromatography. Unsaturation PC was calculated as the total PC minus the ovalbumin-insensible PC. Gas chromatography analysis of fatty acid methyl esters derived from the unsaturated PC fraction indicated that most unsaturated PC were mono rather than di-unsaturated.

Linear regression analysis showed that P in amniotic fluid samples (n=46) correlated with L/S ratio, saturated and unsaturated PC (r=0.90, 0.90, -0.08). However, using the cutoff values of P>0.26, L/S>3.0, P disagreed with L/S ratio in 8 out of 46 cases. Moreover, the presence of PC has little effect on P. In 11 unincubated samples obtained by abdominal tap, and with L/S ratios between 3 and 4, PC was present in 3 samples, all of which had fluorescent polarization results greater than 0.26, indicating fetal lung immaturity. Fluorescent polarization may be useful only as a screening test. More studies are needed to determine the relationship between microviscosity measurement, by fluorescent polarization and the amount of fetal lung surfactant present in the amniotic fluid.

**ELECTROLYTES, METABOLITES AND URNAISALYSIS**

**POSTER SESSION 3:30pm-5:30pm**

**Jan S. Brouwer (Ciba Corning Diagnostics, G3 North Street, Medfield, MA 02052) (Spon: P. D'Orazio)**

We evaluated the Ciba Corning 634 iCa++/pH analyzer. The auto-calibrating 634 iCa++/pH meter contains ion sensors with micro electrodes in 35 ul of whole blood, plasma or serum. Results, reported within 60 seconds, include the option for pH adjustment. A total of 135 whole blood, 76 plasma, and 156 serum samples were run in duplicate on both the 634 and Radiometer ICA1 analyzers over seven days. Due to the limited range of the patient samples (0.8-1.7 mmol/l for iCa++ and 7.10-7.64 for pHe), paired sample analysis rather than linear regression was used. Comparison results for whole blood and precision for a control (in the above range) showed:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean Diff</th>
<th>95% CI</th>
<th>Precision+</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-0.025mmol/l</td>
<td>-0.04 -0.06</td>
<td>2.5VC 2.5%</td>
</tr>
<tr>
<td>iCa++</td>
<td>0.003</td>
<td>-0.005 -0.005</td>
<td>0.007 0.008</td>
</tr>
</tbody>
</table>


1234 CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988
The infrequent use of Magnesium reagents during the working day makes the preparation of calibration methods difficult because the CO2 absorption contributes to a spectral shift. The addition of cyanide for stabilization was not acceptable due to reagent toxicity. We investigated the use of Xylydyl Blue and formulated stable reagents which can be adapted to most automated instruments.

Our reagent contains 0.2 mM Xylydyl Blue, 0.045 mM EDTA, TRIS-carbonate buffer to a pH of 11.6 and surfactant.

The reagent was optimized to negate the effects of calcium, iron, bilirubin, and lipemia. Serum levels up to 10 mg/L responded linearly with 0.001 M hydrochloric acid to avoid precipitation. Calibration is independent of the matrix used.

The method was applied to RA-System, ASSIST, COBA-BIO, ANALYZER II, DEMAND.

**Correlation data of RA-1000 with Atomic Absorption were as follows:**

<table>
<thead>
<tr>
<th>METHOD (x)</th>
<th>SLOPE</th>
<th>INTERCEPT</th>
<th>r</th>
<th>Syx</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA-1000</td>
<td>0.997</td>
<td>0.137</td>
<td>1.000</td>
<td>0.09</td>
</tr>
</tbody>
</table>

We conclude that the Xylydyl Blue method is suitable for routine analysis of Magnesium levels in biological fluids.

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**401 MAGNESIUM CONTENT AND CONCENTRATION OF MONONUCLEAR BLOOD CELLS AND T LYMPHOCYTES COMPARED.**

C.M., Huang, Thomas A. Flesher and Ronald J. Elin (C.Clin. Pathol. Dept., NIH, Bethesda, MD 20892) (Sponsor: Sam Steinhach)

Magnesium (Mg) has been implicated in cardiac arrhythmias, hyper tension, atherosclerosis, tetany, and premenstrual syndrome. The mononuclear blood cell (MBC) Mg may be a better indicator of Mg status than either serum or red blood cell Mg. MBCs are a heterogenous population composed of lymphocytes (primarily T lymphocytes) and monocytes. A significant difference in the Mg concentration or content between lymphocytes and monocytes could alter the result for Mg Mg.

Therefore, we determined the Mg content and concentration of MBCs and T lymphocytes. MBCs were isolated from the buffy coat of normal donors by a discontinuous gradient using Ficoll-hypaque. T lymphocytes were separated by the anti-human F (ab')2, monolayer technique. The purity of T lymphocytes (T + NK cells) was 98±2% by flow cytometric analysis. Mg in a lysate of MBCs and T lymphocytes was determined by atomic absorption spectrophotometry.

The Mg content and concentration of MBCs (n=12) were 61.9±10.3 fg/cell and 10.6±1.5 mmol/L (mean ± SD), respectively, and of T lymphocytes (n=12) 60.8±9.2 fg/cell and 11.8±1.5 mmol/L, respectively. The Deming de-biased regressions between Mg (x) and T cells (y) were $y = 0.88x + 4.4$ for Mg content, and $y = 0.99x + 1.3$, $r = 0.85$ for Mg concentration. We conclude the Mg concentration of T lymphocytes is significantly higher (p < 0.001) than MBC. However, there was no significant difference for Mg content between MBC and T cells. Thus, the composition of MBCs may affect the result for the Mg concentration.

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Ficoll-hypaque gradient separation of mononuclear blood cells (MBC) from healthy normal volunteers consists primarily of lymphocytes (L) and monocytes (M) with relatively few granulocytes (G). We reduced M and G (phagocytic cells) in the population of cells by treatment of the blood with carboxyl iron particles (CIP). We determined the content (mmol/cell) and concentration (mmol/L) of magnesium (Mg) in MBC from 50 volunteers (21 males and 29 females, ages 17-75, mean 33.3 years) with and without CIP. Four mL of a suspension (1:1 CIP, 15 bovine serum albumin, 0.2% buffered saline, 0.5% formalin) was added to achieve units of concentration. Cyto centrifuge smears were made and a differential count performed. The results (mean ± SE) are the following:

<table>
<thead>
<tr>
<th>Content</th>
<th>Concentration</th>
<th>L($\ell$)</th>
<th>M($$)</th>
<th>O($$$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mmol/cell)</td>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CIP</td>
<td>3.4±0.09</td>
<td>10.5±0.3</td>
<td>86.5±0.7</td>
<td>13.6±0.7</td>
</tr>
<tr>
<td>CIP</td>
<td>2.3±0.05</td>
<td>9.5±0.3</td>
<td>96.1±0.2</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.002</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

---


The relationship between age and blood magnesium (Mg) parameters has not been defined. Three blood Mg parameters (plasma, red blood cell (RBC) and mononuclear blood cell (MBC)) were determined in 84 normal volunteers (32 male and 52 female, ages 11-75 years). The method of separation of MBC has been described previously (Clin Chem 1985, 31:377) with the additional measurement of the total volume of cells in the lysate (to achieve units of concentration). The mean value (± SEM) of 84 normals was as follows: Plasma Mg 2.3±0.3 mmol/L, RBC Mg 2.32±0.31 mmol/L, MBC Mg concentration 3.0±0.48 mmol/L. We compared these data with age (intervals of ten years) using analysis of variance with the following results:

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Plasma Mg</th>
<th>RBC Mg</th>
<th>MBC Mg</th>
</tr>
</thead>
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<td>70</td>
<td>4</td>
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<td>0.8±0.06</td>
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</tbody>
</table>

We found no significant relationship based upon age of the individual among the three Mg parameters (ANOVA p>0.05). Thus, plasma, RBC and MBC Mg parameters do not vary significantly between the ages of 11 to 75 years.

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**404 ADAPTED SERUM POOLS FOR THE STUDY OF THE INFLUENCE OF SODIUM AND ALBUMIN VARIATION ON IONIZED CALCIUM MEASUREMENTS BY ISE Yves Gourselin, B. Gougat, A. Truchaud. Biochemistry Dept., Neum Hospital, 7104 MEAUX FRANCE (Sponsor: Gourselin)**

Ionic strength and albumin content variations in a sample are known to modify respectively activity and binding of ionized calcium (Ca$^{2+}$). To study the response of Ion Selective Electrodes (ISE) analyzers for Ca$^{2+}$ measurements we prepared serum pools using dialyzed bovine serum pools for low sodium concentrations (total calcium, K, glucose, proteins and creatinin were in normal ranges). Sodium Chloride was added to increase ionic strength (Na from 100 to 200 mmol/L). On the other hands were prepared by dilution of human albumin for infusion (200g/L) and addition of electrolytes and metabolites in normal ranges to get solutions with albumin from 20 to 120 g/L and other parameters constant. Serum pools and solutions were buffered with HEPES at 10 mmol/L and pH adjusted at 7.40. The pools and solutions were frozen in vacuum tubes. We controlled these pools with a NOVA SPI blood gas analyzer permitting Na, K, Ca$^{2+}$ and pH measurement on a single sample. Seven different analyzers (NOVA B, RADIOMET ICAI, AD, CIBA CORNING 634, FRESENIUS Ionometer, AVL 984, KONE Microlot) were tested with these pools. Results showed:

- Increase of ionized calcium of 1.00 mmol/L when albumin varied from 40 to 120 g/L.
- For sodium variation, all the instruments gave increasing results of 0.15 mmol/L of ionized calcium for a variation of sodium from 120 to 200 mmol/L. The AVL 984 gave the same response that the other analyzers despite a correction of ionized calcium as a function of sodium concentration.

Conclusion: adapted solutions used as specimens are a very simple way to test ISE instruments and permit to know what kind of bias is obtained with albumin or ionic strength variations.
BLANK CORRECTED MAGNESIUM METHOD FOR THE TECHNICON SMAC. Sue Brown and W. Greg Miller, Department of Pathology, Medical College of Virginia, Richmond, VA 23298-0597 (SPON: S. Brown).

We have adapted the calmagite magnesium procedure reported by Abermethy and Fowler for the SMAC (Clin Chem 1982;28:520-5) which used Empigen BB detergent to enhance the sensitivity and the adaptation of Liebke and Kroon to the Cobas-Bio (Clin Chem 1984;30:1801-4) employing specific detection of the chelate with EDTA to correct for serum blank absorbance. Chelating reagent concentrations were 0.9 mmol/L calmagite, 0.1% Empigen BB, 0.4 mmol/L EGTA, 0.4 mmol/L triethanolamine, 4.8 mmol/L strontium chloride, 0.1 mol/L 2-amino-2-methyl-1-propanol, pH 11.5. Blank reagent was 18.8 mmol/L EDTA, 0.1% Empigen BB, 0.1 mol/L 2-amino-2-methyl-1-propanol, pH 11.5. Pump tubing was 492 + 56B μL/m in for chelating reagent, 37 μL/min for blank reagent and 37 μL/min for sample from the riser. The manifold was designed using polyethylene tubing and measured the chelate absorbance 16 sec after mixing, then added EDTA and measured the blank absorbance 185 sec after mixing. The method was linear to 70 mg/L. The within day precision (CV) using control material was 2.1% at 22 mg/L (N=69) and 1.9% at 105 mg/L. There was no clinically significant interference due to bilirubin (160 mg/L), calcium (134 mg/L), iron (6mg/L), hemoglobin (6.2 g/L) and triglyceride (15 g/L using intralipid). A comparison of the SMAC method to atomic absorption spectroscopy (AAS) gave SMA = 0.96 AAS + 1 mg/L; N=60, range 9-69 mg/L, standard error of the estimate 1 mg/L, SMAC mean 23 and AAS mean 23 mg/L.

COLOMETERS FOR THE DETERMINATION OF SERUM CALCIUM ON BOEHRINGER MANHERR|HITACHI ANALYZERS. Yuh Shiong Wu, N. Simons, M. Cecile, P. Nolan, W. Collinsworth (Boehringer Mannheim Diagnostics, 915 Hague Road, Indianapolis, IN 46250) and Lawrence Kaplan (University of Cincinnati Medical Center, Cincinnati, OH 45267). (SPONS: B. Site)

A colorimetric method for determination of serum calcium using o-cresolphthalein (OCPC/EDTA) has been developed for Hitachi 737 analyzers. Calcium reacts with OCPC in the presence of 8-quinolinol-sulfate to form a purple chromophore. Total absorbance at 570/660 nm is measured and EDTA is then added to reverse the complexation of calcium with OCPC. A second absorbance reading provides an accurate sample and reagent blank. The difference of the two absorbances is proportional to the amount of calcium in the specimen. Performance parameter statistics for the Hitachi 737 are as follows: 1) linearity up to 20 mg/dL, excellent total imprecision (16.6 mg/dL, CV = 1.3%; 8.6 mg/dL, CV = 1.9%; 4.7 mg/dL, CV = 2.3%), and excellent accuracy [OCPC/EDTA = 1.001 ± 0.108 mg/dL, r = 0.9981, n = 105; OCPC/EDTA = 0.98 ± 0.04 (mg/dL, r = 0.995, n = 60).

Another colorimetric assay (OCPC/GABA) for serum calcium using OCPC in gamma-aminobutyrate (GABA) buffer (pH 10.5) has been developed for Hitachi 704, 70-5 and 717 analyzers. Calcium reacts with OCPC in the presence of 8-quinolinol to form purple chromophore. The intensity of the final reaction color is measured bichromatically at 570/660 nm and is proportional to the amount of calcium in the specimen. Performance parameter statistics for the Hitachi 704 are as follows: linearity up to 20 mg/dL, excellent total imprecision (16.3 mg/dL, CV = 1.9%; 9.8 mg/dL, CV = 1.3%; 5.3 mg/dL, CV = 1.9%), and very good accuracy [OCPC/GABA = 0.98 ± 0.09 (mg/dL, r = 0.993, n = 60). No significant interference was observed from magnesium spiked, lipemic, icteric or hemolyzed sera.


An improved calcium method using Arsenazo III offers the following advantages over the existing CPC method: 1) It functions at a slightly acidic pH which decreases the absorption of carbonate. This allows for better on-line (open-tube) stability and less frequent calibration. 2) Better precision of measurement is achieved by doubling the sensitivity as compared to the CPC method.

The reagent consists of two liquids, a dye reagent (Arason III) and an EDTA reagent. The assay methodology functions by first complexing the sample with Arason III and then removing the calcium ion from the complex with a strong chelating agent, EDTA. This eliminates sample interferences and reagent pipetting inaccuracies, since absorbance change caused only by calcium removal is observed.

The assay is performed at 600 nm on COBAS MIRA and 650 nm on the COBAS B104 and COBAS PARA with a total analysis time for a single sample of two minutes. The linear measuring range extends from 0 to 15 mg/dL. Precisions for normal and abnormal controls have coefficients of variation of less than 5%. Correlation with the Roche CPC methodology yields a slope of 0.938, an intercept of 0.43, and a correlation coefficient of 0.993, n = 100.

In summary, this Arsenazo III reagent produces accurate and precise results with better sensitivity and stability than the CPC method. The buffer system offers greater pH stability by eliminating carbonate absorption. The use of EDTA eliminates interferences caused by non-specific absorption, measuring only absorbance change due to the binding of calcium ions.

ABSTRACT WITHDRAWN


A new calcium reagent has been formulated for use on the Monarch Analyzer. The new procedure for the determination of calcium is based on the formation of a purple complex between calcium and o-cresolphthalein (OCPC) in an alkaline solution. The changes to the formulation include: an increase in the CPC concentration, removal of surfactant, and the addition of a blank corrector. The following 30°C data were obtained using NCCLS guidelines.

DETERMINATION OF SODIUM AND POTASSIUM IN BIOLOGICAL FLUIDS BY ION SELECTIVE ELECTRODES BASED ON CROWN ETHER COMPOUNDS. Takenori Goto, Toru Sakaki (Fujiwara Research Lab, Tokuyama Soda Co., Ltd., 205 Endo Fujisawa-shi, Kanagawa, Japan), Hiroshi Yamagami (Analytical Instruments Co., 320-11 Hino, Hino-shi, Tokyo, Japan) Kinya Kawanoto, Mitomo Sekiguchi (Dept. of Clin. Lab, Nihon Univ. Hosp., 30-1 Oyamaguchi Kamisaki, Itabashi-ku, Tokyo, Japan) (SPONS: Yulchi Miura)

We have developed a rapid electrolyte analyzer(PVA-4) using novel sodium and potassium selective electrodes. (Chloride determination is carried out by coulometric titration. These electrodes are based on cyclic polyethers(crown ethers) instead of conventional NAR glass or valinomycin. The quick response of these electrodes(2sec) permits rapid determination of sodium and potassium in biological fluids(100 samples per hour).

Typical evaluation results were as follows. Within-run precision(mean, n=50)

<table>
<thead>
<tr>
<th>Na</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>X</th>
<th>Y</th>
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<tr>
<td>4.39</td>
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These results indicate that our crown ether based electrodes have sufficient applicability for clinical analysis.
Sodium tetraptophylborate has been the basis of a quick, sensitive, specific turbidimetric assay for Potassium in serum. We refined the test and adapted it to an automated system.

Chemically, the potassium ion rapidly forms an insoluble precipitate with the tetraptophylborate ion, with a solubility product constant of 2.25 x 10^-33. The reaction occurs over a wide range of pH, however a pH of 9.0 appeared to be best suited for this assay. Common components in serum such as triglyceride, sodium, magnesium, calcium, and ammonium yielded essentially no interference.

The EMD Potassium assay for the EMD 88 EASY ST analyzer is formulated into a powder blend of reagents in a single cuvette. The assay is conducted as endpoint reaction, with measurement of turbidity at 423 nm. The interassay variance CV is 2.8% and the intra-assay variance CV is 3.3%. Correlation with flame photometry (n = 50) is 0.984 with the linear equation being Y = 0.960X + 0.242. For instrument to instrument variance CV is 3.3%; the linearity of the test is up to 8.8 mg/dL. The test is used with its ease of performance appears to be ideally suited for the physician's office.

Anions

Biobol bicarbonate reagent provides linearity up to 50 mmol/L for fifteen days after reconstitution at 2-8°C. The % drift of the reagent baseline after 15 days reconstitution allows for a calibration frequency of once every 3 days.

The reagent is a three minutes end point method utilizing the phosphogluconate dehydrogenase coupled enzyme system. The decrease in absorbance of NADH measured at 380 nm is directly proportional to the concentration of bicarbonate in the sample.

Correlation with BMD Bicarbonate reagent on Hitachi 7150 using 59 patient serum samples yielded regression line of Biotrol = 0.98 BMD + 3.02, with a correlation coefficient of 0.97. The total imprecision CV were 3.23, 3.08, and 2.53 for bicarbonate level of 20.7 mmol/L, 32.0 mmol/L, and 43.4 mmol/L, respectively.
The new electrode was evaluated on the 736 analyzer. The correlation between the new chloride (Y) and coulometry (X) for patient samples ranging from 90 to 116 meq/L is excellent (Y = 1.00 X + 0.90, N = 50, R = 0.9682, Syx = 0.62). Within-run precision gave a total CV of 0.64%; total precision gave an SD of 0.52 meq/L at 105 meq/L. The dynamic range of the new electrode is 40 to 160 meq/L. The correlation between the old and new chlorine (Y) and coulometry (X) for 52 commercial control sera was determined:

Old electrode: Y = 0.82 X + 25.4, R = 0.9350, Syx = 10.8
New electrode: Y = 0.99 X + 0.69, R = 0.9836, Syx = 2.30

The average recovery using the old electrode was 105% and ranged from 94 to 138%. The average recovery using the new electrode was 98% and ranged from 95 to 103%.

416 HIGHLY ELEVATED LACTATE AFFECTS CARBON DIOXIDE LEVELS IN THE RAT

The correlation between the new chloride (Y) and coulometry (X) for patient samples ranging from 90 to 116 meq/L is excellent (Y = 1.00 X + 0.90, N = 50, R = 0.9682, Syx = 0.62). Within-run precision gave a total CV of 0.64%; total precision gave an SD of 0.52 meq/L at 105 meq/L. The dynamic range of the new electrode is 40 to 160 meq/L. The correlation between the old and new chlorine (Y) and coulometry (X) for 52 commercial control sera was determined:

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Nitrogen Reagents

417 DETERMINATION OF SERUM L-KYNURENINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Earle W. Holmes (Loyola Univ. Med. Ctr., Maywood, IL 60153) (Spon.: Earl W. Holme)

Increases in the concentration of L-kynurenine (KYN) in serum often result from the induction of tryptophan degrading enzymes and/or indoleamine dioxygenase by various physiological and pathological stimuli. Thus, the serum KYN concentration is a useful index of oxidative tryptophan metabolism in vivo. I have developed a simple, direct assay for the determination of serum KYN based on a fluorometric reverse-phase HPLC. Protein-free supernatants (50 μl) prepared by treating serum with 1/10 vol of 2.4 M perchloric acid were analyzed on a 4.6 x 15 cm column of Ultrasphere ODS (Beckman Instruments, Berkeley, CA). The mobile phase, consisting of 2% acetonitrile in 0.1 M acetic acid, 0.1 M ammonium acetate, pH 4.7 was pumped at 1.5 ml/min and KYN was detected by its absorbance at 274 nm at a sensitivity of 0.009 AUEFS. Serum concentrations were determined based on peak height relative to those of aqueous KYN standards.

Recovery of KYN from human serum averaged 101 ± 2% (n=9) in the concentration range of 0.38 - 1.4 μg/ml. There was a linear relationship between KYN peak height and concentration in the range of 0.25 to 3.0 μg/ml. Recovery of KYN for the assay averaged 7.7% and 9.3% at mean serum KYN concentrations of 0.45 and 1.28 μg/ml, respectively. Serum KYN was normally distributed in a group of 40 apparently healthy, fasting adults (x = 0.47 μg/ml; SD: 0.10; median: 0.46; range: 0.27-0.69). Serum KYN was significantly elevated in pre-dialysis specimens from 20 patients with chronic renal failure (x = 0.83 μg/ml; SD: 0.35; median: 0.80; range: 0.28-1.38; t=6.15, p<0.0001 vs. reference population). Since KYN appears to compete with tryptophan for carrier mediated uptake into brain and also possesses convulsant activity in experimental animals in vivo, an increased serum KYN may be one of the factors responsible for the encephalopathy that is frequently associated with the uremic state.

418 HIGH-PRECISION UREA NITROGEN DETERMINATION ON DISCRETE ANALYZERS

S. Klotzsch and M. Ruman, Technicon Instruments Corp., MAD, Tarrytown, NY 10591. (Sponsor: M. Ruman)

Endpoint determinations are usually more precise (lower CVs) than kinetic techniques, even if frequent calibration is added. The reason is that the precision noise ratio in the optical systems when the endpoint mode is used.

Most discrete analyzers cannot pre-dilute samples. The sample to reagent ratio is too high for an endpoint determination of urea nitrogen at 340 nm, following the urease-glutamate dehydrogenase reaction. For those instruments which have a "bichromatic" filter in the 275 nm region, measurements can be made at the spectral shoulder. We developed an endpoint method with significantly improved precision which we applied successfully on RA-1000, COBAS-BIO, and OLYMPUS DEMAND. The total CV for RA-1000 was 2.05 at a level of 30 mg/dL. Comparison of the kinetic versus endpoint method gave the following data:

METHOD(1) H SLOPE INTERCEPT F SYX
Endpoint, 376 nm 50 1.0 0.43 0.99 1.0
Y = Kinetic, 340 nm

Our working reagents were stable for 6 weeks without recalibration. The endpoint method has the benefit of better precision and smaller calibration stability needed to known Reagents. However, extreme high levels of free ammonia and certain abnormal globulins may affect the recovery of the true value.


In 1981, the uric acid methods on Technicon BMA 19 28 38 were converted to uricase to improve specificity. These methods utilized a base-enzyme cell. Recently Technicon has introduced flavocytochrome BMA Uric Acid and BMA Uric Acid-B. These methods are modified to use free uricase in place of the base-enzyme cell. In addition, the new reagents incorporate absorbance endpoint and are intended to reduce interference as well as a new substrate, 5,8-dimethylxanthine. The color reaction also uses 4-aminopyrazole and paraamido. The uricase forced is read at 290 nm. Each system has a new substrate which use a shorter K-line path length, a direct sample and improved reagents.

The proposed candidate reference method for uric acid is that described in NCLLM TEC-B by Dennis, P., et al. Development and Evaluation of a Candidate Reference Method for Serum (or Plasma) Uric Acid. The reaction is as follows:

Uric Acid + H2 = H2O + 02 MII03383 Assay: H2O + 02

Uricotinometric measurements of serum and plasma are taken before and after incubation with Uricase. Preparations are precipitated by 5% trichloroacetic acid and the supernatants are standardized with an aqueous solution of N,N,N trimethyl uric acid.

Forty-eight human samples were analyzed by each method. The sample concentrations range from 2.7 to 7.7 mg/dL and are distributed as described in NCLLM in EPS-P.

Method Regression Parameters (Patel) Y= b0 + b1 X
Reference BMA Y (Pur) y = 1.05x - 0.10 0.48 0.19 48
Reference BMA Y (Pur) y = 1.05x - 0.10 0.48 0.19 48
Reference BMA Y (Solv) y = 1.05x + 0.08 0.38 0.19 48
Reference BMA Y (Solv) y = 1.05x + 0.08 0.38 0.19 48

The new reagents for BMA and BMA show good correlation with the Proposed Candidate Reference Method.

420 URIC ACID "ENDPOINT" DETERMINATION ON DISCRETE ANALYZERS, USING 1- AND 2-REAGENT FORMULATIONS, M. Ruman and S. Klotzsch, Technicon Instruments Corp., MAD, Tarrytown, NY 10591. (Sponsor: A. Toth)

Reagents for Uric Acid determinations on discrete analyzers were formulated in one-reagent or two-reagent configurations using the enzyme Uricase coupled to the Trinder reaction sequence. Refrigerated working reagents are stable for at least 30 days and no recalibration is needed.

The degree of Millirubin interference varies with the ratio of its free to conjugated bilirubin in the specimen, the free bilirubin has a greater effect: Levels above 10 mg/kg can decrease the results.
The CV on RA-System was 2.4% at a level of 2.4 mg/dL and 1.5% at a level of 9.6 mg/dL. Linear regression analysis provided the following data:

**METHOD**<sup>a</sup>  | **S**  | **Slope**  | **Intercept**<sup>b</sup>  | **r**<sub>S</sub>  | **Sxy**  |
---|---|---|---|---|---|
1. RA-systems | 102 | 0.887 | 51.198 | 0.969 | 0.857 |
2. SNAC-II | 79 | 1.041 | 0.112 | 0.996 | 0.257 |

<sup>a</sup>Y = RA-reagents, one-reagent formulation

<sup>b</sup>Y = RA-reagents, two-reagent formulation

**MULTIPLE**

We conclude that the endpoint method for uric acid is advantageous for attaining high precision and accuracy, making this formulation the best choice for routine laboratories.

**Effect of 5-Fluorocytosine on Serum Creatinine Concentration of a Patient Receiving Drug Therapy with a Cephalosporin, a Carboxypeptidase Inhibitor, and Cytosine Antagonists**

Fluorocytosine is an antifungal drug used for the treatment of Candida and Cryptococcus. This drug is excreted primarily by the kidney and not metabolized significantly by man. Accurate evaluation of kidney function is necessary to correctly adjust the dosage in order to assure efficacy and avoid adverse reactions. Treatment may be prolonged and its necessity may be dependent on changes in renal status. Serial serum creatinine concentrations are measured over a month from a patient receiving 5-fluorocytosine and ceftazidime (a cephalosporin) among other drugs for cryptococcosis and candidiasis. Creatinine concentrations are measured by the Jaffe<sup>†</sup> reaction on the Beckman AXA VIII, ACA III, Hitachi 705, and Kodak Ektachem 700 according to manufacturers' instructions. Results are compared to those obtained by high performance liquid chromatography (HPLC). Jaffe<sup>†</sup> methods are significantly higher than by HPLC. Kodak enzymatic method results are five times higher than creatinine concentrations obtained by HPLC and do not follow the trend shown by the Jaffe<sup>†</sup> methods and HPLC. Within the same day, concentrations varied as much as 20 mg/L during the month, variation varied from 43 to 147 mg/L. This interference with the Kodak method obscured the patients changes in renal function. The patient also received ceftazidime, as well as other drugs during this time. Precise interference of cephalosporin antibodies with the Jaffe<sup>†</sup> reaction can also obscure changes in renal function of these patients. Data obtained from this patient illustrates the complexity of interpreting laboratory tests when dealing with multiple drugs. In this case the cephalosporin interfered with the Jaffe<sup>†</sup> reaction and the 5-fluorocytosine interfered with the enzymatic reaction. The HPLC method shows no interference from these drugs when they are added to serum or water.

**Evaluation of Interference of Cephalosporin Antibiotics with Serum Creatinine Concentrations**

Changes in serum creatinine concentration in hospitalized patients is used by physicians to evaluate changing renal function. Numerous drugs used in the hospital setting interfere with creatinine measured by the Jaffe<sup>†</sup> reaction. Cephalosporin antibiotics are commonly listed as interferences in this assay. Ten cephalosporin antibiotics are evaluated for interference with the Jaffe<sup>†</sup> reaction on the Beckman AXA VIII, DelPha, ACA III, Hitachi 705, Kodak Ektachem 700, and high performance liquid chromatography (HPLC) creatinine assay methods according to the NCCI<sup>2</sup> 's Proposed Guidelines for Interference Testing in Clinical Chemistry. Serum creatinine concentration in a group of patients receiving cephalosporin antibiotics with and without concomitant renal disease was evaluated and compared to summed concentrations obtained by HPLC. Our data show that the cephalosporin tested, cefadroxil, cephalaxin, and cefoxitin when added to normal serum at therapeutic levels result in positive interference of up to 30 mg/L as measured by instruments utilizing the Jaffe<sup>†</sup> reaction. Serial serum creatinine concentrations from patients receiving these drugs 18 to 28 mg/L higher as compared to concentrations measured by HPLC. None of the cephalosporins tested interfered with the Kodak Ektachem enzymatic creatinine method.

**Kinetic Method for Determination of Serum BUN Using Bio-Rad Mannheim/Hitachi Analyzers**

Yih Shiong Wu, Melissa Rode, Cindie Nee, Mary Cecill Jean Johansson, Mark Simmons and Harrison Site, (Bio-Rad Mannheim Diagnostics, 9115 Hague Road, Indianapolis, IN 46250-0100) (Spons: Yih Shiong Wu)

A kinetic BUN reagent kit (2 vial system) using glutamate dehydrogenase (GLDH)/urease as the coupling enzyme was developed for Roche Mannheim/Hitachi 704, 705, 737, and 737 analyzers. The reagent has been optimized such that the GLDH-catalyzed reaction is the rate limiting step. In this case, the GLDH has a large Michaelis constant with respect to its respective substrate concentration (Km). The reaction follows pseudo-first-order kinetics. The amount of urea nitrogen in a sample is calculated by determining the OD of the reagent at each rate using a microprocessor controlled microcomputer. The performance characteristics on Hitachi 705 include the following: Linearity to 200 mg/dL, total imprecision (mean = 15.0 mg/dL; CV = 2.5%) to 29 mg/dL, CV = 1.3%; mean = 69.7 mg/dL; CV = 1.5%; comparable accuracy | (Y'[(kinetic on Hitachi 705) = 1.005X (kinetic on Hitachi 705) - 0.447 mg/dL, r = 0.9988, n = 85 on Hitachi 705; (kinetic on Hitachi 704) = 1.009X (kinetic on Hitachi 705) - 0.009 mg/dL, r = 0.9988, n = 85 on Hitachi 704].

**Frequency of Drug Interference in Jaffe Creatinine Assay**

Vukas N. Subbarao and J. Robert Swanson (Clinical Pathology, Oregon Health Sciences Univ., Portland, OR 97201) (Spons: J. Robert Swanson)

It is well known that many drugs and other compounds interfere with the Jaffe creatinine method. Enzymatic methods are becoming available commercially that should be subject to fewer interferences but the reagents are considerably more expensive. We attempted to determine the frequency of drug interferences in the rate Jaffe creatinine assay performed on the Hitachi 737 in order to decide whether our routine assay method should be changed. All samples submitted to our lab for creatinine assay for several days were also assayed by a creatinase method (Kodak) on a Cobas analyzer. We assumed that any drugs interfering with the assay would affect one method and not the other since the chemical reactions of the two assays are very different. The linear regression equation calculated for the 1305 pairs of values was Enzymatic - 0.973(rate Jaffe) - 0.250; r = 0.998; 572.80 to -0.103. Points (8 values) that were more than 3 SD from the regression line were considered outliers. Those samples giving outlier results were reassayed by both methods. In all cases the repeat assay results brought the points back within the original 3.0 SD limits of the regression line. We conclude that the original points were outliers because of random analytical variation of the methods or undetermined analytical errors. We interpret these results to mean that drug interference with the rate Jaffe assay is an infrequent occurrence in this university hospital patient population. Changing to more expensive enzymatic assays to avoid drug interferences may not be warranted.

**Significance of Low Serum Creatinine Concentrations in Hospital Patients**


Serum creatinine measured by the Jaffe reaction includes a non-creatinine component of about 0.3 mg/dL and serum creatinine values of 0.4 mg/dL or lower are rarely observed. Jaffe methods, even in the presence of clinical conditions which would be expected to lower the serum creatinine significantly. Using a creatinase-specific method, Kodak Ektachem, we evaluated serum creatinine measurements in a unscreened hospital population were 0.4 mg/dL.

Serum creatinine was measured with a Kodak Ektachem 700 analyzer by colorimetry of ammonia enzymatically released from creatinine. There were 682 values 0.4 mg/dL among 16,626 serum creatinine results in the laboratory over a 24 month period. The clinical significance of low serum creatinine concentrations, records of 85 patients were randomly selected for review. In 32% of the 85 patients (49 of whom were female), a value 0.20 mg/dL was obtained on repeat analysis, but the remaining 68% had persistently low values. Clinical conditions associated with a persistently low creatinine concentration included low body mass, pregnancy, insulin-dependent diabetes mellitus and total immobilisation. In pregnancy, the modal value was 0.4 mg/dL. The most markedly decreased values were found in emaciated patients. 12% of the 85 patients, all female, had no obvious cause for the persistently low creatinine concentration.

Physiologically decreased serum creatinine concentrations may be the result of increased muscle mass, decreased muscle activity, although low serum creatinine concentrations are not in themselves clinically significant, clinicians must be aware that more accurate analytical methods may produce valid results which historically might seem pathophysiological improbable.
426 BINDING PROTEIN ASSAY FOR HYALURONIC ACID, Kenji Chichibu(Fujisawa Pharmaceutical Co., Ltd., Tokyo Japan), and Takashi Matsuura, Shigeiki Chichijo and M. Mitsu Yokoyama(Kurume University, School of Medicine, Kurume, Japan)(Spon. M. Usuki)

Hyaluronic acid(HA) is suggested to be a new marker for rheumatoid arthritis(Annals Rheumat. Dis. 83-88, 44, 1985).

We have developed a new binding protein assay for HA using hyaluronic acid binding protein(HABP), which is derived from calf cartilage, and avidine-biotin system. Assay procedure is as follows: Fifty(50) pg/ml of HABP containing 20 pg/ml is coated on microtitration plate, incubated for 2 hours at room temp. and the plate is blocked with 3% BSA. The assay is carried out by using biotin-labeled HABP, avidine-labeled peroxidase and o-phenylenediamine. Dynamic range was found to be 2 to 500 ng/ml.

Serum samples of 99 patients with rheumatoid arthritis were tested by this new method and the results were compared with the samples of healthy controls(49 persons). The following results were obtained.

Samples RP R X(ng/ml) SD(ng/ml)
Nor. + 49 3.9 2.4
R. arth. - 53 33.9 62.6
R. arth. - 46 47.7 40.4

HA levels in sera were not correlated with presence of rheumatoid factor(RF) and this method is valuable for clinical diagnosis and therapeutic monitoring of rheumatoid arthritis.

427 SCREENING FOR HYPERBILIRUBINEMIA: AN ASSESSMENT OF DIAGNOSTIC UTILITY IN OUTPATIENTS, Frederick Van Lente(Dept. Biochem., Cleveland Clinic Found., Cleveland, OH 44106) (Spon.: Frederick Van Lente)

We evaluated 3641 adult males undergoing health maintenance examination for hyperbilirubinemia as part of a chemical profile to assess the diagnostic utility of this screening test. Total bilirubin was determined by a modification of the method of Jendrassik and Goff on a SMA II Analyzer (Technicon). Individual exhibited increasing concentrations of serum total bilirubin were classified as exhibiting Gilbert's Syndrome, hemolysis or liver dysfunction according to additional laboratory determinations. These induced bilirubin fractionation, gamma-glutamyl transferase activity, reticulocyte count and haptoglobin determinations. Clinical examination findings and medical history were evaluated in all patients. One-hundred thirty-seven (3.76%) of individuals had hyperbilirubinemia. Of these, 111 (81%) were classified as having Gilbert's Syndrome, 7 (5%) exhibited evidence of hemolysis and 19 (14%) had evidence of liver dysfunction. Bilirubin concentrations ranged from 16 to 37 mg/L and all increases were due to un conjugated bilirubin. Sixty-five patients classified as Gilbert's Syndrome had previous bilirubin determinations, and 50 (76.9%) had repeatedly supranormal concentrations but only 10 were aware of this fact. Screening for hyperbilirubinemia as part of a biochemical profile in an ambulatory populations appears to contribute little diagnostic yield unless additional laboratory data is available that allows distinction between the more frequently occurring Gilbert's Syndrome and potentially more important causes of hyperbilirubinemia. We have found that this negative interference is brought about by sodium dodecylsulfate (SDS), a promoter for the oxidation of bilirubin by BO, and a newly analyzed reaction mixture. Although SDS has no effect on the absorbance of either Hb or BIL alone at 456 nm, it causes a suppression of the absorbance of the BIL-Hb complex in the reaction mixture. This suppression is responsible for the decrease in the apparent BIL concentration when Hb is present in sera.


The clinical determinations regarding the pharmacology and exchange of bilirubin in neonates are dependent upon bilirubin fractionation. Elevated serum unconjugated bilirubin values (Bu) are associated with anemia, with exchange transfusions of the neonate. Exchange transfusions have associated morbidity and mortality, accurate bilirubin fractionation in the 20 mg/dL range is essential. The Ektachem system for bilirubin uses a multimeter to measure Bu and conjugated bilirubin (Cb). The analysis utilizes dual wavelength reflectometry of Bu and Cb when bound to a sorbent. To the assay of the bilirubin system for Bu measurement in the neonate, we analyzed human serum samples with added Bu to form a dilution series over the range of 0.2-5 mg/dL Bu. Since normal human neonate serum contains 2-5% of the total bilirubin as Bu (Pediatr. Res 20:47, 1986) we also varied the ratio of Bu/Cb in the samples (0-83%) utilizing diatrizoate conjugated bilirubin. If samples contained Bu > 15 mg/dL and Bu/Cb < 30, the Ektachem system gave no interference and no numerical results. Dilution of samples containing Bu > 15 mg/dL with either water or bilirubin-free human serum over a 6-fold range resulted in acceptable Bu values. However, dilution with water gave a greater percentage error (7%) than the serum (4%). The Ektachem Bu results in the dilution series were linear yielding a correlation coefficient of 1.0 and slope of 1.0.

Conclusions: 1) Serum samples with elevated Bu levels must contain Bu with Bo/Bu > 30 for the Ektachem to give acceptable numerical results. 2) The Ektachem system provides reliable data for elevated Bu values when the sample is diluted with water. 3) The Bu values obtained in the Ektachem system are acceptable for the clinical management of neonates.


We detected two types of interferences using the manufacturer's procedure for total bilirubin on the Olympus AU 5000. Samples were compared using the Olympus procedure, (a method utilizing 2.5 dichloro-phenyl-diazonium-tetrafluoroborate (DCP) and two, more traditional Hb was utilizing azo dyes: sulfanilic acid (BSA): one on the Technicon RA-1000 and one on the DuPont ACA. Even slightly hemolyzed samples produced a 25-50% negative Interference with the DCP method versus the RA-1000 method. Both of these interferences have previously been described for other analyzers using DCP and related diazo compounds.
To minimize these interferences we adapted our RA-1000 DSA method (Clin. Chem. 33:1670, 1987) to the AU 5000. Addition of a hemolyzate resulted in significant negative interference (Excess color interference caused the DCP Olympus method to report bilirubin concentrations of 0.2-0.5 g/L. In contrast, our new DSA Olympus procedure, as well as the other DSA methods, did not show interference until bilirubin concentrations were 2-4 g/L. Our DSA method for the Olympus AU 5000 showed no positive interference with samples from patients with chronic renal failure. This DSA method, which was routinely used in our laboratory, is linear to 214 mg/dL total bilirubin and gives excellent correlation with our DSA RA-1000 method, y = 0.99x - 0.039, n = 50. We recommend that the DCP method not be utilized on the Olympus AU 5000 analyzer and that its use on other analyzers be carefully scrutinized for interference due to hemolysis and/or patients with chronic renal failure.

QUANTITATION OF INDIVIDUAL SERUM BILE ACIDS IN THE RAT
FOR DETECTION OF IRREVERSIBLE HEPATIC DISEASES. J.E. Mathen, D.J. Creighton, and S.W. Looney (The Clinical Research Laboratories, The Upjohn Company, Kalamazoo, MI 49001) (Spon.: L.K. Wathen)

A reverse phase, high performance liquid chromatography (HPLC) assay was developed to quantitate 17 bile acids found in rat serum. This bile acid profile was investigated as a tool for early detection and identification of specific types of hepatobiliary damage. In rats treated with hepatotoxins, we demonstrated detectable changes in the total bile acid levels and in the bile acid profile composition in alpha-naphthyl acetate-treated rats, glycoconjugated bile acids in drug-treated rats. The bile acids in rat serum were separated on a C18 silica column, and the individual bile acids were detected and identified by comparison with standards. The assay was sensitive to about 200 picomoles of each acid. This permitted quantitation of individual bile acids representing less than 1% of the total bile acid concentration present in normal rats.

We conclude that this HPLC separation technique offers a method for demonstrating specific hepatotoxicity in rats. The distinct differences in serum bile acid profile between hepatotoxins suggest that this assay may differentiate between centrilobular and periportal and biliary duct necrosis.

NON-REVERSING JUNDICE: BILIRUBIN COVALENTLY ATTACHMENT TO SERUM ALBUMIN CIRCULATING IN THE METABOLIC HALF-LIFE OF ATTACHED BILIRUBIN. H.J. Bjerregaard, H.A. Feten, E. Christensen, S. W. Petersen, and M.等领域. (Medical Research Institute, The Mary Imogene Bassett Hosp., Cooperstown, NY 13326) (Spon.: Roberta G. Fend)

In conjugated hyperbilirubinemia bilirubin may become covalently attached to serum albumin to produce a non-dissociable complex. The protein bound bilirubin may react with detoxifying reagents to contribute to both total bilirubin and the direct reacting bilirubin fraction.

To learn whether this covalently bound form of bilirubin remains attached to albumin for the entire time the albumin molecule remains in the circulation, the metabolic clearance of bilirubin-albumin complexes was measured. Radicallabeled form of free bilirubin, rat albumin and covalent complexes of bilirubin and albumin were injected into the circulation of Sprague Dawley rats and serial samples of serum were analyzed for the injected compounds. Trinitiated bilirubin covalently attached to rat albumin at 1 to 3 mol/mol was cleared from the circulation with the same half-life as isolated rat albumin. Trinitiated free bilirubin, on the other hand, was cleared from the circulation with a half-life of only 6.2 min. We conclude that bilirubin covalently attached to albumin is cleared from the circulation with the long half-life of albumin rather than the short half-life of bilirubin.

For patients, the half-life of albumin is 19 days. A 19-day half-life for bilirubin to explain the albumin-bound bilirubin explains why, in patients treated for hepaticobiliary disease or obstructive jaundice, serum bilirubin often remains elevated when other indicators suggest return of normal hepaticobiliary function.

(Supported by USPHS Grants HL 02751 and DE 32581 and the Stephen C. Clark Research Fund.)

A NEW LIQUID TOTAL BILIRUBIN REAGENT WITH MINIMAL INTERFERENCE FROM LIPEMIA. Zaid Hissem and Sandra Pile (Abbott Diagnostics Division, Irving, TX 75015) (Spons.: Andrew M. Ruland)

Measurement of total bilirubin in lipemic samples can result in inaccurate determination of analyte. Several commercial reagent manufacturers, therefore, suggest that lipemic samples not be tested or that special sample handling be employed, thereby limiting the diagnostic utility of these assays. In this report, we describe a modified ABBOTT SPECTRUM Liquid Total Bilirubin Reagent, which is accurate in the presence of lipemia while retaining the performance characteristics of the original reagent (linearity: 20 mg/dL, working reagent stability: >4 weeks on-board). The data below shows results (in mg/dL) obtained with the original and reformulated Liquid Total Bilirubin Reagents using samples spiked with a turbid lipid suspension (triglyceride value in [mg/dL] in brackets).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Original LCT-Bilirubin</th>
<th>New LCT-Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>0.50</td>
<td>0.62</td>
</tr>
<tr>
<td>Serum 2 (5000)</td>
<td>7.19</td>
<td>7.19</td>
</tr>
<tr>
<td>Serum 3</td>
<td>0.67</td>
<td>0.70</td>
</tr>
<tr>
<td>Serum 4 (2800)</td>
<td>3.88</td>
<td>3.88</td>
</tr>
<tr>
<td>Serum 5</td>
<td>0.60</td>
<td>0.56</td>
</tr>
<tr>
<td>Serum 6 (1400)</td>
<td>2.12</td>
<td>2.12</td>
</tr>
</tbody>
</table>

In summary, the new Liquid Total Bilirubin Reagent can accurately determine total bilirubin in lipemic samples, thereby expanding the sample application and overall efficacy of this reagent.

HEMOGLOBIN INTERFERENCE ON REFLOTRON® BILIRUBIN AND A DIGITAL ROUTINE METHOD WITH THE ILEX-MEDICS REAGENT. P.U. Koller, R. Reimerberger and R. Reiter (Boehringer Mannheim GmbH, Evaluation Department, 680 Mannheim, W-Germany) (Spon.: P.U. Koller)

When we evaluated Reflotron® Bilirubin we noted its susceptibility to interference by hemoglobin. Thus we studied possible implications on test performance of Reflotron® Bilirubin and the dichrochlorphenyl diazonium (DPD) routine method.

The addition of hemoglobin as erythrocytes lysate to human venous sera resulted in a concentration dependent underestimation of bilirubin by the DPD method, but an increased recovery by the Reflotron® system. At 1 mg/dL bilirubin the presence of 45 mg/dL hemoglobin led to a -7 and 10% deviation by the DPD method and the Reflotron® system respectively. Even minor hemolysis that was barely visible to the eye resulted in significant differences of the results, obtained by both methods.

The practical consequences of this interference on the use of capillary blood specimen, which are frequently used by the Reflotron® system were further studied. When 200 - 300 μL capillary blood was collected in Microtainer® tubes consistently 0.1-0.2 mg/dL higher bilirubin concentrations in comparison to the DPD method were obtained (y = 0.13x + 0.15, r = 0.964). In a second procedure 30 μL of capillary blood were transferred from the skin puncture site to the reagent carrier with glass capillaries. The results show a good correlation and a negligible intercept between bilirubin determinations in capillary blood by the Reflotron® system and the results by the DPD method (y = 0.00442x + 0.996, r = 0.992). This points out the importance of proper handling and therefore we recommend the use of glass capillaries for Reflotron® Bilirubin.

DEVELOPMENT OF A BICHROMATIC TOTAL BILIRUBIN ANALYSIS FOR HOSPITAL SERA ON THE REFA 1000. B.G. Bilshoven, H.A. Roodt and B. Leijnse (Academic Hospital Rotterdam, The Netherlands) (Spon.: G.J.M. Boerman)

Bichromatic analysis of total bilirubin is very popular in the neonatal field despite the sometimes moderate attention paid to the accuracy and the precision of the analysis. (1). The methods involved, direct serum measurement or measurement of bilirubin interaction with buffer, are easy to perform.

Through the courtesy of the Technicon Company we got the opportunity to develop a total bilirubin determination for a RA-1000 by installing a new set of interference filters especially made for this study (457 and 550 nm). By using a phosphate buffer (150 mM), pH 7.4 for dilution and a sample volume of 20 μL we studied the following items:

a. Calibration graph: linear up to 500 μmol/L.

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We have modified the subject method by using a 33% ethylene glycol (EG) matrix instead of water (AQ) as a solvent for the two reagents used. We have used these modified reagents for the assay of aceto-acetic acid in urine samples and obtained quantitative recovery of the added ketone. These recovery studies were performed on urine to which had been added aceto-acetic acid at 90 mg/dL. Recovery in the AQ reagent was 82 mg/dL (103%) and in the EG reagents was 86 mg/dL (106%). An aqueous solution at 90 mg/dL of the lithium salt of aceto-acetic acid (LAA) was used as a standard.

Linearity studies were performed to a level of 200 mg/dL (18.6 mmol/L). AQ equation is Y = 0.989X + 0.969 and EG equation is Y = 1.099X + 0.670 with r = 0.969.

Precision studies (within day) at a level of 80 mg/dL (7.4 mmol/L) demonstrated a CV (AQ) of 1.2% while the CV (EG) was 1.0%.

Using this modified method we have found that the present use of EG confers two advantages over the original method. These are:

(1) An increase of about 50% in sensitivity of the color reaction of the EG reagents over the AQ reagents

(2) An increase in stability of the working reagents from a single day (AQ) to over one month with EG reagents.

The observed increased stability of these reagents allows one to more conveniently run quantitative analyses of urine for aceto-acetic acid.

**LAB MANAGEMENT**

**Lab Management and QC**

**TIME SERIES MODELING FOR CLINICAL CHEMISTRY QUALITY CONTROL**, Michael G. Bissell and Layth C. Alwan (City of Hope National Medical Center, Duarte, CA 91010-0569, and University of Chicago Graduate Sch. Business, Chicago, IL 60637) (Spon.: N. G. Bissell)

Autocorrelation of clinical chemistry quality control measurements causes one of the basic assumptions underlying the use of Levey-Jennings control charts to be violated and their performance to be degraded. This is the requirement that the observations be statistically independent. We present a proposal for a new approach to statistical quality control which removes this difficulty. We propose to replace the current single control chart of raw QC data with two charts: 1) a common cause chart, representing a Box-Jenkins ARIMA time series model of any underlying nonrandomness in the process, and 2) a special cause chart of the residuals from the above model, which, being free of such persisting nonrandomness, fulfills the criteria for use of the standard Levey-Jennings plotting format and standard control rules. A comparison of our proposed approach with that of current practice was carried out on 4 months' control data on 28 analytes run on Ektachem 700 analyzers at the Univ. of Chicago Hospitals. We defined the discordance ratio (D/T) as the number of disagreements (D) in classification between approaches, over the total number of warnings and rejections classified by both approaches (T). The standard Levey-Jennings chart with all six Westgard control rules gave discordance ratios of 64% for both high and low level controls when compared with the time series special cause chart. In practice, when implemented on commonly available microcomputer software, the special cause chart would represent a statistically filtered Levey-Jennings chart, transparent to the user at the bench. The common cause chart can be used by management to provide visual representation of persisting global effects on the whole system (equipment, operating procedures, or the working environment) which are beyond the scope of on-line operators to change.

**EVALUATION OF A NEW MICROCOMPUTER QUALITY MANAGEMENT SYSTEM (OMS)**, John Ross, MD, B. McCook, MFT/(ASCP) (Clinical Laboratory, Kennestone Hospital, Marietta, GA 30060) (Spon.: S. Chappell)

We evaluated a beta test version of an IBM-PC based quality management system (QMS) developed by Frederick Smith, MD. QMS was found to be a full featured, easy to use QC program. Initial system required some care and thought before full use could be made of macros. However, system flexibility allowed expansion of the system at a later date.

A highlight of OMS is programmed structures for up to ten basic rule types. A set of rule types can be combined to make a test/analyze
specific set of control rules. We evaluated a modified Westgard rule set using one month of SMAC data from each of the laboratories. The results were identical to those made using our in house control evaluations.

Graphic reporting features of QMS allow rapid assessment of laboratory control status. The interpretative test specific help feature, along with a built-in set of test specific rules, made documentation more complete and accurate.

Successful implementation of statistical and clinical decision limits added in results reporting.

QMS was found to be helpful system. Several wanted features were added during beta test. No major wanted features were noted. It is felt the full power of QMS will be realized when the newer rule types are evaluated and fine-tuned using simulated data, and then these rules implemented. QMS has many features beyond those found in present systems.

### 441 FORECAST COMPARISONS IN INVENTORY MANAGEMENT

Steven Noel (Greater Balt. Med. Ctr., Baltimore, MD 21206), and John Snyder (Ohio State Univ., Columbus, OH 43210) (Sponsor: Stephen A. Knisely)

Cost-effective management of an appropriate inventory of supplies for the laboratory requires the ability to forecast demands and make decisions about how much and when to order. The purpose of this study was to compare retrospectively three forecasting methodologies, empirical smoothing and linear regression analysis, with an existing method of minimal quantity on hand for five items in a clinical chemistry laboratory. Other variations studied were order interval length and means of order generation. We compared order intervals of one and four weeks for each forecast method. Order generations included, first, a computer using safety stock (SS), forecast (F) and current inventory (CIV) = (SS + F) - CIV = Amount Ordered. Second, a reorder point (ROP) was calculated as ROP = SS + (F + Lead Time). When the CIV falls below the ROP, a pre-determined quantity of stock (SS + F) is ordered. Cost analysis comparisons included supply cost, purchasing cost and carrying cost (30% of the supply cost).

The most cost-effective method (annual savings of $1757 for five items) was the moving average forecast using monthly review intervals, order generation based on the equation (SS + F) - CIV, and a safety stock equal to the lead time. Substantial annual savings of $466 and $645 occurred using exponential smoothing and linear regression, respectively. The ROP/EOO ordering technique also produced a cost savings ($261 per year), but less significant than the computation method. Regardless of the forecasting method, one week ordering intervals increased cost ($125 < $1747). Management of inventory cost is controlled more effectively by computing the order quantity as opposed to estimation. To simplify this process a computer program, written in AppleSoft Basic (NE SOS Version 3.0), has been developed.

### 442 STAT TURNAROUND TIME AS A MEASURE OF QUALITY ASSURANCE

JE McCaig, LH Hibberson, JA Repinski, PJ Howesitz, GS Smith (Clinical Laboratories, UCLA Medical Center, Los Angeles, CA 90024) (Sponsor: L Hibberson)

We evaluated the turnaround time (TAT) for 29 stat laboratory tests over a 25 day period using our hospital's Community Health Computing (CHC) laboratory computer. Data was transferred from the LIS to floppy diskettes using Supersearch supplied by CHC. The data was then analyzed by an IBM-AT personal computer using the SAS statistical package (SAS, Inc. Cary, NC). TAT was determined for six separate work areas including the main hospital chemistry, coagulation and hematology laboratories, and satellites in the emergency room, the perinatal intensive care unit and an adult outpatient clinic. TATs were also divided by day, evening and night shifts. TAT was defined as the difference between the documented in-lab time and the completion time. Data was excluded for those specimens completed during regular computer maintenance, or when there was evidence that the receipt and completion times may have been altered due to subsequent requests on the same specimen. Blood gas analysis was not included.

We obtained 22,417 stat observations over the 25 day period. Results indicate more expeditious TATs for satellite laboratories as well as those tests performed during less busy evening and night shifts. We have instituted measures to improve stat TAT from these baseline intervals.

In summary, we recommend clinical laboratories extend the concept of quality assurance beyond analytic accuracy and precision to include timeliness of result reporting.

### 443 REAL TIME QUALITY ASSURANCE PROGRAM FOR MONITORING CONTROL RESULTS ON THE SYRCHROM CATHS. M.H. Williams, S. Bruce, J. A. Kiely, J. D. Rangel, B. B. Rasooly (Beechman Instruments, Inc., Ca 92621) (Sponsor: M.H. Williams)

The Synchro C3X quality control program is intended for use as an aide in monitoring quality control results generated exclusively by the C3X. The control program is capable of maintaining statistics for 5 chemicals on 5 different control materials.

The Synchro C3X QC program performs 5 major functions.

1. Daily Log - lists all controls run for the current day (up to 25 per control level) in chronological order per chemistry and control level. Applied to the control results are 3 Westgard rules 12s, 13s, and 22s. Control results that are outside of limits are flagged and the Westgard Rule book broken is indicated. 2. Daily Summary & (C) Monthly Summary: 4) Computed using confidence interval for results in real time. The statistical summaries consist of the Mean, R.D., S, and C.V. The summaries are calculated by maintaining the summations of the Individual values & the number of times the control was assayed. 5) Levey-Jennings plots can be displayed on the CRT or printed for data points within a 4 day time frame. 200 chemistry results per control level can maintained for the Levey-Jennings plots. The program is self editing; results that are >3 S.D. from the average are shown in red. The control results were also displayed in the Daily Log & Levey-Jennings plots. To verify the performance of the quality control program 5 levels of controls were identified (3 sets a day, 4 times a week). The reported control materials were analyzed 5 to 25 times a day for one week. The accuracy of the program summaries were verified by comparing the C3X statistical summaries with those calculated by the program.

In conclusion the statistical calculations performed by the Synchro C3X Quality Control Program accurately compared to the statistical calculations performed by the laboratory. The Synchro C3X QC Data is used to verify the quality control program provided the technologist with a means to evaluate the day to day precision and accuracy of the C3X.


We have been studying the performance of physician office testing when a hospital laboratory is involved with quality assurance. The study comprises several physician offices with no previous experience in the BMD Reflron as but have been relying on reference laboratories for tests available on the Reflron. The tasks of the hospital laboratory staff include: (1) To assure that the analyte has the proper technique after training by the manufacturer; (2) To establish and monitor a quality control program; (3) To perform comparisons on the two sites, (4) To establish the physician office; (4) To conduct bi-weekly visits initially and monthly visits subsequently of the physician office. We have performed, (a) sample processing, precision, accuracy, turnaround time).

Satisfactory performance was found at our 1st site, where testing had been performed by a person with a B.A. degree from a 2-year educational program:

- **Glucose**: 145.5, 125.2, 104.0 1.04, 0.4
- **BUN**: 41.0, 5.0, 0.974 1.5 3.3-3.9
- **AST**: 44.0, 3.0, 0.983 3.0 3.2-4.3
- **Uric acid**: 43.0, 1.0, 0.008 0.052 0.5 3.8-4.7
- **Two-month precision; **mg/dL; **U/L; **K**: 737, hospital lab; **KA**: 737, physician office.

Preliminary findings for our 2nd site (tests performed by 2 nurses) showed performance comparable to our 1st site. We conclude that quality performance can be assured with laboratories assistance.

### 445 REDUCING COST BY MAXIMIZING AUTOMATION IN A RAPIDLY GROWING CLINICAL LABORATORY. Cate Buba (Delta Memorial Hospital, Antioch, CA 95609) (Sponsor: Nancy A. Blessor)

The objective was to document the effects of automation and computerization during a three year period of rapidly increasing volumes using comparative financial data, statistics and ratios. Also, to establish further goals for automation and develop criteria to measure the effects. At a time when hospital automation has been curtailed by many fronts, Delta Memorial Hospital decided to adopt an outreach program to increase outpatient revenues. In 1983, it became clear that the lab either had to expand, or decrease its scope of operations to reduce its overhead, in

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order to reduce costs. With tools such as improved instrumentation, and
the use of microcomputer lab information systems (LIS), it may be
possible to make the small laboratory competitive with larger, more complex
organizations.

An outreach program was established in 1983. Due to automation and
growth, the outpatient portion of the operations has had the following
favorable results:

1. Outpatient revenue has increased 120% despite a drastic reduction in
costs to service patients.

2. Outpatient test volume has increased 90%.

3. The contribution (gross revenue minus direct expenses, including
overhead and rent, divided by cost of capital) has increased 60%.

An examination of the increased contribution not only reflects the
increased revenues, but a corresponding decline in expenses. Direct expenses
per statistic test (tests) went down an average of 10% per year over the period.
The productivity statistics (productivity hour per test) declined 10%
indicating an overall increase in labor. Most of the

444 USE OF P-HYDROXYPHENYL (PHP) & RED 28 REAGENTS TO
HOMOGENIZE LIVER & HUMAN LIVER: CAROL HERNANDEZ, Susan
Carol Hernandez)

We used the AUDIT (LESS, Scottsdale, AZ) verification procedure
and kit containing solutions of PHP and direct re 28 with

known concentrations to check pipetting and wavelength
accuracy of RA-XT (Technicon) and NCA (Inst. Labs). Wavelength
verification is based on the linear regression calculations of the
measured AUDIT absorbances. An example of the analysis shows the following results:

<table>
<thead>
<tr>
<th>Slope (m)</th>
<th>Intercept (y)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA-XT</td>
<td>0.0154</td>
<td>0.0017</td>
</tr>
<tr>
<td>NCA</td>
<td>0.0977</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Acceptance limits are: m: 1.0±0.1; y: 0±0.5; and r: 0.972.0.

Known concentrations of PHP are used for checking the the
pipetting volume. The volume dispensed is calculated using the
regression results obtained from the wavelength check for each
analyzer.

Actual Volume = x Abs of Pipettor - y)/PHP Concentration
Dispensed

A linear regression model was calculated using the
regression results obtained from the wavelength check for each
analyzer.

For example: The NCA’s 6 ul pipettor volume was calculated as:

\[ \frac{0.5674 - 0.0135}{0.0918} = 6.032 \text{ ul (showing a 0.5% error).} \]

AUDIT’s ready-to-use reagents provide a reliable time-saving
procedure that can be used routinely. The statistical
evaluation provides an ideal model for assessing instrument
performance.

447 OPTIMIZING USE OF PANEL TESTING IN THE
CHEMISTRY LABORATORY. Peter V. Roco, Stanley J. Podsiadak and
(Spons: T.J. Dill)

In order to develop a more efficient strategy for ordering serum
chemistry determinations we have analyzed the results of serial
individual analyte test results obtained from patients within one
week of the initial panel. Forty-two patients had an initial panel plus
at least one subsequent panel for a total of 143 panels (792 individual
test results and 1,782 repeat results). Each analyte was
classified according to whether the change from previous to new result
exceeded a limit of the reference range. The sum of these conversions
was used as an index of individual test liability. There was a maximum
of 99 changes that could have occurred for each analyte. We set the
criterion for low test liability at less than 11 changes per analyte. In
addition each test was also classified based on whether the percentage of
abnormal results for each analyte was greater than 25%. According to
these criteria, the analytes were distributed in four groups as follows:

Greater percentage of normal results and more labile:
Potassium, Phosphorous, Aspartate Aminotransferase,
Alanine Aminotransferase
Greater percentage of normal results and less labile:
Creatinine, Direct Bilirubin, Total Bilirubin

Lesser percentage of normal results and more labile:
Glutathione, Sodium, Creatinine, Lactate Dehydrogenase, Total Protein, Calcium

Lesser percentage of normal results and less labile:
Blood Urea Nitrogen, Albumin, Alkaline Phosphatase

These findings can be used to guide the choice of repeat testing after
initial screening panels in order to reduce unnecessary analyses while
maintaining the likelihood of detecting significant changes that
might affect patient management.

448 PURIFICATION OF ALANINE AMINOTRANSFERASE FROM
HUMAN LIVER AND ITS EVALUATION AS A PROFICIENCY
TESTING MATERIAL, Daniel A. Meaham, Daniel A. Meaham,
Robert L. Habig, and Kenneth A. Schneider (Dept.

In 1986 the New York State Department of Health ordered that all donor blood in New York State be
screened for alanine aminotransferase (AlaAT) activity, a surrogate test for non-A, non-B hepatitis.
Implicit in this mandate is that the laboratory's proficiency in measuring this enzyme must be regularly
evaluated. The purpose of this study was, therefore, to develop a human AlaAT material suitable for use in the
New York State proficiency testing program.

The AlaAT purification procedure takes advantage of the low isoelcetric point of the enzyme and involves,
sequentially, CM-Sepharose CL-6B fractionation at pH 6.0 and preparative isoelectric focusing. The final
preparation, with a specific activity of 303 U/mg, presented as a single band on Coomassie Blue stained
polyacrylamide gels. Overall AlaAT recovery was 12.3%. This preparation was diluted in a matrix of 50
mM/L Tris, 54 (w/v) BSA, 0.01 mM/L α-ketoglutaric acid, 0.05 mM/L pyridoxal 5'-phosphate, 10 mM/L H3
acetyl-L-cysteine, and 1 mM/L EDTA, pH 7.0 to produce three different lyophilized pools of enzyme
activity. Recovery of enzyme activity after lyophilization was 95-97%. There was no loss of enzyme activity
within 5 h after reconstitution and storage at room temperature. During storage at 4°C for
over the last 5 months, the lyophilized material has lost 4-8% of its original enzyme activity.

The highly purified AlaAT is an appropriate proficiency testing material, it is stable and of
human origin.

449 AN EVALUATION OF THE PROPOSED CDC MINIMUM
STANDARDS FOR PROFICIENCY TESTING (PT) PROGRAMS:
THE ABILITY TO CORRECTLY ASSESS INTRALABORATORY ERROR.
Ronald H. Laessig, Sharon S. Ehrenyr (University of Wisconsin,
Center for Health Sciences, Madison, WI 53706) (Sponsor: Ronald H.
Laessig)

The CDC has proposed new minimum performance standards for providers of PT programs in clinical chemistry. These standards will
establish the minimum acceptable levels of performance for PT programs provided to laboratories licensed under Medicare or CLIA.
Our study uses a previously described computer model which enables us to predict the quantitative relationship between minimally
acceptable performance in an interlaboratory PT program and actual intralaboratory performance.

The proposed PT standard for cholesterol defines acceptable performance as results within +/- 10% of the true value on four of six
consecutive challenge tests. On an intralaboratory basis, the corresponding level of performance would allow an internal
coefficient of variation of 16%. For a patient with a 220 mg/dL cholesterol, the laboratory would report a result greater than 255 or
less than 185 on one out of three specimens. Clearly this laboratory, which would be certified as passing PT, would not be
providing clinically useful laboratory data.

Ross and Skendzel have defined minimum intralaboratory CVs for medically useful results for 13 analytes in six cases the CDC
criteria allow errors larger than needed for medical usefulness, in
eight cases they are approximately correct and in one, Urea Nitrogen, it is tighter than necessary.
CSF TRAP ("Transport and Rapid Access for Additional Procedures") is a laboratory protocol providing: 1) a mechanism for appropriate storage of CSF, at -70°C; and 2) rapid access to this reserved fluid through the hospital’s computer. A major purpose of the CSF TRAP is to enhance patient care by allowing clinicians to order tests retrospectively, thus decreasing the need for repeat lumbar punctures. Further, the CSF TRAP provides reserve fluid for: 1) repeat of questionable test results; 2) replacement of lost or deferral due to laboratory accident; and 3) deferral of low-yield, high-cost assays pending review of initial CSF results. CSF TRAP samples have been utilized on 44 patients during the 8-month period since inception of CSF TRAP, and for 247 of 2076 specimens (11.9%) collected in the same 8-month period last year.

Although CSF TRAP alone does not modify physicians' test ordering behavior, it clearly provides the framework for restructuring the approaches to CSF testing.

### 453 BIOLGIC VARIABILITY AS DETERMINED FROM BLINDED SPLIT SAMPLE ANALYSIS

**R. W. Forrester, G. G. Klee, L. K. Colborn**  

Data collected for 10 years from a blind split sample program was studied in order to determine biologic variability for 23 analytes. This information is useful in establishing performance criteria for the split sample quality control and to assess analytical precision.

Blood obtained from healthy volunteers was pooled for each individual, aliquoted and submitted for analysis with two prolific identifications. Results were obtained twice monthly for each analyte from 3 to 14 different donors. The standard deviations of the average of the two results were multiplied by 2.2 to estimate total variability for each donor. Also, the standard deviations of the differences were divided by 2.2 to estimate analytic variability. Results were expressed as S coefficient of variation. The biologic component was calculated from the formula: Biologic = \( \frac{\text{vmean} - \text{vanalyte}}{\text{vanalyte}} \). The median biologic % CV for each test is shown in the table.

<table>
<thead>
<tr>
<th>MEDIAN TEST</th>
<th>Biologic CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>48.3</td>
</tr>
<tr>
<td>T3</td>
<td>34.8</td>
</tr>
<tr>
<td>T4</td>
<td>32.9</td>
</tr>
<tr>
<td>T3G</td>
<td>31.5</td>
</tr>
<tr>
<td>CK</td>
<td>30.3</td>
</tr>
<tr>
<td>ACP</td>
<td>29.1</td>
</tr>
<tr>
<td>T3G Free</td>
<td>28.2</td>
</tr>
<tr>
<td>T3G ACT</td>
<td>28.4</td>
</tr>
<tr>
<td>T3G ALT</td>
<td>17.6</td>
</tr>
<tr>
<td>T3G LD</td>
<td>12.8</td>
</tr>
<tr>
<td>T3G PK</td>
<td>11.3</td>
</tr>
<tr>
<td>Creat</td>
<td>10.5</td>
</tr>
<tr>
<td>ALP</td>
<td>9.8</td>
</tr>
<tr>
<td>Urea</td>
<td>8.8</td>
</tr>
<tr>
<td>Chol</td>
<td>7.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>7.3</td>
</tr>
<tr>
<td>Na</td>
<td>7.1</td>
</tr>
<tr>
<td>K</td>
<td>6.9</td>
</tr>
<tr>
<td>Protein</td>
<td>4.4</td>
</tr>
<tr>
<td>Ca</td>
<td>2.5</td>
</tr>
<tr>
<td>Mg</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Retrospective analysis of split sample data can yield information regarding analytic, biologic and total variability. This data may be used to establish quality control limits and determine where improved analytic precision may or may not improve the clinical utility of a test.

### 454 THE APPLICATION OF WESTGARD RULES TO INTERLABORATORY QUALITY ASSURANCE PROGRAMS

**S. Ehrnmer, Ronald H. Laessig, and James O. Westgard** (University of Wisconsin, Center for Health Sciences, Madison, WI 53706) (Sponsor: Sharon S. Ehrmmer)

The Westgard rules have resulted in a major restructuring of intralaboratory quality assurance; we examined through the use of our previously described computer model the applicability of this multipurpose approach to interlaboratory quality assurance programs. Currently, proficiency testing (PT) programs use grading algorithms based on the interlaboratory equivalent of the 12S rule, i.e. group mean ± 2 group standard deviations. Our previous work demonstrated the limitations of this rule and that other rules are better able to detect unacceptable as well as acceptable intralaboratory performance. An improved PT system design is possible by using up to 3 PT challenges and by applying more than one rule to differentiate between various types of intralaboratory errors. Rules where their standard deviation exceeds a specified group standard deviation and fall on different sides of the mean will detect random error. Rules where the standard deviation exceeds a specified group standard deviation are capable of detecting systematic error. The number of PT challenges incorporated the rule and the magnitude and nature of the error become critical factors in determining the type of intralaboratory error detected in interlaboratory quality assurance programs.
The evaluation of the turnaround time (TAT) for 18 clinical chemistry analytes where both stat and routine tests are frequently requested. Data were transferred from our Community Health Computing (CHC) laboratory computer to diskettes using the SCX program. Data were analyzed with an IBM-AT personal computer using the SAS statistical package (SAS, Inc., Cary, NC). We define TAT as the difference between specimen receipt and completion times. Data were excluded for specimens completed during computer maintenance, or when there was evidence receipt or completion times may have been altered due to subsequent requests on the same specimen.

In a representative one day period, we received 1,922 requests for the 18 analytes studied. Average of the median TATs was 33.5% faster for stat tests compared to tests ordered routinely. When examined by shift, average TAT is considerably faster for tests ordered stat than for those ordered routinely during the day (35%) and evening (51%) shifts when our workload is greatest. Average TAT for stat tests ordered on the night shift is similar or in some cases longer than routinely ordered tests (-3%), although all tests on slower shifts are reported faster than routine requests on the day shift. The lack of improved TAT on night shifts may result from disruption of the normal workflow when staffing is decreased. Information on stat and routine TATs is now being utilized by scheduling staff so they may follow season productivity and make modifications in section staffing as necessary.

We recommend that clinical chemistry laboratories include time of day and priority reporting as part of their quality assurance programs. Laboratories may wish to investigate the utility of stat requests during slower shifts since such requests may interrupt the normal flow of specimens without expediting reporting of results.

The goal of this study was to find the most cost-effective quality control procedure with which to operate our Hitachi 737. Cost-effective QC options would simultaneously minimize false rejections of runs and ensure detection of medically important errors. Our previous practice had been based on our instrument, the Technicon SMAC, an analyzer with some completely different characteristics. Medical usefulness requirements for some tests are defined for each test on our Hitachi 737 to guide the design of these QC procedures.

The sizes of medically important random and systematic errors were calculated from the analytical quality requirements (defined for our laboratory) and the precision characteristics for each test (determined under routine operating conditions in our laboratory). Because of the small standard deviations (s) for many of the tests, the medically important errors were often large multiples of s. Performance objectives for QC procedures were set as 90% (or greater) detection of the medically important systematic errors while keeping the false rejection rate as low as possible. The actual probabilities for detecting these critical sized errors were assessed from power function graphs for the various control procedures studied. Tests could be divided into two groups based on the QC procedures needed to provide the desired error detection. Control limits of 2s and 2.5s were effective for chloride, bicarbonate, calcium and albumin. Control limits of 3.5s and two control measurements per run were effective for the remaining tests. These simple, single-rule, individualized designs provided medically useful QC procedures that reduced false rejections and required less operator intervention than in the past, while maintaining the necessary detection of medically important errors.
The trend in methodology is toward using an assay based on measuring the 4-nitrophenyl anion liberated by the action of sample alpha-amylase from a defined substrate, particularly 4-nitrophenyl-D- maltotetraose. Such a trend toward methodological uniformity can but have only a positive effect on interlaboratory comparisons and enzyme standardization.

**USE OF IMMUNOCHEMISTRY SURVEY DATA TO ESTABLISH PERFORMANCE CRITERIA**

Linda Stead & David Platt, Baxter Healthcare Corp., Miami, FL

We have taken data for 33 tests on 6 pools of freeze-dried serum from national interlaboratory assurance programs and analyzed it in 3 ways:

1. Average and ranges of CV's (Current and Cumulative) for labs reporting a given instrument-reagent pair on 3 levels of controls.
2. Below are representative CV data for method-constituent pairs (a complete set of data will be handed out at the session).

<table>
<thead>
<tr>
<th>Digoxin</th>
<th>ACA (n=10)</th>
<th>Stratus (n=10)</th>
<th>TDx (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low control</td>
<td>11.9</td>
<td>12.9</td>
<td>12.3</td>
</tr>
<tr>
<td>min</td>
<td>6.6</td>
<td>7.4</td>
<td>7.0</td>
</tr>
<tr>
<td>max</td>
<td>12.3</td>
<td>15.9</td>
<td>10.7</td>
</tr>
<tr>
<td>Normal control</td>
<td>21.1</td>
<td>25.4</td>
<td>25.5</td>
</tr>
<tr>
<td>min</td>
<td>4.5</td>
<td>5.6</td>
<td>5.4</td>
</tr>
<tr>
<td>max</td>
<td>22.1</td>
<td>27.5</td>
<td>18.3</td>
</tr>
<tr>
<td>High control</td>
<td>21.4</td>
<td>25.4</td>
<td>25.5</td>
</tr>
<tr>
<td>min</td>
<td>2.8</td>
<td>5.6</td>
<td>4.5</td>
</tr>
<tr>
<td>max</td>
<td>25.4</td>
<td>32.1</td>
<td>20.0</td>
</tr>
</tbody>
</table>

From the data we examined, we conclude that:

- Some methods, on the average, are more precise than others; but distinct differences do exist between labs using the same instrument-reagent system.

There is little, if any, systematic relationship between the number of points submitted in a given data month and a lab's precision.

When the system is operating "in control", precision varies little with time.

**SURVEY OF SPECIAL PRACTICES ASSOCIATED WITH PROFICIENCY TESTING IN THE COMMONWEALTH OF PENNSYLVANIA, George S. Cembrowski, Park Nenickell Medical Center, Minneapolis, MN 55416 and Raymond E. Vanderlinde, Dept. Path. Lab., Med., Nahmussen University, Philadelphia, PA 19102**

A questionnaire was sent in three different time mailings to the Chemistry Supervisors, Hematology Supervisor and Chief Pathologist of 190 hospital laboratories in Pennsylvania. The questionnaires covered practices for handling proficiency test specimens in their laboratories, with assurances of confidentiality of the respondents. Responses were received from 156 of 190 hospitals (82.1%) and included responses from 131 Chemistry Supervisors, 106 Hematology Supervisors and 92 Pathologists. The median hospital size was 258 beds. Virtually all respondents participated in CAP surveys (97% in chemistry and 100% in hematology). These surveys meet Pennsylvania licensure requirements. The vast majority of respondents (95 to 99%) indicated moderate to great pressure for scoring acceptably. Thus, it is not surprising that the survey showed a high prevalence of special practices including analysis of controls prior to survey specimens (23 to 42%), analysis in duplicate on a single instrument (52 to 68%), analysis on more than one instrument (17 to 31%), analysis on two or more instruments (20 to 30%) and delay of testing until an instrument is "working better" (24 to 34%).

Approximately 63% of chemistry results and 72% of hematology results are reported as averages or medians. Pathologists consistently reported a lower prevalence of special practices than the laboratory supervisors and reported only 41% of results being reported as averages or medians.

In summary, the observed high prevalence of special handling of proficiency specimens, both important and otherwise, is surprising and divergent implication. These include:

1. Artificially improved test proficiency scores;
2. Artificially narrowed test proficiency limits and
3. Inability to correctly identify both good and bad performing laboratories.

**CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988 1247**
A LOTUS 1-2-3 TEMPLATE WITH MACROS FOR LABORATORATORY STATISTICS. Kenneth J. Blich and Clayton Fuller (Department of Pathol., U. of Oklahoma Health Sci.Ctr., Oklahoma City, OK 73126) (Sponsor: John W. Soper)

We have developed a macro driven LOTUS 1-2-3 spreadsheet program to automate laboratory statistics including mean, standard deviation, correlation coefficient, and linear regression. Macros included on the spreadsheet provide for data entry and editing, calculation of standard statistical parameters, data storage and retrieval, printing of raw data and statistics, graphing of data including the regression line, and spreadsheet finalization. A macro for on-screen user help is also included. The spreadsheet can accommodate up to 150 pairs of data elements for correlation analysis.

The "Release 2" version of LOTUS 1-2-3 was used to develop the statistical program on an IBM PC AT (468 K RAM, 30 MB hard disk). Several preliminary ideas with respect to spreadsheet layout were obtained from a template downloaded from the CAP bulletin board.

Paired data for statistical analysis was conveniently preselected and stored in our Lotus statistical template from Dbase III files and other Lotus spreadsheets. Also, downloaded data from a laboratory information system can be conveniently imported into our statistical spreadsheet, thus facilitating data analysis without rekeying. Graphical output for plotted data was stored as ".PIC" files, then plotted on a multiscreen Hewlett-Packard plotter Model 7475A.

A GRAPHICAL TECHNIQUE FOR PREDICTIVE VALUE ESTIMATION: A RAPID METHOD FOR CALCULATING AND COMPARING TEST VALUES. Richard T. Todd Light and Mary Carole Smith. (Vanderbilt Univ., Path. Dept., and Radiation Admistration Medical Center, Nashville, TN 37212) (Spon.: Richard Todd Light)

Test predictive value is increasingly used to assist in clinical decision making including the selection of follow-up testing and therapy. In an effort to make predictive value (PV) estimation more convenient we have devised a pair of graphs that can be used to estimate the predictive value of a positive (PV+) or negative (PV-) result for any clinical test for the entire range of sensitivity, specificity, and prevalence. The method requires the sequential use of two graphs. Using the sensitivity and specificity as coordinates, the first graph estimates a dimensionless parameter which, for the positive predictive value is the inverse of the likelihood ratio. The second graph is a plot of PV versus prevalence (for PV+) or versus 1 - prevalence (for PV-). The dimensionless parameter used with the second graph is used to select one graph from a family of curves which plot the PV as a function of prevalence. The same pair of graphs can be used to estimate both the PV+ and the PV-. The first graph permits a convenient comparison of tests with different specificities and sensitivities which is independent of the prevalence of the disorder being sought. The second graph emphasizes the importance of prevalence in determining predictive value and can be used to educate medical professionals in the importance of pretest prevalence in test selection.

The technique reduces predictive value calculations to the sequential use of two graphs. For given values of specificity and sensitivity a single graph of PV versus prevalence can easily be generated on a computer to permit the physician to make his own estimate of PV dependent only on his estimate of pretest prevalence.

AN EVALUATION OF THE BECKMAN INTERLINK SYSTEMS DIRECTOR INTERLINK WITH THE SYNCHRON AS6 AND THE BMD HITACHI 705 CLINICAL CHEMISTRY ANALYZERS. I. Matsuda, P. Rothe, J. R. Pearson (University Hospital, Denver, CO 80262) V. Lauderidge, M. Fox, F. Thompson, B. Haden, D. Dickey (Beckman Instruments, Brea, CA 92621) (Spons: J. R. Pearson)

We evaluated the use of the Beckman Interlink Systems Director (Interlink) to interface with analyzers from two manufacturers; the Beckman Synchron AS6 and the BMD Hitachi 705 (70S).

The Interlink consists of an IBM PC-AI, color monitor, printer and special instrument interfacing boards and cables. The Interlink allows two-way communication with the AS6 and one-way communication from the 705. Patient sample information for both instruments was entered into the Interlink. The system was tested by using it to re-assay patients' samples which were received in the laboratory that day. 1029 samples were assayed over six weeks by one operator. One to twenty assays were run per sample. Over 30 assays of each of three control sera were run to give the appropriate patient information; the 705 results were collated by the operator at the Interlink with the COLLARE RESULTS function. All results and patient identification information printed by the Interlink were checked against instrument data tapes and instrument load lists for errors in transmission or translation; none were found. The reports on the control sera and the Levy-Jennings Charts provided similar information to that from the quality control system presently in use in the laboratory. The patient results were judged to be satisfactory.

The results of this study show acceptable performance of the Interlink when interfaced with AS6 and 705 analyzers to assist with the evaluation of the results from an analytical run. To produce quality control reports and to produce an integrated patient report.

COMSYS: A Telecommunication System Operated by the Laboratory Information Systems Division of the AACC. Jerome A. Johnson, Ph.D. (Digestive Disease Center, Grant Medical Center, 111 South Grant Ave, Columbus, OH 43215) (Spon.: Jerome A. Johnson)

COMSYS is an experimental telecommunication system sponsored by the Laboratory Information Systems (LIS) Division for Division and AACC members. COMSYS can be used for posting of clinical laboratory data and results for use by any user, and for the exchange of test and program files. COMSYS requires no programming knowledge to use and is designed to be transparent to the user. Message entry and retrieval use simple techniques with on-line help available at all times.

COMSYS is operated on a Kaypro-10 personal computer using a customized version of the remote operating system (ROS) software package written by Steve Fox. A U.S. B. R. modem answers calls at 350-3400 bps over a standard telephone line.

Text, data, and program files can be uploaded to the system using the XMODEM file transfer protocol.

During the first year of operation, 125 different users connected to COMSYS on average of 4.5 times each with an average connect time of 27 minutes per call. Most users (85%) were local users calling over personal telephone lines. Over 850 messages were exchanged among all users while only 15 program/data files were uploaded to the system during its first twelve months.

The major improvement to COMSYS in 1988 is the installation of a toll free (800) number to the system which will allow free access by all Division and Association users. Grants to support this feature have been provided by a number of clinical laboratory computer vendors.

COMSYS user manuals will be available at the poster session and include the necessary sign-on procedures to access the system.

Microcomputer Based Models for Assessing Laboratory Productivity
Paul S. Belyeu, G. Behn, D. Malpede and D. O'Bryan, SmithKline Bio-Science Laboratories, King of Prussia, PA 19406 (Spon: Paul S. Belyeu)

An accurate assessment of laboratory productivity is a critical concern to managers directing laboratory operations in today's fiscal environment. The ability of a laboratory to evaluate its productive capacity and adjust its operating systems to balance capacity with workload is also essential.
We will present a microcomputer based system that will permit the laboratory to collect data and provide them with an immediate indication of departmental productivity. Lotus 1-2-3 and a microcomputer based model using CAP unit values are used for the calculation of productivity. Laboratory management can determine a laboratory's productivity for workload performed using existing methods and instrumentation. Management can also evaluate laboratory requirements using alternate methods to perform the same volume of specimen load.

The use of a microcomputer spreadsheet model provides a convenient vehicle for the daily collection of test volumes. Further, it provides an easy way to record labor hours spent analyzing specimens and allows an immediate evaluation of laboratory productivity.

**470**

**BREAST CANCER ESTROGEN RECEPTOR ASSAYS: ASSESSMENT OF A DIRECT HYPERBOLIC FITTING TO ANALYSE MULTIPONT BINDING DATA.**

J.Y. Bournaud, M.P. Bournaud, T. Metaye, P. Regon (Laboratoire de Biophysique Cellulaire, Service de Medicine Nucleaire, Hopital Jean Bernard, BP 577, 86021 POITIERS Cedex, FRANCE).

(Spon: A. Thompson)

Analysis of breast cancer estrogen receptor multipoint binding assay is performed by fitting experimental data to an hyperbolic model derived from the law of mass action. The calculations performed on a personal computer are carried out from the total bound and free ligand concentrations. The parameters estimated by hyperbolic fitting, receptor concentration N and constant of dissociation Kd, are in good agreement with those obtained by Scatchard's transformation. N and K derived from hyperbolic analysis are much less susceptible to the influence of experimental errors.

The model is more reliable at low receptor concentrations. The main advantage of the hyperbolic fitting is to simplify the technical methodology in clinical laboratory practice; there is no need to determine the non-specific bindings experimentally.

Calculations can be easily automated on any laboratory microcomputer. Assays of any kind of receptor can be analysed by the hyperbolic fitting when the physical-chemical equilibrium between receptor, non- saturable component and ligand can be approximated by a hyperbolic component model.

**471**

**AN EVALUATION OF THE BECKMAN INTERLINK**

**SYSTEM ORTECK INTERFACING TWO SIECERON AS3's, ONE SIECERON CE3 AND A NELWITT PACKARD 1000 HOST COMPUTER.**

Steven A. Stichel, Peter R. Anderson, Dept. Pathol., St. Vincent Hospital and Medical Center, Portland, OR 97239 (Spon: F. B. Anderson).

The Beckman Interlink System Interface was interfaced bidirectionally to two SIECERON AS3's, one SIECERON CE3 and a Hewlett Packard 1000 host computer in a clinical laboratory setting.

The Interlink allowed the operator to program chemistry requests and demographics for the three attached instruments at a single work station. The programming information was then sent to the instruments via connecting cables. After chemistry analysis, the instruments sent the results to the Interlink whereas they were automatically collected into the appropriate patient file. During the evaluation, 5440 results obtained at the Interlink were verified against the instrument result printouts.

The major Interlink functions were tested including Process Samples, Send Data, Edit/Approve, Special Functions, Print Reports and Quality Control. The various pathways possible to complete each function were tested as well as FACT and ORGAC (Overrange Detection and Correction) capabilities.

The computer interface capabilities was established following the Interlink specifications. Once the Interlink received the programming information from the host, the PROCESS DOWNLOAD function was used to assign these samples to Interlink trays. This information was then sent to the instruments and analyzed; then results were transmitted back to Interlink and on to the host computer. From data 3000 results were verified at all stages of transmission.

This study demonstrates that Interlink successfully bidirectionally interfaced the attached instruments and the host computer.

**472**

**DIAGRAMATIC REPRESENTATION OF QUANTITATIVE LABORATORY RESULTS.**

Dietrich Geisseler, Manfred Eggstein (Dept. Biofisica, Universidade Federal do Piaui, Recife/Brasil 61 Med. Universitatsklinik Tuebingen Abt.IV/West-Germany) (Spon: Dietrich Geisseler)

Evaluation of quantitative test results in clinical chemistry requires both information about the biological variation of the test variable and the analytical variation of the applied test method. The conventional report of laboratory tests as numerical values only neglects the ratio between normal range and analytical deviation which may suggest the misconception of a "point" on the scale of values for this variable.

In increasing biological and analytical variation in units of the normal range (2SD), the results of all variables can be presented on the same standardised scale. The mean of the normal range has the value of 0 and all normal values fall within the range of -0.5 to 0.5. After appropriate mathematical transformation to produce a normal distribution, a certain test value appears on this scale in length of a methodology error. The situation of the line on this scale indicates the relative position to the normal range.

Using this data reduction, the significance of a numerical test result in regard to the extensions of normal range and analytical precision is obvious.

The normal values for all test variables are identical, so that multiple tests can be plotted on the same conditions.

Test values are not subject to changes in determination conditions.

**DRUGS—PART A**

**473**

**EFFECT OF DIGOXIN-LIKE IMMUNOREACTIVE FACTOR ON THE REFORMULATED ABBOTT TDx DIGOXIN II ASSAY.**


Digoxin-like immunoreactive factor (DLIF) is known to interfere positively with a large number of digoxin immunoassays. Recently, Abbott has reformulated its Digoxin II assay to reduce interference from DLIF. We tested Digoxin-free sera from neontates (age 2-25 weeks), patients with renal failure (creatinine >15 mg/L), hepatic failure (bilirubin >20 mg/L) and combined renal and hepatic failure, along with normal patients (creatinine <10 mg/L; bilirubin <20 mg/L).

Digoxin (µg/L)

<table>
<thead>
<tr>
<th></th>
<th>Reformulated assay</th>
<th>Original assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (range)</td>
<td>0.75 (0.51-1.29)</td>
<td>0.52 (0.49-0.64)</td>
</tr>
<tr>
<td>Renal Failure</td>
<td>0.06 (0.00-1.28)</td>
<td>0.05 (0.00-1.28)</td>
</tr>
<tr>
<td>Heparin Failure</td>
<td>0.07 (0.00-0.28)</td>
<td>0.07 (0.00-0.28)</td>
</tr>
<tr>
<td>Renal &amp; Hepatic Failure</td>
<td>0.53 (0.10-1.29)</td>
<td>0.40 (0.19-3.04)</td>
</tr>
<tr>
<td>Neutones 0-2 weeks</td>
<td>0.58 (0.05-1.14)</td>
<td>1.23 (0.34-2.48)</td>
</tr>
<tr>
<td>3-25 weeks</td>
<td>0.36 (0.05-1.16)</td>
<td>0.85 (0.22-1.86)</td>
</tr>
</tbody>
</table>

Twenty-four of the renal and hepatic patients samples whose apparent digoxin value by the reformulated assay was greater than 0.5 µg/L were analyzed by the Corning Magid DigoxinRIA and the Sysmex Column Digoxin enzyme immunoassay.

**474**

**APPLICATION OF THE CEDIA™ DIGOXIN ASSAY TO THE EPOS™ CHEMISTRY ANALYZER.**

Walt Klotzsch, Joy Metzler, Piere L.Kaasen (Microgenics Corporation, Costa Mesa, CA 92626) (Spon: Donald Roedel).

Microgenic's CEDIA™ Digoxin Assay has been adapted to the EMDIS™ EPOSTM Chemistry Analyzer. The CEDIA™ assay is based on a genetically engineered 5-galactosidase enzyme which is split into two enzyme portions, one a galactosidase and one a digoxin antibody fragment. The fragments can spontaneously recombine to form active enzyme. The degree of recombination is controlled by binding of anti-digoxin antibodies to the digoxin labeled peptide, which is in turn controlled by the concentration of digoxin in the sample. Adaptation of the CEDIA™ Digoxin Assay to the EPOS™ allows for both batch and batch testing capabilities. Reagents are reconstituted and used for up to seven consecutive runs. Calibrators, controls, and serum samples are transferred to sample positions and the run is initiated. All sampling is done in single point. The assay is linear to 4 mg/L and the standard curve is stable for three days with daily zero span.
The administration of Digoxin Immune-Fab (Digibind®), as an antidote for severe digitalis intoxication, renders all total digoxin immunosassay methods useless. Depending on the stoichiometry between digoxin and Digibind, digoxin concentrations measured by immunosassay methods will vary from highly elevated to undetectable.

During the administration of Digibind, the free digoxin concentrations often become essentially zero. One of the severe complications of Digibind therapy is that patients with intrinsic poor renal cardiac function cannot tolerate withdrawal of the isotropic action of digoxin.

Methods are presented for measuring total digoxin, free digoxin, and Digibind concentrations in serum during Digibind therapy. Measurement of these compounds during Digibind therapy should facilitate the elimination of digitalis toxicity while preventing over-dosing and withdrawal symptoms.

We have found that one immunosassay system (SYVA) actually measures free digoxin rather than total digoxin as advertised. This system was found to be unaffected by Digibind and can be used to measure free digoxin throughout the course of therapy. Free digoxin can be measured by other immunosassay systems by using free-drug filters, but other factors affect the accuracy of the filtrate concentrations. Total digoxin can be measured in the presence of Digibind by placing the serum specimen in a boiling bath for 5 minutes to destroy the Fab fragments. Digibind can be measured by adding excess digoxin to a specimen, and measuring digoxin on the "discards" from a SYVA isolation column.


Concentrations of substance with digoxin-like immunoreactivity (DLI) in plasma or serum samples of normal adult subjects are frequently close to the limit of sensitivity of RIA methods, while DL1 concentrations in urine samples are four- to five-fold greater (Clerico et al. Clin Chem 1987, 33:340-1). Urine samples could be preferable for clinical investigations. However, studies on a large number of normal subjects, and on the variation of 24-hour urinary excretion of DLI have not been reported.

We studied 91 normal subjects (46 men and 45 women; mean age 50.2, SD 10.1 years; body mass index 22.7±5.30), who collected their urines during the night at rest for the determination of urinary excretion rates of DLI (pg/mg) by means of a sensitive RIA method (Bozi et al. Clin Chem 1984; 30:450). The creatinine (mg/mg) and salicylates (mg/mg) urinary excretion rates were also calculated on some of these subjects. DLI excretion rates in men were significantly higher than in women (73.7±4.27 pg/mg vs 51.6±2.14 pg/mg, P<0.0006). We found significant and positive correlations between the values of DLI excretion rates and those of creatinine (r=0.567, P<0.0001, n=96, r²=0.283, P=0.0048, n=56) and K⁺ urinary excretion rates (r=0.32, P=0.0162, n=56), and also with weight (r=0.335, P=0.0015), height (r=0.291, P=0.0063), body mass index (r=0.248, P=0.0208), and systolic blood pressure (r=0.285, P=0.0047).

In addition, to verify whether there is a variation of 24-hour urinary excretion of digoxin-like immunoreactivity, we studied 16 normal adults, who collected their urines for 24-h in several fractions. The mean DLI urinary excretion rate calculated in the complete 24-h collection was 84.6±31.3 pg/mg. The mean DLI urinary excretion rate calculated for overnight collections was significantly lower than those of afternoon collections (P<0.01) and the 24-h collections (P<0.05). Significant positive correlations were found, in 16 of these subjects, between urinary DLI and creatinine rates for creatinine (r=0.347, P=0.0016), Na⁺ (r=0.292, P=0.038) and K⁺ (r=0.323, P=0.003).

Our data suggest that urinary secretion of DLI is higher during "active" hours of the day, and dependent to body mass and renal glomerular filtration.

477 CONCURRENT QUANTITATION OF FLECAINIDE ACETATE AND PROPRANOLOL BY NORMAL PHASE LIQUID CHROMATOGRAPHY, Cheryl L. Rogenrodt and Cheng-Nan Ou (Department of Pathology, Texas Children's Hospital and Baylor College of Medicine, Houston, Texas 77030) (Spon.: C. L. Rogenrodt)

Propranolol and flecainide acid are two antiarrhythmic agents whose preferred methods of quantitation at present involve HPLC with fluorescence detection. The ability to determine concurrent concentrations of these drugs in serum may be a cost-effective use of the relatively expensive equipment involved.

In this procedure, 100 ul of serum or plasma is mixed with 200 ul internal standard [N-(2-piperidinylmethyl)-2,3-bis(2,2-trifluoroethoxy) benzene hydrochloride], 200 ul 0.2 M sodium carbonate, and 200 ml is then drawn through a primed C18 Bond Elut column (Analytichem Intl). The column is washed with acetonitrile. Two milliliters of the mixture is then passed through the column, collected, and evaporated to dryness. Reconstitution is made in 200 ul methanol. A 50 ul aliquot is injected onto a 5.9 mm x 15 cm Waters Resolve 5 u spherical silica column. The mobile phase is methanol and 7.3 M acetate buffer (pH 7.123) with a flow rate of 0.9 ml/min. The fluorescence is monitored at an excitation wavelength of 225 nm and an emission wavelength of 340 nm. Retention times are 5.3, 6.0 and 7.0 min for propranolol, flecainide and the I.S., respectively. Quantitation is based on peak area ratios compared with serum based standards for flecainide and aequous standards for propranolol.

Absolute recovery for both drugs is 80-85% at all levels. The method has sensitivity to detect as little as 25 ng/ml of flecainide and propranolol at 10 ng/ml. It is linear to at least 2000 ng/ml for both drugs. Precision within run is 1-3% and between runs is 2-4%. Other cardiac agents as well as commonly encountered drugs do not interfere with this assay.

The method is rapid, sensitive and cost-effective. The small sample volume requirement makes it an ideal method for pediatric drug monitoring.
The digoxin level in an ultrafiltrate without ethanol or nifedipine in ethanol was 1.73 +/- 0.15 ng/mL; with ethanol, 1.66 +/- 0.06 ng/mL, and with nifedipine in ethanol, 2.22 +/- 0.32 ng/mL and 1.87 +/- 0.04 ng/mL, respectively. Thus, no interference with the assay was seen.

Free digoxin was determined as follows: Sample Total Digoxin Nifedipine Free Digoxin % Free (ng/mL)(SD) (ng/mL) (ng/mL)(SD) 1a 3.15 0 2.33 74.0 1b 100 2.36 74.9 500 2.30 75.9 2a 1.07 0 0.75 74.8 2b 500 0.79 73.8 3a 2.01 (.07) 0 1.56 (.21) 77.6 3b 500 1.96 (.10) 75.0 4a 4.40 (.23) 0 3.76 (.18) 85.5 4b 500 3.67 (.08) 83.4 (* endogenous digoxin; 1 hr incubation at room temperature, SD not calculated due to limited availability of patient specimen. ** added digoxin; 22hr incubation) We conclude that nifedipine does not increase the unbound fraction of digoxin in the blood and should not contribute to digoxin toxicity by this mechanism.

** Determination of free quinidine by fluorescence polarization immunoassay, Bai Haimin Chen, E. Howard Taylor, and Alex A. Pappas (Dep. Path., Univ. Arkansas Med. Sci., Little Rock, AR 72205) (Spon. by Haimin Chen)

Serum quinidine results for both total and free drug, determined by fluorescence polarization immunoassay (FPIA, Abbott TDx), were compared with results from high performance liquid chromatography (HPLC). Patients with ventricular arrhythmias or the presence of at least 5 consecutive complexes of inducible ventricular tachycardia received 5 mg/kg intravenous quinidine gluconate. We found good agreement between FPIA and HPLC for total quinidine [FPIA = 0.956 (HPLC) + 0.234, r = 0.993, n = 36], and for free quinidine [FPIA = 1.126 (HPLC) + 0.064, r = 0.917, n = 32]. For FPIA, the within-run coefficient of variation (CV) was 1.6% and 1.7%, and day-to-day CV was 1.9% and 1.6% for total and free quinidine at concentrations of 3.25 and 0.33 mg/L, respectively (n = 4 for each).

Measurement of standard solutions in 0.9% NaCl before and after ultrafiltration revealed that only 5.2%, 3.1% and 5.4% were retained, respectively, for quinidine, 3-hydroxyquinidine and dihydroquinidine. The proportion of free quinidine averaged 8.6 +/- 3.8% (range 4.4 - 17.2%), and the proportion of free 3-hydroxyquinidine averaged 47.1 +/- 13.3% (range 18.2 - 64.6%). The quinidine fraction was not influenced by 3-hydroxyquinidine, since there was no correlation between the proportion of free quinidine fraction and the total 3-hydroxyquinidine concentration. Thus, FPIA is a rapid, reliable method for the determination of free, as well as total, quinidine.

** Solid phase fluorescence immunoassay for serum digoxin, Wee Lee T. Wong and Naomi Komada (SOLAG INC. West Coast, Sunnyvale, CA 94089) (Spon.: G. L. Howley)

A totally automated fluorescence immun assay for serum digoxin utilizing IMPULSE® instrumentation has been developed. The IMPULSE reagents for digoxin and samples are placed in the instrument. The digoxin assay configuration of the instrument is initiated. Automatically the 50 µL serum sample and 10 µL fluorometric reagent are pipetted into an antibody coated reaction vessel and the competitive binding reaction takes place between the labeled and serum digoxin. The unbound components are removed, the vessel washed, and the bound components solubilized. The bound fluorescent labeled digoxin is measured for each sample and the concentration obtained from a calibration curve.

The IMPULSE reagents are stable for one year, all in ready-to-use formulations, and the calibration curve can be stored for use for the life of the kit. The instrument is compatible with pipetting, washing, reading, calculating and tabulating.

The quantitation of serum digoxin is linear over the concentration range of 0.25 ng/mL to 60 ng/mL and the assay sensitivity is 0.25 ng/mL with 95% confidence. The precision is 5.0 to 7.0% coefficient of variation for both inter and intra assay runs. The average recovery of spiked digoxin from serum specimens is 101.1% with a range of 92.3 - 110.5%. Cross-reactivity with diploptin is 3.69% and correlation with RIA (Diagnostic Products, Inc.) is 0.97, r = 0.02, 0.797, and with FPFA (Abbott TDx) is 0.96, n = 68, 0.07 + 1.68.

** Comparison of four non-radioimmunoassay digoxin methodologies to radioimmunoassay, R. T. Chamberlain, K. Y. Chamberlain, Sheila White, and Judith Upton (Toxicology Lab., VA Med. Ctr., Memphis, TN 38104) (Spon.: R. T. Chamberlain)

Four digoxin non-RIA methods were compared to an RIA method presently in use. Compared to the Becton Dickinson RIA assay was CEDIA (Microgenica, Concord, CA), TDx Digoxin II (Abbott Labs, Irving, TX), Du Pont ACA and Du Pont Extend (Du Pont, Wilmington, DE). A total of 84 samples were analyzed by all methods. Included were hemodialysis patients, most of whom were not on digoxin. These patients were included to see if any of the test methods would exhibit a significant amount of Digoxin Like Interfering Factor (DLIF). When compared to RIA the following results were obtained from linear regression analysis:

<table>
<thead>
<tr>
<th>B</th>
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<th>Slope</th>
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</tr>
</thead>
<tbody>
<tr>
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<td>0.115</td>
</tr>
<tr>
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<td>0.176</td>
</tr>
<tr>
<td>ACA</td>
<td>84</td>
<td>1.42</td>
<td>0.924</td>
<td>0.323</td>
</tr>
<tr>
<td>Extend</td>
<td>84</td>
<td>1.28</td>
<td>0.767</td>
<td>0.266</td>
</tr>
</tbody>
</table>

A few discrepancies in digoxin concentrations in dialysis patients were seen. These were seen only in Extend and ACA methods. Other methods showed no significant amount of interference.

Due to lack of pretreatment the CEDIA method was less labor intensive. The TDx and ACA methodologies required a short pretreatment while the Extend method was time consuming. The CEDIA and Extend Reagent cost were the lowest. The Cobas Bio (Roche Diagnostics, Montclair, NJ) was utilized to run the CEDIA and Extend assays. Both of these methods are adaptable to multireagent compatible analyzers.

** Abstract Withdrawn

** Simultaneous analysis of five new class I antiarrhythmic drugs, Richard E. Scott, Peter Johnson, and Thomas P. Moyer (Toxicology Lab., Mayo Clinic, Rochester, MN 55905) (Spon.: R. E. Scott)

A method is presented for the simultaneous analysis of tocainide, mexiletine, encainide, flecainide and propafenone in serum by reversed phase high performance liquid chromatography using gradient elution. Monitoring of these new antiarrhythmics is necessary due to their narrow therapeutic ranges and propensity for undesirable side effects at supratherapeutic levels. A single liquid-liquid extraction technique, employing a butyl chloride/isopropanol solvent mixture, allows all drugs to be rapidly and quantitatively removed from 1 mL of alkalized plasma or standard solution (in calf serum). Samples are dried, reconstituted in mobile phase, and 40 µL injected onto a 30 cm x 4.6 mm Lichrosorb RP-18 column (Waters, Milford, MA 01757).

The drugs elute in the k' range of 3.45 (tocainide) to 13.68 (propafenone) and are detected by absorbance at 210 nm. Analytical recoveries vary from 29% (mexiletine) to 72% (encainide). Intra-assay precisions from 1.0% to 3.7% and inter-assay precisions from 2.4% to 12.6% (drug and concentration dependent) were observed. The assay is linear over the therapeutic ranges of these drugs, and the lower limit of detection is at least 1.0 µg/mL for tocainide and 50 µg/mL for the remainder. No significant interference was found after analyzing 98 other drugs. Comparison with our existing isocratic HPLC methods for tocainide, mexiletine, and encainide showed good correlations (r = 0.946, 0.852, 0.924, respectively).

While the recoveries are lower than optimal in some cases, the observed precision, sensitivity, and correlation with established procedures suggests it is a good substitute, with the advantage of simultaneous measurement of these five antiarrhythmic drugs.

An immunometric assay for digoxin in serum or plasma has been developed. This assay uses an excess of a monoclonal antibody of 8-galactosidase with the Fab fragment of a monoclonal antibody against digoxin to bind the digoxin in the sample. The excess conjugate is removed by mixing the sample-conjugate solution with an affinity resin containing covalently coupled digitoxigenin and separating the resin with a small particle filter. The enzyme activity of the conjugate in the filtrate is determined on a reagent strip containing the substrate 7-D-glucopyranosylxoyethyl-ethyl-5-ethyl-3-amin-2-one using an Ames SERALYZE1 Photometer. The rate of color development between 40 and 60s at 630 nm is directly proportional to the digoxin concentration in the sample. The assay uses a 30 second calibration and is linear between 0 and 5 ng/mL. The precision of the assay was found to be as follows:

<table>
<thead>
<tr>
<th>Target Value (ng/mL)</th>
<th>Mean Within Run (n=60)</th>
<th>Mean Between Run (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>0.04</td>
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</tr>
<tr>
<td>0.06</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>0.20</td>
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</tr>
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<td>1.00</td>
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</tr>
<tr>
<td>4.00</td>
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<td>4.03</td>
</tr>
</tbody>
</table>

Comparative studies were performed against Clinical Assays RIA, NML RIA, and TDX digoxin assays on 100 serum samples. The regression equations were y=0.03x - 0.07 (r=0.96), y=0.06x + 0.06 (r=0.95), and y=0.13x - 0.01 (r=0.96), respectively. Crossreactivity studies indicate no crossreactivity to metabolites and significant crossreactivity only to chemicals used in the assay procedure.

In summary, we have developed an immunometric assay for digoxin use with the Ames SERALYZE1 Photometer which is convenient to use and has very good performance characteristics.

**Cardiac Interference with Abbott TDX Digoxin II FPIA, J. B. Brown, P. Bunting, S. J. Goldin, Dept. of Clinical Biochemistry, Univ. of Toronto, Toronto, Ontario. (Spon.: Judy Stone)**

We describe a case of creatinine (Cr) interference with the Abbott TDX digoxin II FPIA (SSA), investigated with a specific digoxin HPLC-immunoassay (LC-FFIA) method previously described. The patient was a 72-year-old male in acute renal failure. Two days after beginning therapy with 400 mg IV Cr daily, his serum digoxin concentration (SSA) was 3.8 mg/mL as measured by SSA. (SEC three days before Cr was 1.1 mg/mL). Digoxin (DG) was discontinued, but SSA rose to a maximum of 5.0 mg/mL eight days after the final DG dose, at which time DG was discontinued. SSA by SSA then fell to 1.9 mg/mL (15 days after final DG, 7 days after final Cr dose). The patient showed no signs of digitalis toxicity. SSA as measured by digoxin II FPIA (LC-FFIA) were approximately 1/2 those by SSA e.g. 2.0 to 2.1 mg/mL. These same samples had SSA between 0.8 and 1.0 mg/mL when analyzed by LC-FFIA. The patient was then given to on Cr of 0.6, 0.7, and 2.1 mg/mL by LC-FFIA, SSA and TDX respectively. SSA is aigononol (SP) metabolite. SSA itself has a concentration of SSA and TCA of 0.18 at 10 mg/mL. SSA was measured after 3 days of 400 mg Cr daily had SSA of 0.4 mg/mL by TCA, 0.8 mg/mL by SSA. Free txa and 3 volumes on low dose Cr or for shorter times had SSA < 0.3 mg/mL by all methods. We conclude that patients on high dose SSA exhibit a significant positive interference with the Abbott TDX digoxin II FPIA as has been previously shown for other Cr immunassays. Patients on high dose SSA for greater than 3 days may or may not have falsely positive values in the digoxin II FPIA.


It has been suggested that an endogenous factor with activity similar to cardiac glycosides could play a role in the regulation of fluids and electrolytes in animals and humans, as well as the pathogenes of low-renin hypertension. On the other hand, it is well known that hypertension occurs more frequently in diabetic patients than in normal population and that the hypertension of insulin-dependent diabetes mellitus is characterized by a volume expansion and low levels of renin.

We studied the secretion rate (ES) of digoxin-like immunoreactivity (DLIS), during timed overnight urinary collections, in 91 non-diabetic, normotensive subjects and 104 insulin-dependent normotensive diabetic patients (DLIS concentration in urine was measured by a sensitive RIA method DLIS-ER in diabetes was correlated with urinary Albumin Excretion Rate (AER), glycated hemoglobin, patients age and duration of disease, renography, and ER of electrolytes.

The mean DLIS secretion rate (ES) in diabetic patients was significantly higher than that obtained in controls (73 ± 41 vs 63 ± 36 pg/min, p = 0.024). Within diabetes, men had mean DLIS-ER higher than women (87 ± 41 vs 66 ± 40 pg/min, p = 0.01). Furthermore, diabetic males had DLIS-ER higher than control ones (87 ± 41 vs 74 ± 45 pg/min, p = 0.04), as did diabetic patients in insulin treatment with healthy females (66 ± 40 vs 121 ± 49 pg/min, p = 0.03). In diabetics DLIS-ER significantly correlated with AER (r = 0.26, p = 0.0071) and creatinine ER (r = 0.395, p = 0.0001), body weight (r = 0.258, p = 0.0126) and (r = 0.281, p = 0.0185) and fexcretion rate (r = 0.298, p = 0.016). In the multiple regression analysis the DLIS-ER was significantly contributed to the correlation with those of DLIS-ER in diabetic patients (r = 0.517, p = 0.001).

Our findings indicate that increased amounts of a substance (or a group of substances) with activity similar to cardiac glycosides are excreted in urine of some
insulin-dependent diabetic patients. Further, we suggest that our methodology of DLIS is dependent on glomerular filtration rate and physico-chemical properties of glomerular membrane, besides on the body mass.

Anti-Convulsant Drugs

**450**

**EVALUATION OF A PETINIA ASSAY FOR PHENYTOIN ON THE HITACHI 705.** B.G. Penfield and R.B. Feld, (Dept. of Path., Univ. of Iowa, Lowville, IA 52242) (Spon.: Ronald Feld)

We have adapted a PETINIA (particle enhanced turbidimetric inhibition assay) (DuPont) for phenytoin (DHP) to the Hitachi 705. No preincubation with benzamidinium. Reagent preparation was as follows: working solution (R) was diluted with 20 parts phosphate buffer, the antibody solution (Rb) was diluted with an equal volume of normal saline. Instrument parameters were: end point mode with blank, sample volume 4 ul, Rb - 400 ul, Rb - 50 ul, wavelength 640/340 nm. The calibration curve consisted of 6 DPH calibrators ranging from 0.0 to 30 ug/mL. The calibration curve was calculated using a nonlinear least squares computer program. The standard curve was stable with a simple dilution of working particle solution.

Within run precision was determined with 10 replicates from a single pool. A mean value of 19.9 ug/mL was obtained with a S.D. of 0.62, C.V. of 3.1%. Between run precision was determined using a commercial lyophilized control over 24 days. This gave an S.D. of 1.67, C.V. of 12.0% at a mean value of 14.90 ug/mL.

Comparison studies were performed on 62 serum samples covering the analytical range of 0 to 29 mg/mL DHP. Most emphasis was placed on the therapeutic range of 10-20 mg/mL. The comparison method used was an EKT procedure (Syva) performed on a Multistat III analyzer (Instrumentation Labs). The correlation equation was PETINIA = 1.038 EKT - 0.133. r = 0.972, n = 62 with mean values of 13.5 ug/mL PETINIA and 13.1 ug/mL EKT. Cross-calibration of the two methods gave good agreement. Linearity was determined by admixture of a high (30 ug/mL) serum DPH specimen and DPH free serum specimen. The assay was linear to at least 25 ug/mL. The Hitachi 705 gave the same correlation with the EKT assay and acceptable precision.

**401**

**COMPARISON OF FLUORESCENCE POLARIZATION IMMUNOASSAY (Abbott-TDX) AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FOR THE QUANTITATIVE DETERMINATION OF TOTAL AND FREE PHENYTOIN, Maria C. Maas, R.R. Karcher, K. Epstein, and F.L. Kiesche (William Beaumont Hospital, Royal Oak, MI 48072). (Spon.: F.L. Kiesche)

Two methods (ABBOTT-TDX and HPLC) that measure total and free phenytoin were compared. For the phenytoin determinations, serum ultrafiltrates were prepared by centrifugation in disposable filter units (CentriFree Micropartition System, Amicon Corp.). Randomly selected sera were assayed simultaneously by both methods for free and total phenytoin. No significant difference in precision of the two methods was observed. Between run CV's ranged from 2.7 to 3.8% for TDX and from 3.7 to 4.1% for HPLC. Linear regression of TDX (y) values on those from HPLC (x) for total phenytoin was: y = 0.999 x - 0.103, r = 0.975, SEE = 0.22, (p<0.001), by paired t test) and for free phenytoin (n = 60), y = 0.994 x - 0.103, r = 0.975, SEE = 0.22, (p<0.001), by paired t test). Similar analysis of the free fraction (y) vs total phenytoin (x) for each technique (n = 50) yielded for HPLC, y = 0.973 x + 0.046, r = 0.960 and for TDX, y = 1.00 x - 0.210, r = 0.978. Ten patients, excluded from regression analysis of free vs total phenytoin, had the percentage of free phenytoin of 31%. Review of the clinical records of these patients revealed that 4 had low albumin (CL/7 mg/dl), 2 had elevated creatinine (35 mg/dl), 2 had both abnormalities and 2 were on medications that compete with phenytoin for albumin binding.

In conclusion, although a statistically significant bias was observed between the two methods, there is excellent agreement between the two for both total and free phenytoin over the entire therapeutic range. The observed differences are not significant for the clinical use of the methods. Free levels are most useful in patients with low albumin, high creatinine or competitive binding drugs, since free levels do not correlate with total phenytoin in these cases.

**494**

**Comparison of Free and Total Phenyltoin and Carbamazepine Concentrations determined by Abbott TDX and HPLC,** Dobrin Svinarov and C.G. Pippenger (Clinical Laboratory, Medical Academy, Sofia, Bulgaria and Department of Biochemistry, The Cleveland Clinic Foundation, Cleveland, Ohio 44195). (Sponsor: Nancy Solano).

Free and total phenytoin (PRT) and carbamazepine (CBZ) concentrations were quantitated in 211 plasma specimens from 90 epileptic patients. Ultrafiltrates were collected with Amicon Centrifree Filters. All Abbott TDX assays were performed on both plasma and ultrafiltrate according to manufacturers instructions. HPLC utilizing the Svinarov technique (Svinarov and Dimova, 1984) was performed on Clini. Lab. Exp. 10-12 Sofia 1984. 50 ul plasma or 100 ul ultrafiltrate is passed through a micro columns and the extract is eluted with 1.0 ml methylene chloride, evaporated to dryness under nitrogen, reconstituted in 100 ul.

**Inc., Stuart, VA 24171) and R.J. Paquet (Technicon Instr. Corp., Tarrytown, NY 10591) (Spon.: H.N. Rose).** The Technicon RAP Systems turbidimetric immunoassay for Phenyltoin has been adopted to the Monarch 1000. Two reagents, a phenytoin-anticarbamazepine conjugate, are used. Calibration is performed using 6 standards ranging from 0.0 ug/mL to 40 ug/mL and was stable for at least 14 days. The assay is performed using the following parameters:

**IDENTIFICATION PARAMETERS**

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**DATA ACQUISITION PARAMETERS**

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</tr>
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<td>400-500</td>
</tr>
</tbody>
</table>

The Technicon RAP Systems Phenytioin reagents are easily adapted to the Monarch 1000 Chemistry Analyzer. Calibration and reagents require no reconstitution. This procedure correlates well with the Abbott TDX and proves to be an economical approach.

**493**

**The Relationships Between Total and Free Carbamazepine Epoepide and Carbamazepine Concentrations in Plasma of Epileptic Patients,** C.G. Pippenger (Clinical Laboratory, Medical Academy, Sofia, Bulgaria and Department of Biochemistry, The Cleveland Clinic Foundation, Cleveland, Ohio 44195). (Sponsor: C.G. Pippenger).

Total and free plasma carbamazepine (CBZ) and carbamazepine epoxide (CBZE) concentrations were quantitated in 115 specimens obtained from 48 epileptic patients receiving either CBZ mono- (n = 87) or polyantiepileptic drug therapy (n = 28). Plasma ultrafiltrates for 40 patients were obtained using Amicon Centrifree Filters. Both ultrafiltrate and plasma were extracted for HPLC analysis utilizing the Svinarov technique (Svinarov and Dimova, 1984). Total CBZ/CBZE ratios were 2.37 ± 0.21 in all patients; Total CBZ/CBZE ratios in patients receiving CBZ monotherapy were 2.26 ± 1.92; polytherapy patients other than CBZ/CBZE ratio were 2.41 ± 0.94. Free CBZ/CBZE ratios in all patients was 2.73 ± 1.14; monotherapy patients 2.02 ± 0.41; polytherapy patients 2.70 ± 1.05. Regression analysis of total vs free CBZ/CBZE ratios in all patients: y = 1.77 x + 0.37; r = 0.957. Quantoation of total CBZ/CBZE ratios can be utilised to predict free CBZ/CBZE ratios in mono or poly CBZ patients. However, the interpatient variability can be quite large depending whether the patient is receiving mono- or polytherapy. Routine measurement of free CBZ/CBZE ratio is not clinically necessary since they parallel the total CBZ/CBZE ratios.

**492**

**ADAPTATION OF TECHNICON RAP SYSTEMS PHENYTOIN REAGENTS TO THE MONARCH 1000,** L.H. Jones, R. Clement-Noulin, D. Lawless (J. Reynolds-Patrick County Mem Hsp.,

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mobile phase (acetonitrile/water 21:79). 20 ul of reconstituted mobile phase was chromatographed on a Sartorius C6 3.0 μ l column (flow rate = 3.3 ml/min) with UV detection at 208 nm. Results expressed as R x SD were as follows: total PTH (n = 20) TDX 13.2 ± 3.3 μ g/ml; HPLC 14.3 ± 3.4 μ g/ml; r = 0.987. Free PTH (n = 93) TDX 1.2 ± 0.7 μ g/ml; HPLC 1.4 ± 0.8 μ g/ml; r = 0.978. Total CSE (n = 107) TDX 8.2 ± 4.0 μ g/ml; HPLC 7.9 ± 3.7 μ g/ml; r = 0.985. Free CSE (n = 115) TDX 1.0 ± 0.9 μ g/ml; HPLC 1.9 ± 1.0 μ g/ml; r = 0.975. Carbasemizepine epoxide quantified in the IL CS plasma was 1.7 ± 1.2 μ g/ml and in the ultrafiltrate was 0.8 ± 0.4 μ g/ml. No significant differences between total and free concentrations determined by the Abbott TDX as compared to the HPLC technique were found. We conclude, Abbott TDX determinations of total and free PTH and CSE are not significantly different from observed HPLC values. The presence of carbasemizepine epoxide at varying concentrations encountered clinically does not interfere with carbasemizepine determinations by the Abbott TDX immunoassay technique.

LATEX INHIBITION IMMUNOASSAY FOR PHENOBARBITAL ON THE IL MONARCH® CHEMISTRY SYSTEM.


A quantitative latex agglutination inhibition assay for the detection of phenobarbital in serum has been developed for use with the Monarch® Chemistry Analyzer. This procedure, which can be run at either 30°C or 37°C, utilizes phenobarbital-coupled latex which competes with phenobarbital present in the sample to limit the number of antibody binding sites. The observed agglutination inhibition, as measured at 340 nm, is proportional to the concentration of phenobarbital present in the patient sample.

Levels of imprecision were typically found to be less than 5% (within-run) and 8% (total) when measuring samples containing between 15 - 60 μ g/ml analyze. Recoveries ranged from 95% to 109%. There is no interference due to the hemoglobin up to 500 mg/dL, bilirubin (up to 100 mg/dL) and lipemia (up to 20 at 600 mg/dL). Phenobarbital recovery is in excess of 90% in the presence of up to 300 IU/dl rheumatoid factor. There is significant interference with relevant drugs or metabolites. Nephobarbital and hexobarbital, not normally found in patient samples, are potent cross-reactants. Patient correlation studies typically resulted in the following: Y = 1.2 x 0.981X (Syva ENIT; n = 100, r = 0.990), and Y = 1.7 ± 0.981X (fluorescence polarization; n = 100, r = 0.995).

In summary, the IL Phenobarbital Immunoassay is a fully automated, rapid, accurate and precise method for the quantitative determination of phenobarbital in serum.

DEVELOPMENT OF A LATEX-BASED PHENYTOIN ASSAY FOR USE ON THE IL MONARCH® CHEMISTRY SYSTEM.


A quantitative latex agglutination immunoassay for the detection of phenytoin has been developed for use with the Monarch® Chemistry Analyzer. This sequential immunoassay utilizes a patient serum sample containing an unknown amount of phenytoin, which is first allowed to react with anti-phenytoin antibody reagent and subsequently with a phenytoin latex reagent. The extent of agglutination, measured at 340 nm, is inversely proportional to the concentration of phenytoin in the patient sample.

Representative correlation studies (n=32) utilizing IL TDX Cal® component calibrators and Fisher Thermo-Plus Tri-Level TDC™ Therapeutic Drug Controls were found to be: y = 0.0 ± 0.967X (Syva ENIT; r = 0.965); y = 0.5 ± 0.943X (fluorescence polarization; r = 0.940). The effect of interfering substances were evaluated and determined to be ≤ 5% for hemoglobin at 400 mg/dL, bilirubin at 60 mg/dL and serum lipids at concentrations up to 500 mg/dL. In the presence of 90% recovery at 400 IU/mL, was determined for rheumatoid factor. No significant interference was observed with any of the interfering substances at or below manufacturers test limits. The observed precision with the phenytoin assay at 30°C was found to range from 2.22 - 5.40% when assaying Fisher TDC Level 1; 2.30 - 5.38% for Level 3 and 3.35 - 5.30% for Level 3 (33.5, 33.5 and 33.5 μ g/ml respectively). At 37°C it was observed to be 3.22 - 6.50%, 3.23 - 5.40% and 2.45 - 6.24% for Levels 1, 2 and 3, respectively.

In summary, the IL Phenytoin Immunoassay is a fully automated, rapid accurate and precise method for the quantitative determination of phenytoin in serum.

3-METHYL-PHENYTOIN ASSOCIATED WITH GC/MS ANALYSIS OF SEVERE SAMPLES CONTAINING PHENYTOIN, Patrick K. Kaynes, Kathy L. Taylor and Anthony G. Naarallah (DeVor Laboratories, Boardman, OH 44412). (Spon.: Patrick K. Kaynes)

An analysis of a severe sample submitted for emergency toxicology revealed phenytoin (PHN) and a priori screening, discrete, unknown component. Mass chromatogram analysis suggested the unknown component to be a PHT derivative, possibly 3-methyl-phenytoin (3-MPH). Using pure 3-MPH kindly provided by Dr. K.H. Dudley we were able to confirm the unknown material as 3-MPH.

PARAMETER OUTLINE

Column: 30m SPB-1, wide bore (0.75mm ID).

Chromatography: Inject temperature 200°C, 2 min at 200°C then to 280°C @7°C/ min. Hold 7 min.

Spectrometry: Separator temp. 275°C, Manifold temp. 50°C.

The presence of 3-MPH has not, to our knowledge, been previously reported in biological samples. The presence of this material suggested three possible origins: PHT metabolite, drug preparation contaminant or analytical artifact.

Seven therapeutic phenytoin patients submitted urine, serum and drug preparation samples. GC/MS analysis revealed PHT in all samples and 3-MPH in the seven serum samples. Furthermore, when pure PHT was spiked into urine and serum blanks PHT was identified in all samples and 3-MPH in the serum samples. These experiments indicate that 3-MPH can be an analytic artifact of serum phenytoin analysis. The metathesis is dependent on a serum component(s) and probably takes place in the injector. Due to its close structural similarity to PHT, the reaction during an emergency toxicology analysis, it is important to recognize this occurrence and not confuse this entity with an unknown drug.

COMPARISON OF CAPILLARY COLUMN WITH PACKED COLUMN FOR THE ANALYSIS OF ANTI-EPILEPTIC DRUGS (AED) BY GC/MS CHROMATOGRAPHY.


Gas chromatography (GC) has been used for quantification of AED in serum. Packed glass columns have traditionally been used for AED analysis. Pulsed silica capillary columns have provided an alternative with improved resolution. We compared GC analyses of phenobarbital (PBR), phenytoin (PHT), and carbamazepine (CBE) in glass and capillary columns. Packed column spec: 5 ft x 2mm i.d., packed with SP-2100/1% SP-2510 DA on 100/120 Suplecoport (Supelco, Bellefonte, PA); and capillary column spec: 30m x 0.25mm i.d. Fused silica column with 0.25μ coating of DB-17. (J&W Scientific, Cordova, CA 92522)

Patient sera were extracted with methylene chloride, and resuspended in ethyl acetate. Phenobarbital and phenytoin were added as internal standards. Results of analyses show the following:

Reproducibility: Capillary Packed Capillary Packed

PBR (15μg/ml) 3.8% 3.8% 2.8% 3.8%
PHT (4μg/ml) 1.7% 3.8% 2.8% 3.8%
CBE (6μg/ml) 1.3% 3.4% 3.8% 3.6%

Correlation: X = packed column, Y = capillary column

PBR N=25 Y = 1.05 X ± 0.47 r = 0.977

CBE N=27 Y = 1.04 X ± 0.24 r = 0.996

DPh N=47 Y = 0.99 X ± 0.28 r = 0.977

Although the results from capillary columns correlate well with those from packed columns, improved precision, better resolution, minimal interference from other drugs, greater stability, lower maintenance and longer life span make capillary columns preferable for AED analysis.

EVALUATION OF MODIFIED HPLC METHOD FOR THE QUANTITATIVE DETERMINATION OF BHERIDINE-PRIMIDONE.

S.Nanda, C.Patel, Quality Assurance Development, Amersham Company, P.O. Box 70, Elkhart, IN 46515. (Spon.: C. Patel)

Prepared selected commercial samples for the determination of anti-epileptic agents, Primidone, Phenobarbital and Carbamazepine by reversed phase Liquid Chromatography (Clm.Chem. 36:1, 105-106,1990) has been modified to assess specifically 13-Methyl benzodiazepine as internal standard and detection at wavelength 205 nm. Whith these modifications we have data to show that peak sharpness, precision, specificity, resolution and baseline separation have been greatly improved. The
method validation study covered following statistical analysis. The linearity was assessed by analyzing seven levels ranging from 2.5 to 20 mcg/mL produced with intra-day and inter-day coefficient of 0.99996, y intercept of 0.0008±0.0008, slope of 0.103±0.018 and standard error of estimate of 0.095% all at 99% confidence level. The selectivity of the assay was evaluated using the criteria where HPLC detector response was measured in presence of theophylline, phenobarbital, carbamazepine and caffeine. The response was found to be in the range of 0.0 to 10%. The accuracy for the spiked serum was about 100% compared to gravimetrically prepared aqueous standard and precision and within run with the mean level with the confidence level was found 0.17 mcg/mL and total error of 1.2 mcg/mL compared to theoretical values.

A novel competitive immunoassay for the determination of phenytoin in serum was developed for use in the Immunochromatography System (Primary Diagnostic Systems). The assay requires one pipetting and two reagent dispensings and utilizes glass test tubes coated with a complement inactivation reagent (CIR), phenytoin labelled liposome containing a fluorescent dye, serum calibrators, a stabilized complement-antibody (CAB) reagent and plastic tubes coated with stopping reagent. Serum is pipetted into a glass CIR tube which inactivates endogenous complement. The liposome and CAB reagents are dispensed sequentially with Dilutant and the tube is vortexed. The assay tube is coupled to a stopping reagent tube, incubated in the IQ incubator (45°C) for 10 minutes and inverted automatically to stop the reaction. The signal is read and processed in the IQ Analyzer. Within-assay CV's at the therapeutic and toxic levels were 7.52% and 5.48%, respectively. The corresponding inter-assay CV's were 7.21% and 7.55%. Correlation with a nonisotopic method using 77 specimens gave correlation coefficient=0.98, slope=0.98, and y-intercept=0.63. The average spiked recovery was 99% and the linearity in saline diluted ranged from 91% to 103% over the assay range (5-30 ug/mL). Cross-reactivity with major metabolites and related drugs were insignificant. Elevated levels of serum complement and immuno complexes did not interfere. This procedure is simple and sensitive (12 ug/mL). It allows rapid monitoring of phenytoin and other therapeutic drugs in a doctor's office during a patient's visit.

Serum M-Desmethylphenothiazine Determination Using a Modified EMI-ETHOSOXIDE ASSAY PROTOCOLS, Cheryl M. Howells, Michael V. Miles, Robert E. Cross, Michael T. Tennison, Robert S. Greenwood, Robert Hope (Dept. of Path. & Hosp. Lab., Div. of Clin. Chem., NC Mem. Hosp.; Sch. of Pharmacy & Medicine, Univ. of NC, Chapel Hill, NC 27514. (Spon: Robert E. Cross)

There has been renewed interest in methadone (MMD), a second-line antipsychotic drug, because of recent evidence that it may be effective for patients whose partial or generalized tonic-clonic seizure disorders fail to respond to first-line antipsychotic drugs. Monitoring serum levels of M-desmethylmethadone (MDX), the primary metabolite and biologically active form of MMD, is recommended. An immunoassay for MDX is not available, however.

We developed an EMIT assay for MDX using EMIT-ethoxyzide assay reagents (Order #68119, Syva, Palo Alto, CA) and the Coas-Bio (Roche Diagnostic Systems), and compared the results with those obtained using an HPLC method. The EMIT assay parameters used were: vol. = 10 mL, sample volume = 0.5 mL, reagent vol. = 15 mL, reagent vol. = 150 mL, and incubation time = 20 seconds. A 3 point standard curve was calculated using an EMIT reagent blank (correlation coefficient=0.992, detection limit: MDX: 0.083 ng/mL, MDX: 0.13 ng/mL, and FA: 0.44 ng/mL). The EMIT assay was linear from 0.05 to 100 microliters. Good precision was observed in the range of 0.95-0.97, and the recovery of the assay was 100% for MDX and 99% for the first-line antipsychotics, except for ethoxyzide, of course, do not interfere. Only lipids was found to interfere with the assay. The within-run precision (CV%) at 20 was 4.55 and 4.24, and the day-to-day precision (CV%) at 15 was 7.58 and 4.66 at 15 and 45 mg/L, respectively. HPLC comparison studies of 30 patient serum specimens indicate a good correlation (r = 0.93, intercept = -3.1, slope = 1.12). This method makes it possible to monitor MDX serum levels with an EMT system.


Powder formulated EMT reagents have been developed for the determination of theophylline, phenobarbital and phenytoin in serum or plasma on the Coas Mira analyzer. The Cobas MIRA, a random access analyzer, allows the combination of therapeutic drug assays and general chemistry to be performed for the same sample aliquot. To perform an assay, the M150 of antibody/substrate reagent is incubated with 3 μL of sample for 25 seconds. The subsequent addition of 75 μL of enzyme reagent initiates a 50 second read interval. The reaction occurs at 37°C. Throughout is approximately 70 per hour.

The following data indicate that these assays are accurate and precise.

<table>
<thead>
<tr>
<th>Reference (EMIT)</th>
<th>Theoph</th>
<th>Phenobarb</th>
<th>Phenytoin</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cobas Bio)</td>
<td>(AutoLab)</td>
<td>(Cobas Bio)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>79</td>
<td>79</td>
<td>94</td>
</tr>
<tr>
<td>r</td>
<td>0.989</td>
<td>0.989</td>
<td>0.987</td>
</tr>
<tr>
<td>slope</td>
<td>0.988</td>
<td>1.04</td>
<td>0.91</td>
</tr>
<tr>
<td>Inter-Assay</td>
<td>-1.092</td>
<td>-0.42</td>
<td>0.44</td>
</tr>
<tr>
<td>Average recovery</td>
<td>95.5%</td>
<td>105.8%</td>
<td>99.3%</td>
</tr>
<tr>
<td>Precision CV% n=20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within-Run</td>
<td>3.4-7.0</td>
<td>1.7-2.9</td>
<td>3.3-4.2</td>
</tr>
<tr>
<td>Between-Run</td>
<td>5.2-7.1</td>
<td>2.1-4.3</td>
<td>5.5-9.9</td>
</tr>
</tbody>
</table>

The presence of bilirubin (30 mg/dL), hemoglobin (800 mg/dL), or triglycerides (1000 mg/dL) do not interfere with drug quantitation or precision. These assays are highly specific for their respective compounds and cross react minimally with metabolites or commonly co-administered drugs at clinically significant concentrations.

Antibiotic Drugs

Determination of vancomycin concentrations in serum by EMT, Amitava Ranil, Kent E. Opheld, Vidmantas A. Ralsys, Kathryn Dugaw, Dept. of Laboratory Medicine, SB-10, University of Washington School of Medicine, Seattle, WA 98195 (Spon: K. Opheld)

Vancomycin is a narrow-spectrum antibiotic that is used, frequently, to treat infections caused by gram-positive bacteria. Vancomycin can cause concentration-dependent ototoxicity, with possible deafness occurring at serum concentrations > 80 μg/mL. We evaluated the EMIT vancomycin assay (Syva Co., Palo Alto, CA) using the Cobas-Bio analyzer, and report data on precision, accuracy, and comparative analysis of patients' sera, with the Abbott TDx as a reference method. The EMIT assay uses monoclonal antibodies to vancomycin in the Antibody/Substrate. "A" Reagent, and vancomycin labeled with the glucose-6-phosphate dehydrogenase as the Enzyme "B" Reagent. The within-run CV for the low control was 6.5% (X = 7.97 μg/mL; SD = 0.55 μg/mL; n=20) and for the high control was 5.7% (X = 40.8 μg/mL; SD = 2.32 μg/mL; n=20). The between-run CV for the low control was 9.9% (X = 7.16 μg/mL; SD = 0.71 μg/mL; n=20) and 5.9% for the high control (X = 38.2 μg/mL; SD = 2.23 μg/mL; n=20). Recovery of vancomycin from spiked samples averaged 99% for the EMIT method over the concentration range of 5 - 50 μg/mL compared to 92% for the TDx. Carry-over of vancomycin on the Cobas was < 0%. Analysis of 100 patients' serum specimens (spanning a concentration range of 5 - 25 μg/mL) using the TDx (X-method) versus EMIT (Y-method) gave the following regression statistics: Y = 0.876 X - 0.259; r = 0.922. Routinely encountered levels of hemoglobin, bilirubin, or lipase did not result in a significant interference. We conclude that despite a small bias between the EMIT and TDx methods, either can be used to give clinically useful information.

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The principle of this assay system is based on the competitive inhibition of immunoprecipitation from free gentamicin in the patient sample. A drug-protein complex with the antibody (gentamicin) for binding sites on a monoclonal antibody which results in a decrease in the formation of insoluble complexes of antibody-drug-protein. Optimization of the assay for Coulter DadeChemistry Analyzer studies, increased sensitivity by the proper combination of drug-protein ratio (2:801), bridge length (4C-7C) and coupling chemistry for the protein conjugate and antibody. It is not precipitating monoclonal antibody, of purity, affinity and avidity. Patient samples do not require dilution. Performance on DadeChemistry analyzer gives an assay dynamic range of 0-125 μg/L.

Within Run
n=22, Mean=2.63±0.46μg/mL, CV=15.75
Precision
n=22, Mean=5.00±0.45μg/mL, CV=8.92
n=22, Mean=9.16±0.53μg/mL, CV=5.75
Correlation (Syva EMI1): n=169, y=0.92x-0.22μg/mL, r=0.95

Cross Reactivity: (Concentration necessary to produce 10% error at a gentamicin concentration of 3.6 μg/mL)

Amikacin > 250 μg/mL
Erythromycin > 750 μg/mL
Ampicillin > 750 μg/mL
Nalidixic acid > 750 μg/mL
Metronidazole > 750 μg/mL
Tobramycin > 750 μg/mL
Streptomycin > 750 μg/mL

We conclude an accurate assay for gentamicin can be designed using a monoclonal antibody in a turbidimetric rate inhibition assay model system.
and total protein (0.21 ± 0.11) in patients receiving vancomycin and amikacin therapy did not differ significantly from the mean for NAG (10.7 ± 3.1), AAP (33.8 ± 28.7), and total protein (0.18 ± 0.05) in patients not receiving vancomycin. Median AAP and total protein values also did not differ significantly between these two groups when compared on each day of therapy. We conclude that the addition of a recent formulation of vancomycin (Vancocin®, Eli Lilly Co.) to amikacin therapy did not enhance clinical or tubular nephrotoxicity in these children.


Using investigational reagents supplied by Syva Co. (Palo Alto, CA), we studied the performance of the EMT system for determination of vancomycin. Precision studies using Syva control materials revealed (concentrations in ug/ml): within-run between-run

<table>
<thead>
<tr>
<th>Level</th>
<th>mean</th>
<th>SD</th>
<th>CV</th>
<th>mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level I</td>
<td>6.9</td>
<td>0.5</td>
<td>7.5</td>
<td>7.7</td>
<td>0.8</td>
<td>11.2</td>
</tr>
<tr>
<td>Level II</td>
<td>40.3</td>
<td>2.1</td>
<td>5.2</td>
<td>38.1</td>
<td>2.3</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Recovery studies using Syva spiked sets spanning a range of 5-50 ug/ml indicated an average accuracy of 95%. Comparison of patient samples to the Abbott TDx system yielded the following regression line:

\[ y(EMT) = 0.856x(TDx) + 0.643 \] (r = 0.976, F = 21.9, n = 111)

Concerned about the relative flatness of the standard curve between the last two calibrators (30 and 50 ug/ml), we assayed, neat and on 1:2 dilution, two patient samples on 20 separate EMT runs:

<table>
<thead>
<tr>
<th>Patient</th>
<th>1:2 Dilution</th>
<th>Neat</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.04</td>
<td>5.04</td>
</tr>
<tr>
<td>B</td>
<td>5.20</td>
<td>5.20</td>
</tr>
</tbody>
</table>

We conclude that there is a proportional bias between the two methods that requires further study, and that samples whose EMT values exceed 30 ug/ml should be assayed on dilution to avoid reporting clinically significant falsely low results. Given these two caveats, the EMT assay for vancomycin can be quite useful, and may be a satisfactory alternative to the TDx system.


Protein precipitation by methanol or acetonitrile used to measure chloramphenicol by HPLC was marred by interference in premature, neonatal and pediatric sera or plasma. Other solvent extractions were also found to be unreliable due to either instability or insensitivity of the chosen internal standards. An HPLC procedure overcoming these problems was developed.

200 ul serum or plasma (as little as 50 ul could be used) was extracted with 1 ml ethylacetate containing 5 ug p-nitroacetanilide as an internal standard. 0.8 ml supernatant was dried under a stream of nitrogen, the residue reconstituted with 200 ul methanol and 25 ul of final solution injected into a column of u-Bondapack C18, 30 cm X 4 mm (Waters Associates, Inc.) at room temperature. A linear program was used in some methods in pH 3.5 of 0.01M acetic buffer (45:55 by vol) at the flow rate of 1.3 ml/min. The drug was detected at 275 nm.

The linear range of this procedure was 2 to 50 ug/ml serum. The recoveries of chloramphenicol and p-nitroacetanilide were 94% and 101%, respectively. The day to day coefficient of variation was less than 8.5%. The correlation coefficient with EMT assay was 0.98 (Y = -1.13 + 1.21 X, n = 38).

Using this procedure, no interference was encountered in sera of premature, neonatal and pediatric patients.

We conclude this HPLC procedure for chloramphenicol is rapid, accurate, inexpensive, without interference, and suitable for premature, neonatal infants and pediatric patients.


The pharmacokinetics of sulfamethoxazole and trimethoprim were studied in end-stage renal disease in five subjects treated with continuous ambulatory peritoneal dialysis (CAPD) and peritonitis-free. Plasma and dialysate levels of sulfamethoxazole and trimethoprim were determined for one exchange following a single dose of sulfamethoxazole/trimethoprim given either p.o. or i.p., and were fit using the NONLIN4 nonlinear regression program to a two-compartment model which took into account the equilibrium nature of CAPD by including return from the peritoneum in p.o. studies and return from the systemic compartment in i.p. studies. Clearances were calculated using NONLIN4 and an analysis of variance using the SAS statistical program. There was a significant directional effect (p < 0.01) with plasma-peritoneal clearances greater than peritoneal-plasma clearances for both drugs. There was also a significant difference (p < 0.0001) between sulfamethoxazole and trimethoprim clearances, with trimethoprim clearances greater in both directions. Differences in protein binding and molar mass were accounted for by model significance; therefore, differences in lipid solubility, polarity, and metabolism of sulfamethoxazole and trimethoprim are considered to afford explanation for the observed behavior of the two drugs.

GENTAMICIN REAGENT KIT DESIGNED FOR USE ON THE COBAS MIRA ANALYZER. S. Pankey, J. Wood, B. Chung, B. Graham, M. Delaurentis (Syva Co., Palo Alto, CA 94304) (Spons.: A. Jakliitsch)

A convenient gentamicin enzyme immunoassay has been developed for the Roche Cobas Mira random access sequential analyzer. The two-reagent test kit needs only a two-step reconstitution. This Eria® kit "Convenience Pack" is designed to fit directly into the Cobas Mira Reagent 5 Rack. The 37C assay requires a 325 ul total reaction volume. Antibody reagent (150 ul) and 3 ul sample are incubated for 25 seconds. The 75 ul enzyme addition then initiates a 50 second read time. The assay range is 0.6 to 10.0 g/mL. EDTA and
oxalate plasma samples are equivalent to serum. Excellent recovery (Between-run and within-sample precision (3-55) are maintained despite high levels of bilirubin (30 mg/dl), hemoglobin (800 mg/dl), or triglycerides (1000 mg/dl).

The comparative assays are summarized below:

<table>
<thead>
<tr>
<th>Reference (EmT):</th>
<th>Autolab</th>
<th>Cobas Bio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Data:</td>
<td>Time-EMT</td>
<td>Cobas Bio</td>
</tr>
<tr>
<td>slope:</td>
<td>1.08 1.00</td>
<td>1.01 1.02</td>
</tr>
<tr>
<td>Intercept:</td>
<td>0.0 0.3</td>
<td>0.1 -0.1</td>
</tr>
<tr>
<td>N:</td>
<td>77 80</td>
<td>75 80</td>
</tr>
</tbody>
</table>

Within and between-run precision (N=20 each) of four different gentamicin control samples gave CV’s of 2.1-3.9% and 3.3-10.3%, respectively. Average recovery throughout the curve was 103%. No carryover was observed.

The only significant crossreactants observed in the gentamicin assay were staphylococci and netilmicin. This new assay configuration is accurate, precise and practical for use on the Cobas Mira analyzer.


To better reflect levels seen in current patient populations, we redesigned the EmT® Gentamicin and Tobramycin Assays to quantitate in the 0 to 10 mg/dl range. Two carbonate SPE columns were added. A 1 ml of patient sera, calibrators, and controls. Mixtures were then applied to C-18 columns (bond-Elut). Columns were washed twice with distilled water and eluted. Drugs were eluted by adding sequential 300 μL and 100 μL aliquots of eluting solvent (25 mL ether, 10 mL 2-Methyl propanol, and 65 mL dichloromethane). After drying drugs for minutes, 1 μL of each was reconstituted with 10 μL methanol, and 1 μL injected into the gas chromatograph (Perkin-Elmer Sigma 2000; 67 m SPB-1 capillary column, 0.1 mm ID; column temperature 320 °C; nitrogen/phosphorus detector, range 1; bead setting, 1). The assay possesses linearity up to 5.0 μg/mL, sensitivity to at least 0.25 μg/mL, average recovery of 5.8%. We conclude that the method reported here is ideally suited for monitoring therapeutic and toxic levels of tobramycin.


Alprazolam is a member of a new class of 1,4-benzodiazepines which are characterized by a unique triazole structure, high potency, oral activity and low toxicity. It is prescribed for the treatment of anxiety, panic attack and for the anxiety-associated with depression. The objective of this study was to optimize the reactivity of the EMIT tox benzodiazepine assay for toxicological screening of alprazolam. The standard EMIT protocol was extensively modified so that therapeutic concentrations of alprazolam could be detected. Modifications for methods A and B included: single dilution of serum specimens, dilution of EMIT reagents A and B (1:3), increasing incubation temperature to 37°C, and increasing specimen volume from 50 μL to 100 μL. Method B also included supplementing reagent A with glucose-6-phosphate and NAD. Using serum standards, the modified EMIT assays have detection limits of 25 μg/mL (A) and 20 mg/L (B). Both methods were linear up to 200 μg/mL. Method A had a linear range of 2.5 μg/mL. Both methods was less than 25 μg/L using diluted (1:16) EMIT calibrators (n=6). Serum collected from patients receiving various benzodiazepine antidepressant (e.g. alprazolam) methods and alprazolam was found using the modified EMIT procedures and gas chromatography with electron capture detection. (SPA-I column, 30 μm 0.75 mm I.D.). Any specimen with an EMIT reactivity of >1.0 or greater the negative calibrator was considered positive. Using this criteria, all 28 specimens were positive for method A and 24/28 specimens by method B. All 28 specimens were positive for alprazolam (>10 mg/L) by 6.C. In conclusion, the EMIT tox benzodiazepine assay can be modified to screen for alprazolam in serum at therapeutic concentrations.


We have developed a gas chromatographic method for the determination of serum levels of the antidepressant drug trazodone. This method uses a method from the internal standard 8-hydroxyloxipines (5 μg/mL) and 10 μL of the 0.1 mol/L sodium carbonate were added to 1 mL of patient sera, calibrators, and controls. Mixtures were then applied to C-18 columns (bond-Elut). Columns were washed twice with distilled water and eluted. Drugs were eluted by adding sequential 300 μL and 100 μL aliquots of eluting solvent (25 mL ether, 10 mL 2-Methyl propanol, and 65 mL dichloromethane). After drying drugs for days, 1 μL of each was reconstituted with 10 μL methanol, and 1 μL injected into the gas chromatograph (Perkin-Elmer Sigma 2000; 67 m SPB-1 capillary column, 0.1 mm ID; column temperature 320 °C; nitrogen/phosphorus detector, range 1; bead setting, 1). The assay possesses linearity up to 5.0 μg/mL, sensitivity to at least 0.25 μg/mL, average recovery of 5.8%. We conclude that the method reported here is ideally suited for monitoring therapeutic and toxic levels of trazodone.

517 DEVELOPMENT OF A COST-EFFECTIVE EMIT® d.a.u. COCAINE METABOLITE ASSAY USING THE ROCHE COBAS BIO, Charles Welch, Steve Dubun, Bruce Smith, and Diane Hebert (Emit Lab, Research Med. Ctr., Kansas City, Mo 64132) (Spon: Charles Welch).

Various characteristics of the Syva EmT® d.a.u. Cocaine Metabolite assay were studied in order to develop a more cost-effective means of performing this assay on the Roche Cobas Bio analyzer. The EmT® d.a.u. optimization protocol of Sung and Weely (Clin. Chem. 31:1210, 1985) was used in preparing the Cocaine Metabolite reagents: Reagent A was diluted 70-fold with Emit buffer and supplemented with 366 nmol/L glucose-6-P (A) (1.0 μL/50 μl diluted R). Reagent B was diluted 7-fold with Emit buffer. Study of various parameters of the Cocaine Metabolite assay yielded the following optimal analyser settings: Temp: -37.0. Analysis Type: 3(Kinetic), Sample Vol.-10 μL, Diluent Vol.-30 μL, R. Vol.(A)-150 μL, Incubation Time-10 sec. Start Ry. Vol.(B)-15 μL, Plate Height-0.5 mm, Time Interval-10 sec, # of Readings-12. These Cocaine Bio settings allow over 2800 tests to be run from a 100-test Emit® d.a.u. Cocaine Metabolite kit.

Within-run precision (n=5) of a negative urine pool’s absorbance change gave a CV=1.1% while that of a urine pool positive for cocaine metabolite was 0.6%. Between day CV’s (n=5) for the absorbance changes of negative and positive urine pools were 1.0% and 0.5%, respectively. One hundred urine samples were assayed for cocaine metabolite by our optimized EmT protocol and by the standard Cobas Bio. The absorbance change was defined by an absorbance change equal to or greater than the Low calibrator. There was 100% agreement between results obtained with both EmT and Cobas Bio samples. The absorbance change negative for cocaine metabolite by both procedures while twenty-one urine samples tested positive. All positive samples were confirmed by GC. When stored at 0 degrees C, C diluted A and B reagents were stable at least 30 days.

Our optimized EmT Cocaine Metabolite protocol is sensitive, precise, rapid, and very cost-effective.

1258 CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988
I. (trimipramine) and 100 µL of 0.2 N HCl, drawn through the cartridges by applying positive pressure. The cartridges were washed with 2 mL aliquots of water and 1 mL of acetonitrile/60 (10/90) before eluting to the AASFP for automated analysis. The entire extraction procedure took less than five minutes to extract ten samples.

The drugs eluted from the AASFP cassettes were chromatographed on a 25 cm, 5 µm particle size cyanopropyl HPLC column (Analytichem International) and detected at 254 nm. The total analysis time was 16.0 minutes. The detector response was linear over the concentration range of 3-3000 ng/mL with a practical detection limit of 10 ng/mL. The day-to-day precision over 12 successive weeks was 2-3% (CV) over the concentration range of 3-1200 ng/mL. A second assay tested over the concentration range of 40-400 ng/mL were 100%. Analyses from four different batch productions of the C2 bonded phase showed no variation in results. Patient samples supplied by two independent sources were analyzed and the results correlated to within 20%. A study on the effect of possible interfering drugs on the extraction, carried out by an independent analyst, showed that of the 18 drugs tested no interfering peaks were observed.

We conclude that the combined use of scan and SIM GC/MS yields valuable information that would be missed by derivatized SIM GC/MS alone.

**URINE OPIATE CONCENTRATION USING TOXI-LAB EXTRACTION TUBES AND A COMBINATION OF SCAN AND SIM GC/MS**

Sheldon Brunk and Mary Raggette (Baptist Regional Lab, Memphis, TN 38105)

Spon: S. Brunk

We evaluated the success rate of qualitative urine opiate concentration using TOXI-LAB A (TOXI-LAB, Analytical Systems) and a combined scan/SIM GC/MS protocol. 200 consecutive BMD

I. (trimipramine) and 100 µL of 0.2 N HCl, drawn through the cartridges by applying positive pressure. The cartridges were washed with 2 mL aliquots of water and 1 mL of acetonitrile/60 (10/90) before eluting to the AASFP for automated analysis. The entire extraction procedure took less than five minutes to extract ten samples.

The drugs eluted from the AASFP cassettes were chromatographed on a 25 cm, 5 µm particle size cyanopropyl HPLC column (Analytichem International) and detected at 254 nm. The total analysis time was 16.0 minutes. The detector response was linear over the concentration range of 3-3000 ng/mL with a practical detection limit of 10 ng/mL. The day-to-day precision over 12 successive weeks was 2-3% (CV) over the concentration range of 3-1200 ng/mL. A second assay tested over the concentration range of 40-400 ng/mL were 100%. Analyses from four different batch productions of the C2 bonded phase showed no variation in results. Patient samples supplied by two independent sources were analyzed and the results correlated to within 20%. A study on the effect of possible interfering drugs on the extraction, carried out by an independent analyst, showed that of the 18 drugs tested no interfering peaks were observed.

We conclude that the combined use of scan and SIM GC/MS yields valuable information that would be missed by derivatized SIM GC/MS alone.
Further studies proved that these five specimens had cannabinoid content of around 508.5 ng/ml. We conclude that the procedure is reliable for practical purposes and, unless highly specific results are required, can be used as a reasonably sensitive and economic alternative for the screening and quantitation of cannabinoids in urine.

We developed a sensitive RIA (CYCLO-Trac SP) that specifically measures cyclosporin A in serum or plasma and whole blood of transplant patients with no significant crossreactivity of the major metabolites. The assay incubation is performed at room temperature for one hour followed by a 20 minute centrifugation. The monoclonal antibody specific for cyclosporin A used in this assay was developed by Sandoz Corp. (Basel, Switzerland) and is the same one used in their iodinated material for the iodinated tracer was obtained from Sandoz.

### 523

**ADAPTATION OF TECHNICON RAT SYSTEMS THEOPHYLLINE REAGENTS TO THE MONARCH 1000**


The Technicon Systems Turbidimetric immunoassay for theophylline has been adapted to the Monarch 1000. Two reagents, a theophylline-ficolll conjugate and an antibody-ficolll conjugate, are used. Calibration is performed using 6 standards ranging from 0.0 to 60 mg/ml and was stable for at least 14 days. The assay is performed using the following parameters:

**IDENTIFICATION PARAMETERS**

- **LOADING PARAMETERS**
- **Optical Mode**
  - **Absorbance**
- **Reference Type**
  - **Dil**
- **Response Algorithm**
  - **Slope**
- **Sample Volume**
  - **3 µL**
- **Result Algorithm**
  - **Non-linear**
- **Linear Sample Diluent**
  - **0 µL**
- **DATA INTEGRITY PARAMETERS**
  - **Reagent Diluent**
  - **0 µL**
- **Slope**
  - **Positive**
  - **1st & 2nd Reagent**
  - **85 µL**
- **DATA ACQUISITION PARAMETERS**
  - **Analysis Type**
  - **Mix Run Temperature**
    - **30°C**
  - **Delay Time**
    - **30 Sec**
  - **Interval Time**
    - **15 Sec**
  - **No. of Data Pts.**
    - **6**
  - **Correlation with the Abbott TDx using Abbott reagents was**: N = 6
  - **Slope**
    - **0.038**
  - **Intercept**
  - **-17.97**
  - **Sy Rx**
    - **0.999**

We have developed a rapid and sensitive immunomassay (PPIA) which detects various Amphetamine Class drugs (amphetamine and amphetamine-like) in urine on the TDx Analyzer. The assay's standard curves range from 0 to 6 mg/ml based on d-1, amphetamine and the calibrators' average % recovery between urine and buffer is 100.0% ± 2.1. The standard curve is stable for at least 2 weeks and its 2 week C.V. data for 3 concentration levels are:

- **Threshold Low**
  - **Target CONC. (ug/ml)**: 0.50
  - **Mean found (ug/ml)**: 0.498
  - **Within C.V. (%)**: 5.28

- **Between C.V. (%)**: 6.14

169 clinical samples which contain various commonly abused Amphetamine Class drugs were compared with 150 d-1, amphetamine and the calibrators' average % recovery between urine and buffer is 100.0% ± 2.1. The standard curve is stable for at least 2 weeks and its 2 week C.V. data for 3 concentration levels are:

- **Threshold Low**
  - **Target CONC. (ug/ml)**: 0.50
  - **Mean found (ug/ml)**: 0.498
  - **Within C.V. (%)**: 5.28

- **Between C.V. (%)**: 6.14

We concluded that TDx Amphetamine Class assay is a rapid method to detect various amphetamines in urine.

### 524

**CYCLOSPORINE : HPLC VS RIA IN INDIVIDUAL PATIENTS WITH SPECIFIC ORGAN TRANSPLANT, Ching-Nan Ou, Cheryl L. Rognerud and Philip J. Migliore (Department of Pathology, Texas Children's Hospital and The Methodist Hospital, Baylor College of Medicine, Houston, Texas 77030) [Spon.: C.N. Ou]

Until the recent introduction of monoclonal antibodies (Clin Chem 33, 2223, 1987), all radioimmunoassays (RIA) for cyclosporine measurement relied on a polyclonal antibody reagent. Cross reactivity of these antibodies with cyclosporine metabolites was known to produce problems in the assessment of therapeutic cyclosporine concentrations and therefore, resulted in controversy as to which methodology was appropriate for monitoring cyclosporine levels in transplantation patients. To investigate the utility of these assays, we measured cyclosporine concentrations in individual patients having undergone heart, liver, kidney or bone marrow transplantation over a three month period. Both the Sandoz polyclonal radioimmunoassay and a liquid chromatography method developed by Moyer et al (Clin Blochem 19(4): 83, 1986) were used. Linear regression analysis of the data showed the following: patients with transplanted livers had a correlation coefficient ranging from r = 0.2183 to 0.7971; patients with transplanted hearts, range from r = 0.2827 to 0.9079; patients with bone marrow transplantation, range from r = 0.9706 to 0.9891, and had variation in the slope ranging from 1.03 to 0.69. Only a single patient with kidney transplantation was evaluated and had a correlation coefficient of 0.9998. Good correlation can be found in patients with bone marrow or kidney transplantation. Although moderate correlation can be seen in patients with heart transplantation, the significant correlation can be obtained in patients with liver transplantation.

As expected, patients with liver transplantation show the greatest degree of variability in the absorption, metabolism and clearance rate of the parent drug and/or metabolites as indicated by the correlation data. Therefore, polyclonal radioimmunoassay should not be used for the monitoring of cyclosporine in these patients. Results obtained from polyclonal radioimmunoassay for other groups including heart, bone marrow and kidney can be useful when evaluated against a relevant data base and the patient's clinical condition.

### 525

**SPECIFIC MEASUREMENTS OF CYCLOSPORIN A IN TRANSPLANT PATIENTS**

John S. Sehgal, J. L. Yamin, M. A. Ducob, H. Mumbak-Schendel, J. S. Jackson, D. W. Hall, and W. S. Orf (IMSTAR Corp., P.O. Box 265, Stillwater, MN 55082) [Spon.: Sung C. Lee]

We screened five specimens of urine from transplant patients using the Abbott TDx analyzer. The TDx assay was performed according to the directions of the manufacturer. We screened the urine specimens for the presence of cyclosporine and its metabolites using the TDx analyzer. The results of the screening were then confirmed using HPLC analysis. The results of the screening were found to be negative for all five specimens.

### 526

**Screening of Amphetamine Class Drugs in Urine by Fluorescence Polarization Immunoassay**


We have developed a rapid and sensitive fluorescence polarization immunoassay (FPIA) which detects various Amphetamine Class drugs (amphetamine and amphetamine-like) in urine on the TDx Analyzer. The assay's standard curve ranges from 0 to 6 mg/ml based on d-1, amphetamine and the calibrators' average % recovery between urine and buffer is 100.0% ± 2.1. The standard curve is stable for at least 2 weeks and its 2 week C.V. data for 3 concentration levels are:

- **Threshold Low**
  - **Target CONC. (ug/ml)**: 0.50
  - **Mean found (ug/ml)**: 0.498
  - **Within C.V. (%)**: 5.28

- **Between C.V. (%)**: 6.14

We concluded that TDx Amphetamine Class assay is a rapid method to detect various amphetamines in urine.
Because no interference from metabolite cross-reactivity is expected because of high dose saturation of metabolizing enzymes. We tentatively conclude that serum pentobarbital can be monitored using the Abbott TDX urine abuse drug barbiturate assay if the procedure is modified as described above.

528  COMPARISON OF THREE IMMUNOASSAYS FOR THE DETERMINATION OF CYCLOSPORINE AND ITS METABOLITES IN SERUM. Randall T. Roberts, Peggy Duly, Michael J. Keiner, Nicholas N. Alexander (U.S. National Institute of Health, Department of Pathology, U.C.S.B., Medical Center, San Diego, CA 92103). (Sponsor: N.H. Alexander)

In an effort to identify a more precise and labor effective assay for cyclosporine (CSA) and its metabolite in serum, we evaluated the performance of two recently developed polyclonal immunoassays for CSA and its metabolites, an InsetStar radioimmunoassay (RIA) and an Abbott Fluorescence Polarization Immunoassay (FPIA). These two methods were compared to our current method, a Sandos polyonal RIA. In terms of precision, correlation, and interobservability, Precision results were:

<table>
<thead>
<tr>
<th>Method</th>
<th>CV (x1)</th>
<th>CV (x2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandos</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Abbott</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>InsetStar</td>
<td>4.5</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Correlation of the InsetStar and Abbott methods with the Sandos polyonal RIA using sera from renal transplant patients yielded the following:

<table>
<thead>
<tr>
<th>Correlation</th>
<th>N</th>
<th>R</th>
<th>Slope</th>
<th>Intercept</th>
<th>S/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>InsetStar vs Sandos</td>
<td>52</td>
<td>.82</td>
<td>.96</td>
<td>-25</td>
<td>42</td>
</tr>
<tr>
<td>Abbott vs Sandos</td>
<td>49</td>
<td>.88</td>
<td>1.20</td>
<td>-37</td>
<td>43</td>
</tr>
<tr>
<td>InsetStar vs Abbott</td>
<td>49</td>
<td>.93</td>
<td>1.10</td>
<td>4</td>
<td>33</td>
</tr>
</tbody>
</table>

Average labor requirements were 24 min/test with Sandos assay, 6 min/test with the InsetStar & 3 min/test with the Abbott assay. (The results show that the InsetStar and Abbott, approved for CSA and its metabolites correlate well with the Sandos polyonal RIA but offer significantly improved precision and labor effectiveness.


Monitoring of serum cyclosporine (CA) by radio-immunoassay (RIA) was initially used to establish therapeutic ranges at many transplant centers. This procedure has many disadvantages. As a result, we evaluated the Abbott TDX cyclosporine and metabolites FPIA procedure which utilizes 50 ul of serum or plasma. Protein precipitation was used as a pre-treatment step and an aliquot of the centrifuged supernatant was analyzed by the standard TDX procedure to determine the CA. The calibration curve was linear over the range of 0 to 1000 ng/ml with a sensitivity of 0.5 ng/ml. Precision studies at 3 control levels of 75, 250, and 700 ng/ml gave a C.V of 3.3-4.2% for within-run precision and 2.3-4.7% for between-run precision. 307 serum specimens from 6 renal transplant patients were analyzed and the Abbott TDX as well as Sandos (RIA) and High Performance Liquid Chromatography (HPLC).

**Correlation**

<table>
<thead>
<tr>
<th>HPLC vs TDX</th>
<th>TDX vs CA (HPLC)</th>
<th>TDX vs RIA</th>
<th>TDX vs HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>3.8</td>
<td>3.9</td>
<td>3.7</td>
</tr>
</tbody>
</table>

The poor correlation between the HPLC and the immunoassays reflect the poor sensitivity of the HPLC procedure and potentially great differences in metabolite to parent compound. This assay offers a rapid, reproducible alternative to RIA. However, clinical correlations are needed to establish therapeutic and toxic ranges.

530  QUANTITATION OF IOTHALAMATE IN PLASMA BY A MICRO-SAMPLE HPLC ASSAY USING IODIDE (EIO) AS AN INTERNAL STANDARD. K. K. Cheng, David R. Piller, and Clemente Glirado. Metabolic-Nutrition Laboratory, Children's Hospital Medical Center of Northern California, 747 52nd Street, Oakland, CA 94609. (Spon: K. Cheng)

Determination of glomerular filtration rate (GFR) is useful in the management and evaluation of renal function in critically ill patients as well as in the pharmacokinetic and pharmacodynamic studies of therapeutic agents. The most common means of estimating GFR is by measuring the renal clearance of creatinine or inulin, however, these methods are laborious and the colorimetric assays used for GFR measurements may not be specific. The introduction of Iothalamate as a substitute for inulin has been appealing, but the use of radioactivity has obvious drawbacks. We have developed a simple and rapid HPLC assay for the quantitation of non-radioactive iothalamate in plasma using iodide monogluconate (32.5 pg/ml), EIO, as the internal standard. Both volumes (200 ul) of plasma and internal standard are treated with 400 ul cold absolute ethanol. The clear supernatant is dried and redisolvized into 120 ul deionized water. The extracted sample is then injected onto a TDX column at a flow rate of 1.0 ml/min, and monitored by a UV detector at 229 nm. The HPLC system consists of a Bio-rad solvent delivery pump, a Spectroflow 757 UV detector, and an Alltech Econosphere C8 column (25 x 0.46 cm, 3 um) coupled with an Alltech C18 guard column. The mobile phase compositions of 0.5% phosphoric acid and acetonitrile (95:5, v/v). This assay is linear up to 100 pg/ml and has a detection limit estimated at 0.5 pg/ml. The average recovery of the method is 96.9%. The within run precision ranges from 2.4% (mean = 3.3 pg/ml) to 12% (mean = 31 pg/ml). The retention times for iothalamate and iodide are approximately 4 and 6 minutes respectively. No significant interferences were determined in the plasma samples tested at the described settings. The small sample size (20 ul) of this method enhances its usefulness and applicability to the pediatric patients.

531  EVALUATION OF COLONY REAGENTS FOR THEOPHYLLINE AND PHENYTOIN USING THE ABBOTT TDX ANALYZER. Jane Hardie, Melanie Grill, Maryann W窗nert, and Cynthia Schumaker. 7474 WINTERNET CENTER, Sepulveda, CA 91343) (Spon: S. Farber)

Correlation studies were performed comparing Colony reagents to Abbott reagents using the Abbott TDX and to Syva reagents using the Cobas Bios.

**Patient Correlations:**

<table>
<thead>
<tr>
<th>Colony vs Abbott TDX</th>
<th>n</th>
<th>r</th>
<th>b</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theophylline 11</td>
<td>0.67</td>
<td>0.01</td>
<td>1.04</td>
<td>3.91</td>
<td>0.725</td>
</tr>
<tr>
<td>Phenytion 13</td>
<td>0.99</td>
<td>0.02</td>
<td>1.06</td>
<td>2.05</td>
<td>0.717</td>
</tr>
</tbody>
</table>

**Colony using Abbott TDX vs Syva on the Cobas Bios**

<table>
<thead>
<tr>
<th>Colony</th>
<th>n</th>
<th>r</th>
<th>b</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theophylline 43</td>
<td>0.99</td>
<td>0.52</td>
<td>1.07</td>
<td>1.59</td>
<td>0.664</td>
</tr>
<tr>
<td>Phenytion 49</td>
<td>0.99</td>
<td>0.58</td>
<td>2.57</td>
<td>2.85</td>
<td>0.664</td>
</tr>
</tbody>
</table>

**Colony: Recovery of Carb Probes Using Survel and**

<table>
<thead>
<tr>
<th>Colony</th>
<th>n</th>
<th>CAP target sample</th>
<th>sd</th>
<th>cv%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theophylline 20</td>
<td>21.0</td>
<td>0.84</td>
<td>4.1</td>
<td>97.8</td>
</tr>
<tr>
<td>Phenytion 27</td>
<td>14.6</td>
<td>0.22</td>
<td>1.6</td>
<td>93.9</td>
</tr>
</tbody>
</table>

**Colony: Run to Run Precision Using commercial control sera**

<table>
<thead>
<tr>
<th>Colony</th>
<th>n</th>
<th>mean</th>
<th>sd</th>
<th>cv%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theophylline 49</td>
<td>4.19</td>
<td>4.95</td>
<td>0.19</td>
<td>3.8</td>
</tr>
<tr>
<td>Phenytion 42</td>
<td>46.57</td>
<td>39.27</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Phenytion 39</td>
<td>29.93</td>
<td>0.89</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Phenytion 32</td>
<td>12.54</td>
<td>0.19</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Phenytion 28</td>
<td>15.55</td>
<td>0.58</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Phenytion 25</td>
<td>25.26</td>
<td>0.95</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>

The calculated t test value is higher than the critical value of t and indicates that the means are statistically different. However, the difference is less than 0.6 ug/ml in all cases and is not clinically significant. The correlation coefficient is very close to one, the recovery of both drugs using CAP survey samples is about 95%, the observed run to run CVs are all less than 4% and therefore we conclude that Colony reagents can be used on the Abbott TDX with acceptable precision and accuracy.


Measurement of serum lithium in psychiatric patients is essential to insure patient compliance and prevent toxic side effects. In this study, we compared results obtained with the potentiometric selective electrode (ISE) on the Beckman Licates 830 (Brea, CA 92621) and compared t measurements from this instrument with those from a flame photometer (Instrumentation Laboratories).
Control material was used for QC and precision studies, spiked patient serum for linearity studies and serum from patients receiving Li (N = 102) for correlation studies. Linearity was acceptable to at least 5.00 mmol/L in all instruments and to 9.76 mmol/L on the Labyte 830. A within-run precision study (n = 21) on the Labyte 830 showed CV's of 1.7 and 1.1% at Li concentrations of 0.39 and 1.80 mmol/L respectively. A between-run precision study (n = 21) on the Labyte 830 showed CV's of 7.00 and 4.98% at Li concentrations of 0.46 and 1.22 mmol/L respectively.

Correlation studies (N = 102) using y as the Labyte 830, showed the following:

<table>
<thead>
<tr>
<th>x</th>
<th>y</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAS</td>
<td>1.099 ± .021</td>
<td>.993</td>
</tr>
<tr>
<td>Plasma</td>
<td>1.035 ± .032</td>
<td>.993</td>
</tr>
<tr>
<td>ISE</td>
<td>1.099 ± .135</td>
<td>.994</td>
</tr>
</tbody>
</table>

The Labyte 830 lithium electrode for direct potentiometric measurement of Li on the automated instrument is rapid, requires minimal maintenance and only 120 μl of serum, and is useful for both routine and stat monitoring of serum lithium.

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**Thursday Afternoon—July 28**

**Poster Session 12:00m—2:00pm**

**DRUGS—PART B**

**Miscellaneous Other Drugs**

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**535**

**FALSE POSITIVE IDENTIFICATION OF A TRICYCLIC ANTIDEPRESSANT DUE TO CYCLOBENZAPRINE (PLEXERIL)**


(Spon: Alex A. Pappas)

In the last year, we have seen 3 cases in which cyclobenzaprine was falsely identified by four different methods as a tricyclic antidepressant. GC/MS verified the absence of any tricyclic and also quantified the amount of cyclobenzaprine present in urine. The results for 3 patients are shown below:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dupont ACA (EMIT) (+) (+) (+)</td>
<td>Abbott TDX (FPVIA) (+) (+) (+)</td>
<td>TOXI-LAB (TLC) (+) (+) (+)</td>
<td>HPLC (+) (+) (+)</td>
</tr>
<tr>
<td>GC/MS (ng/ml) of 102 542 462</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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**536**

**NORMALIZE URINE THC QUANTITATIVE RESULTS TO CREATININE TO INTERPRET ELIMINATION RATE, E. Howard Taylor, Ray Bell, Bruce Ackerman and Alex Pappas**


(Spon: E. Howard Taylor)

Quantitative results (determined by GC/MS) for 11-nor-delta-9-carboxy-THC (THC-COOH) from an individual are shown below:

<table>
<thead>
<tr>
<th>DAY</th>
<th>THC-COOH</th>
<th>Creat.</th>
<th>THC-COOH na/umol creat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2489</td>
<td>280</td>
<td>889</td>
</tr>
<tr>
<td>13</td>
<td>56</td>
<td>92</td>
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<td>15</td>
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<td>633</td>
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<tr>
<td>20</td>
<td>58</td>
<td>165</td>
<td>373</td>
</tr>
<tr>
<td>29</td>
<td>19</td>
<td>86</td>
<td>439</td>
</tr>
<tr>
<td>32</td>
<td>24</td>
<td>131</td>
<td>189</td>
</tr>
</tbody>
</table>

To verify abstinence of marijuana smoking, one would expect to see a fall in urinary THC-COOH concentrations. This poses a problem at day 15 when the concentration of THC-COOH increases; however, if one normalizes data to creatinine, there does not fall in values. When the last 5 creatinine normalized data points were fit to a one compartment model, a terminal elimination rate constant of 0.0644 reciprocal days reflecting a 10.8 day terminal half-life was calculated. The correlation coefficient was 0.989 with a consistent elimination rate constant among pairs of points indicating linear drug elimination. These data indicated that the half-life of THC in the deep peripheral compartments from this individual was 10.8 days and that normalization to creatinine was necessary to interpret different quantitative THC-COOH values.

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**536**

**CROSS REACTIVITY OF CYCLOSPORINE(CsA) AND METABOLITE "M17" IN FLUORESCENCE POLARIZATION IMMUNOASSAY, Kayla T. Schubert, J.R. Wermeling, T.J. Schroeder, A.J. Pearce, N. Hassan, A. Warner (University of Cincinnati Department of Pathology, Cincinnati, OH 45267-0714) (Spon: F. Stainer)**

In the quantitation of the immunosuppressive drug CsA can now be accomplished by fluorescence polarization immunossay (FPVIA) on the Abbott TDX system utilizing either serum or whole blood. Observed variations between current HPLC and immunossay(Sandoz RIA) methods are due to the crossreaction of CsA and metabolites with the antibody used in RIA. Since each of the Abbott immunossays and the Sandoz RIA utilize different antibodies, it is expected that different values will be obtained for CsA based upon the amount of crossreactivity of each of the three antibodies with CsA. Consequently, a study relating "M17" cross-reactivity to parent drug was performed with the Abbott TDX "Cyclosporine and Metabolites" FPVIA kits for serum and whole blood. The relative concentrations of the pure drug and...
metabolites were determined by UV absorbance of aqueous solutions. Comparative readings were obtained on the TDx for both assays. The cross-reactivity of metabolite "M1" with respect to parent cocaine was 60% and 60% in serum and whole blood, respectively. The percent cross-reactivity was linear over the range of both assays. Thus it can be expected that the TDx may yield lower than expected the RIA values and this difference must be considered when establishing the therapeutic and toxic ranges.

537 PERFORMANCE OF COMMERCIAL BREATH ALCOHOL SIMULATORS - FURTHER STUDIES. Kurt M. Dubowski, and Natalie A. Essay (Dept. of Medicine, The University of Oklahoma HSC, Oklahoma City, OK 73190). (Spon.: Kurt M. Dubowski)

The validity of breath-alcohol analysis is of importance in clinical and forensic situations. We have previously reported on shortcomings of breath-alcohol simulators as calibrating devices and proposed improvements (Dubowski, K., J. Anal. Toxicol. 3:177, 1979). Newer simulators have better temperature control and other enhancements. We evaluated 2 such devices, used in association with current generation breath-alcohol analysers, for their suitability as control devices and calibrators. Methods were as previously (1979) described.

Temperature measurements (N=101) on a factory-adjusted Guth Model 34C simulator (GS) ±1.5°C intervals yielded 34.1°C ±0.05°C (mean±SD) and on a fasttest ABS 120 simulator (TS) yielded 34.3°C ±0.031°C at a 34.0°C target temperature. Accuracy and precision measurements of ethanol by infrared spectrometry with a Model 5000-D Intoxifier for a simulator-effluent target value of 0.100 g/210 L yielded 0.100 g/210 L ±0.0017 (mean±SD) for GS (N=45); and 0.100 g/210 L ±0.0013 for TS (N=42). Forty consecutive control test cycles with a Model 5000-D Intoxifier in the recirculating mode reduced the vapor-ethanol concn. display by 1.6% from the initial 0.101 g/210 L for both GS and TS. The corresponding calculated ethanol depletion without recirculation of simulator effluent was 6.1% for otherwise identical conditions.

Our findings demonstrate substantially improved performance of the device tested over that of earlier simulators; and show that recirculation of simulator effluent in the Model 5000-D Intoxifier can extend the number of acceptable control test cycles of a given simulator ECgH charge by 3.8x.

538 SOLID PHASE EXTRACTION AND HPLC ANALYSIS OF BENZODIAZEPINES. Mohammad Nashar, Steven R. Binder and Mary Biaggi McCracken (Clinical) HPLC Group, Bio-Rad Laboratories, CA 94507 (Spon.: Mohammad Nasar)

We have developed a protocol for the solid phase sample preparation and HPLC analysis of benzodiazepines in serum (plasma) and urine. We used the non-adsorbing form of Sep Pak with or without a urea sample preparation (plasma/serum, urine can also be used) containing chloridiazepoxide,,norcloridiazepoxide, desmethyflurazepam, diazepam, oxazepam and nordiazepam. The reverse phase cleanup columns (Bio-Rad) were conditioned by washing with methanol followed by 1.2% CO2 in acetonitrile. To each column, a mixture of the standard, control or patient with 1 ml of water and 1 mg methylmorphine (I.S.) was added. After aspiration the columns were washed with acetonitrile/water. The benzodiazepines were eluted with a mixture of water/acetonitrile/methanol (2/3/3). Analysis was performed on 100 x 2.1 mm high performance reversed phase column (Bio-Rad) at 35°C with a solvent of acetonitrile/water. The mobile phase consisted of 28% acetonitrile in phosphate buffer with 5 mM o-nitroanilin, adjusted to pH 5.0. All the benzodiazepines were detected at 254 nm with or without the I.S. standard. The mobile phase is linear for concentrations from 25-2000 ng/ml; absolute recovery for all the drugs in the standard was over 96%. Within-run C.V.'s ranged from 1.3-1.3% (N=8) for benzodiazepines analysed at low and high concentrations. Tricyclic antidepressants and phenothiazines are retained by the cleanup columns and thus do not interfere with analysis. The extraction procedure has also been adapted for alprazolam, clonazepam, nitrazepam, triazolam, zolpidem, clorazepate, chloral hydrate and related drugs. In summary, a simple, selective and rapid solid phase extraction procedure was developed for simultaneous analysis of several benzodiazepines by HPLC.

539 ABSTRACT WITHDRAWN

540 ACCURATE AND RELIABLE OPTIMIZATION OF EMT URINE DRUGS OF ABUSE ASSAYS FOR THE ROCHE COBAS FARA, Olivia A. Swager, Ronald H. Hiltoc, Teri B. Peterson (Loma Linda University, Faculty Medical Laboratory, 11370 Anderson #2900, Loma Linda, CA 92354) (Spon.: O.A. Swager)

To establish an accurate, rapid and inexpensive method for urine drugs of abuse (DAU) screening, we evaluated the Sun and Neelley reagent dilution of the EMT daw Assays on the Roche COBAS FARA against the Syva established criteria for satisfactory assay performance and correlation with accepted methodologies. The EMIT daw Assays were performed on the COBAS FARA utilizing a kinetic instrument program, reagents diluted with EMIT buffer by the protocol described by Sun and Neelley (Clin. Chem. 31/7: 1210, 1985), and appropriate negative, low, and medium calibrators. Results of calibrators and samples were printed as ΔA values. A sample that gave a ΔA value equal to or greater than the low calibrator was interpreted as positive. The ΔA between the negative and the low calibrator and between the low and the medium calibrator must meet Syva assay specific minimum ΔA values for performance to be acceptable. The evaluation of the obtained results had the Syva assay for acceptable performance gave results in excess of all minimum acceptable criteria for dilutions with EMIT buffer of 1:70 for amphetamines, benzodiazepines, methadone, and phencyclidine, 1:35 for barbiturates, cocaine and methadone, and 1:4 for cannabinoids' assays.

The correlation with accepted methodologies including Analytical Systems, Toxi-Lab, EMIT daw on Syva AutoCarousel and EMIT Qit system gave sensitivities of greater than 95 percent in all cases. All discrepancies were explained by differences in methodology and sensitivity to drug levels present.

The modified EMT daw methodology on the Roche COBAS FARA gave cost-effective and reliable results as demonstrated by comparison with Syva established criteria and accepted methodologies.


We evaluated the Genetic Diagnostic Corporation (GDC) (Great Neck, New York 11021) Cocaine Assay and compared it to the enzyme immunoassay (EMIT) by Syva (Palo Alto, CA) and to GC/MS. The GDC assay is a competitive ELISA for the qualitative determination of cocaine and metabolites in urine. In this microtiter plate assay, cocaine and cocaine metabolites in a test urine and those immobilized on a solid phase compete for limited antibody binding sites. The reaction is visualized, after addition of an enzyme-labeled second antibody, with a color producing substrate.

One hundred thirty seven (137) urine samples were assayed by each of the three methodologies. The results are presented in the Table.

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC/MS</td>
<td>92</td>
<td>45</td>
</tr>
<tr>
<td>GDC ELISA</td>
<td>92</td>
<td>45</td>
</tr>
<tr>
<td>Syva EMIT</td>
<td>89</td>
<td>48</td>
</tr>
</tbody>
</table>

The manufacturers' recommended procedure was followed for each of the immunoassays. Superior correlation of the GDC ELISA assay with GC/MS was most likely due to the reactivity of the antibody used in the GDC procedure. This antibody recognizes not only benzosylegonine, but also cocaine, methyl ecgonine and ecgonine. The data indicate that the GDC assay compares favorably to the Syva EMIT and correlates well with the reference GC/MS technique. This solid-phase ELISA method should prove to be a reliable, convenient and inexpensive screening technique for the toxicology laboratory.
We compared the Kodak Ektachem Theophylline Assay with the Abbott TDx Theophylline II Assay. One hundred patient samples were assayed by both methods yielding the following linear regression: Let x = TDx response and y = Ektachem response, y = 0.97x + 0.24. 

Theophylline related compounds were assessed for crossreactivity. These were epinephrine, phenylephrine, caffeine, 8-chlorotheophylline, theobromine, dipyridamole, xanthine, 3-methylxanthine, 1-methyluric acid, and phenacetin. The Ektachem assay had significant crossreactivity only with 3-methylxanthine (77% at 10 μg/mL). The TDx exhibited some crossreactivity with 8-chlorotheophylline (20% at 100 μg/mL) while all other compounds tested showed less than 10% cross-reactivity. A recovery/linearity experiment was performed. As reported in the Ektachem Theophylline assay test description, dilutions prepared and run in the Ektachem can be considered approximations (an average of 32% positive bias was observed on four-fold dilutions compared with unlabelled results). Dilutions performed on the TDx showed no recoverable bias.

The day to day precision was evaluated for each method by running a control material. The C.V. for the TDx was 3.7% at 21.6 μg/mL. The C.V. for the Ektachem was 3.5% at 16.9 μg/mL.

Visibly lipemic and hemolyzed specimens were assessed by assay. In this case, the Ektachem assay showed no interference. Moderately uricemic specimens (creatinine > 2.5 mg/dL) showed no interference.

10 drug-free urine samples were spiked with defined amounts of bromazepam (BA) and oxazepam (OX), giving 6 different concentrations 0.2-0.0008 mg/L for BA and 0.06-0.0006 mg/L for OX respectively. Also, 60 specimens for each particular benzodiazepine. These specimens were randomized and analyzed in our routine laboratory with TLC and with FPFA (TDx Abbott).

The tests results were evaluated by the statistical method of KUTTER (Z. Allg. Med., 1975) giving values for "practical sensitivity" (+90 % positive results) and "maximal sensitivity" (+10 % positive results).

We found a practical sensitivity of 0.2 mg/L for BA with a practical sensitivity of 0.02 mg/L and a practical sensitivity of 0.06 mg/L for OX with a maximal sensitivity of <0.006 mg/L. These concentrations ranged beyond the threshold limit (0.2 mg/L) recommended by the manufacturer of the TDx.

Applying the same statistical method for the TDx results, practical sensitivity was 0.07 mg/L (BA) and 0.02 mg/L (OX), showing the greater sensitivity of the TLC method, which has the further advantage of distinguishing among major groups of benzodiazepines.

The analytical reliability of cyclosporin (Csa) assay has been monitored during a national collaborative study starting from 1986. 26 blood quality control (QC) samples have been prepared and sent to each of the 43 participating laboratories. The analytical variability was 15% and no submitter blood. The break-down of the variability into its components indicated that the within-assay, the between-assay and the within-kit CVs were 8.6, 18.8 and 21.8% respectively, suggesting that the kits employed had a poor reliability and that the CVs do not represent the true analytical variability, probably due to the dismissed bound/free separation techniques (charcoal and precipitation) and the double antibody technique. The non-specific binding of the tracer to Csa was assayed on QC samples from treated patients with the kits. The results showed 1.25% tracer irreproducibly higher at the concentration of 400 ng/mL. I. O. C. (International Organization for Standardization) those produced by the kit with 3H tracer (SOZ), while no significant differences were observed on spiked QC samples. These findings suggest that the interferences of Csa metabolites (present in patient samples and not in spiked samples) is enhanced by the use of 125I tracer, due to the reduced affinity of the iodinated Csa towards the antibody.

The precision profiles for the evaluation of radiommunoassays (anticorrelation to the determination of "qualitative" tests for PCP, cocaine and morphine, A.P. Durham and J. Comment, "J. Clin. Pharmacol.," 1986, 26, 1195) were performed using radiommunoassay for PCP cocaine and morphine antibodies as separate antibody-coated tubes and as a 125I tracer, and each spiked with a spectrum of calibrators in human urine. The manufacturer (DPC, Los Angeles, CA) instructions for qualitative and quantitative procedures, differing only in the number of calibrators required. Two precision profiles were constructed for each of the three assay systems, representing within-run coefficients of variation (C.V.) for dose (micrograms per liter) as a function of concentration, and C.V. for response (counts per minute, cpm) also as a function of concentration. Both profiles were constructed using the log-log scale, 1 minute counts per 0.001 mg/L. As the coefficient of variation (counts per minute, cpm) was computed by standard analysis of variance techniques (Durnam, Clin Chem 1986;32,1195), then translated into C.V. based on concentration using the log-log scale. C.V. based on cpm proved to be relatively constant throughout the entire analytical range. For the PCP assay, C.V. = 0.8x log concentration + 1.54. The graph shows that the assay typically yields within-run C.V. (based on cpm) of approximately 10-20% over a range of 125 to 2 μg/L, and a PCP range of 125 to 10 mg/L with a log-log dependence of the concentration (counting time) to the C.V. a little bigger than that observed for the C.V. for dose (counts per minute, cpm) at approximately 1% for 1 minute counts, to approximately 0.1% for 10 minute counts at approximately 20% for 1 minute cpm base.

The precision profiles depict the within-run C.V., based on results in micrograms per liter, to be expected for samples assayed in duplicate. For the PCP assay, the profile indicates a within-run C.V. (based on concentration) approximately 1% at 25 μg/L, the cutoff specified by the manufacturer for use in the qualitative procedure; this corresponds to a 2-standard-deviation confidence band of 25 ± 1 μg/L, at this level.

For the PCP assay, a linear relationship—when a 25 μg/L cutoff is used and samples are assayed in duplicate using 1 minute counts, a "positive" result represents (92% certainty) a PCP concentration of at least 15 μg/L, and a "negative" result represents a concentration of at least 20 μg/L. Analogous results were obtained for the cocaine and morphine assays. The analysis can be expanded using Monte Carlo techniques (Kaye, Nix et al., Stat Med 1986;5:183) to estimate assay C.V., yielding correspondingly more realistic interpretations of "positive" and "negative" results.

Buprenorphine is an opiopeptide derivative with partial agonist activity. It is used for the treatment of chronic pain. In subjects, it is metabolized with glucuronidation and sulfation, using a double-antibody radioimmunoassay with negligible cross-reactivity to other compounds. All samples have been processed directly, without prior hydrolysis.

Between 3 and 7 urine specimens were collected over 2 to 3 days from each of 5 patients receiving either 0.8 or 1.5 mg of buprenorphine sublingually for the treatment of chronic pain. Results were compared directly and after enzymatic hydrolysis with β-glucuronidase and sulfatase. The assay has yielded readily measurable buprenorphine levels, while the increase induced by hydrolysis was relatively small. This suggests that urine samples can be processed directly, without prior hydrolysis.


Fentanyl, a potent analgesic, is used for intraoperative anaesthesia. Its analgesic effects are readily reversible with naloxone. In addition, several synthetic fentanyl analogues ("designer drugs") have been used as heroin substitutes. A quantitative, solid-phase radioimmunoassay for fentanyl in urine and serum has been developed. It is based on antibody-coated tubes, a 141fentanyl tracer, and calibrators ranging from 0.5 to 3.0 μg/L. The procedure involves a 25 μL sample and a single, 60-minute incubation.

Specificity studies demonstrated the following crossreactivities: amphetamine 0.007%, benzoylethorphan 0.022%, fenfluramine 0.021%, and methadone 0.02%. Other compounds were undetectable at levels of 100,000 μg/L. Intra- and inter-assay CVs on samples ranging from 6 to 27 μg/L were 4.6-5.5% and 5.0-7.1%, respectively.

A comparison of the present method to a tritiated (H) fentanyl radioimmunoassay on 55 samples with fentanyl concentrations below 7 μg/L yielded a correlation coefficient of 0.81 and the following relationship:

\[ y = 0.81x + 0.38 \]

where y is the in study human subjects receiving fentanyl for anaesthesia, circulating fentanyl levels ranged from undetectable (less than 0.2 μg/L) up to 11.7 μg/L, as measured over a 8-hour period by this solid-phase radioimmunoassay.


The use of radioimmunoassays (RIAs) with antisera of different crossreactivity profiles has been described for morphine (Hand et al, Ann Clin Biochem 1987;24:153), buprenorphine (Hand et al, ibid. 1986;23:47), and their metabolites. A new method has been used to measure the production of codeine metabolites (Findlay et al, Res Commun Chem Pathol Pharmacol 1977;17:595). Previous attempts to employ specific and sensitive spectrophotometric assays have failed to differentiate heroin and codeine/morphine/morphine based metabolites have not been successful, however (Cassani et al, Giorn Ital Chim Chim 1984;9:27).

This study utilized a combination, antibody-coated tube RIA ("M" and "MC"). (Cost-A-Count Morphine: DPC, Los Angeles, CA) and MC (an in-house procedure) are based on antisera specific for morphine and codeine/heroin/morphine, respectively. Tests for codeine of 180% relative to morphine. Both use human urine-based morphine standards, and both are nonreactive with morphine metabolites.

Urine samples were collected from known heroin abusers, from patients given morphine for cancer pain, and from 18 subjects taking 30 mg codeine phosphate daily. The samples were suitably diluted in drug-free human urine and assayed by both RIAs. The concentration ratio of M/C/M was then calculated for each sample.

For the heroin abusers, the M/C/M ratio ranged from 0.94 to 3.3 (n=47), for the patients on morphine 1.8 to 2.6 (n=13), and for the volunteers on codeine 7.9 to 14.4. The ratios show clear differentiation between subjects taking codeine and subjects on either heroin or morphine.


We report here on several years experience using a dual column system employing an Ultra II (5% phenylmethylsilicone (PTMDS) + 95% dimethylpolysiloxane (DMS)) and a DB-17 (5% phenylmethylsiloxane (PMS)).

Selection of phases is an important aspect of drug identification. Using a methylsilicone, two compounds of a total of 101 compounds co-elute. For PMS, 16 pairs co-elute, whereas for DB-17 only four pairs co-elute. It is important to note that the combinatorial values for DB-17 are less than 4 pairs of compounds co-elute. The temperature program used is 50°C for 2 min; 20°C/min. to 150°C; 5°C/min. to 260°C; 20°C/min. to 300°C with 15 min. hold. Both columns are used on an HP5890. The retention indices of 100 drugs and/or drug metabolites will be reported on both Ultra II and DB-17. The precision of the system is within 0.7 ± 0.1% for the compounds of interest. The reproductibility of the RI is dependent on a number of factors, including carrier gas flow rate. Caffeine and ethambutol co-elute on Ultra II at all flow rates, whereas on DB-17 the resolution of this pair is improved from 0.8 to 1.0 over the flow rate range of 1.3-3.10 m/min. The reproductibility of different lots of DB-17 has presented a problem. Column lifetime has generally exceeded 6 months, however.

In summary, a dual column capillary can be used to identify drugs in serum and urine. Confirmation of drugs by GC/MS is seldom required for serum screens, but the complexity of the urine matrix makes confirmation necessary in about 2% of the samples.


The Abbott TDX® Cyclosporine and Metabolites FIAP procedure utilizes 25 µL of whole blood, a lysing

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988 1265
Review of the findings of preliminary screening vs. confirmatory findings was used to assess the frequency with which various drugs occurred, frequency of false-positives (9%) and false-negatives, and the effectiveness of the TLC system. Due to high assay with TLC is only practical for certain drug classes and concentrations. These findings will be discussed along with additional case findings.


CBZ has been shown to be a useful agent in patients with partial responses to RA; Li has also been suggested. The objective of this study is to evaluate the pharmacokinetic interaction of CBZ or Li on RA. 44 patients were initially treated for 4 weeks with RA dosages adjusted to yield levels between 8 and 25 ng/ml (24-75 mmol/l). 14 out of the 17 patients with refractory symptoms entered an additional two-week trial with random assignment to Li or CBZ with the same adjusted RA dosages. Blood samples were collected weekly for 48 hours over 12 hours from the last RA dose into powdered EDTA VACUTAINER Brand Tubes prior to and while taking both two medications. RA and RHA were separated and assayed as described previously and the procedures previously validated and applied (Clin. Chem. 31, 939, 1985; J. Clin. Psychopharmacol 5, 153, 1986). RA was measured on plasma extracts by using antisera without significant cross-reactivity for RHA, five other metabolites and benztreitol. The limit of det was 0.5 ng/ml, interassay C.V. of 8% and linearity from 0.5 to 25 ng/ml. RA was obtained by subtracting the plasma extracts data from the total plasma values measured by using a different antisera that reacts equally with RA but not with other benzenoids. The limit of det is 2 ng/ml and interassay C.V. of 11.5%. Coomission of RA significantly reduces RA from 21.7 to 12.2 ng/ml (p<0.05) whereas Li did not: 11.5 to 11.1 ng/ml (p=0.62, N=8). The decrease of RA with CBZ is concurrent with a significant increase of RHA indicating stimulation of hepatic microsomal enzymes. Therefore, we conclude that the pharmacokinetic interaction of CBZ on RA must be taken into account by monitoring both RA and RHA.


Geriatric patients, particularly, ≥70 years of age, metabolize NT more slowly and because they are medicated with much greater plasma levels of unconjugated hydroxy metabolites. The objective of this study is to monitor steady state (S-S) levels of NT and to design dosage regimens to achieve a narrow target within the therapeutic window of 50-150 ng/ml (190-570 nmol/L). We have studied 22 patients, mean age 71.2 ± 6.7 (S.D.) for an active period up to 51 days after at least 14 days of drug wash-out. The maintenance dose was obtained by measuring the 48-hour level. After an initial 25 mg oral dose, the S-S dose was obtained through the use of the equation: S-S = 7.78 (48 hr NT plasma level) + 64.7 which has been previously validated (Psychiat. Res. 21, 111, 1987). S-S blood samples were collected into powdered EDTA VACUTAINER Brand Tubes and monitored once a week, stored for 1 week or 12 hours after the single total dose at bed time. The analyses are based on methods described previously (Clin. Chem. 31, 940, 1985). The 48-hour samples were measured in triplicates with sample size of 10-50 microliters by modified RIA which does not cross-react with the metabolites. This method has a detection limit of 2 ng/ml (ranges 1-20 ng/ml) and has a detection limit of 2 ng/ml and mean recovery of 92%. We found that the avg NT level for 22 patients was 95.2 ± 14.6 ng/ml, the correlation between 48-hour and S-S levels (p<0.05) was r = 0.62, the mean dose of NT was 11.8 mg/d (well below than for adults) and the overall successful response rate was 81%. We conclude that predictive TDM for NT in elderly patients achieves suitable dosing earlier during treatment.

TLC VS TLC DRUG SCREENING IN 3,583 POSITIVE PARKLAND MEMORIAL HOSPITAL EMERGENCY PATIENTS, B.L. Potratz, A.B.C. Dossley, R. Boet and P.A. Van Drexel. (Pathology Department, University of Texas Southwestern Medical Center, and Southern Institute for Forensic Sciences, Dallas, Tex.). (Spon: R. Boet)

During an 18 month period 3,583 patient drug blood and/or urine screens were analyzed by both Toxic-Lab (T-L) (Marion Scientific) and other quantitative confirmatory techniques (TLC or TLC screening). The following table summarizes the comparison of T-L vs. confirmatory methods over this period:

<table>
<thead>
<tr>
<th>CLASS OF BLOOD NO. DETECTED URINE NO. DETECTED DRUG SAMPLES BY TOXIC-LAB SAMPLES BY TOXIC-LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antihypertensives</td>
</tr>
<tr>
<td>Anticonvulsants</td>
</tr>
<tr>
<td>Antithrombin</td>
</tr>
<tr>
<td>Analgesics</td>
</tr>
<tr>
<td>Narcotics</td>
</tr>
<tr>
<td>Psychostimulants</td>
</tr>
<tr>
<td>Sedatives</td>
</tr>
<tr>
<td>Stimulants</td>
</tr>
</tbody>
</table>

556 CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988
A novel spectrophotometric method for the quantitation of acetaminophen in serum was developed and evaluated. Free unconjugated acetaminophen was separated from other endogenous compounds containing phenolic groups by extracting with ethyl acetate, followed by hydrolyzing to p-aminophenol. In the presence of sodium periodate, an oxidative coupling reaction between the latter compound and 2,5-dimethylphenol rapidly produced a blue colored dye, which was shown to be stable for at least 18 h at room temperature. The assay exhibited a linear absorption peak at 535 nm. The proposed method has a linearity range from 25-800 μg/mL. Precision studies indicate that day-to-day coefficient of variation for two pooled controls (10) and 204 μg/mL was 4.8% and 4.0%, respectively. Correlation studies between the proposed method and Syva's EMIT procedure show a correlation coefficient of 0.97. Commonly used drugs which may be taken concomitantly with acetaminophen were tested for interference on the proposed method. Only phenacetin will cause significant interference. However, this drug has a short half-life of one hour and is extensively metabolized to acetaminophen. Therefore, the interference exerted by phenacetin can be considered negligible. Moreover, in contrast to nitration method, the proposed method is not subjected to interference by salicylate and salicylamide. Neither icteric nor hemolysed samples showed any interference. Highly lipemic specimens will produce falsely elevated results. However, in such cases, individual sample blank should be obtained for correction purpose. In summary, the proposed method for acetaminophen quantitation is simple and specific.


Current tests for theophylline monitoring are immunological in nature and use antibodies for the recognition of theophylline. Alternatively, GDS Technology's discovery of a microbial enzyme, Theophylline oxidase, which selectively oxidizes theophylline, has enabled the development of a simple, convenient and specific enzymatic test for theophylline monitoring. The Theophylline oxidase oxidizes theophylline in the presence of an electron acceptor, such as ferricytochrome c, as shown in the following reaction:

\[
\text{Theophylline + 1.3 dimethyl uric acid + 2 ferricytochrome c} \rightarrow \text{2 ferricytochrome c}
\]

The ferricytochrome c, thus produced, is measured at 550 nm wavelength and is proportionate to the concentration of theophylline in serum.

The test reagent contains 5-6 units of Theophylline oxidase and 50 mml of ferricytochrome c per ml of reagent. When performed at 30°C with a sample to reagent ratio of 1:20, the test is completed in 15 minutes. As the change of OD at 550 nm is 0.014 per mg/L of theophylline, the test is very sensitive. The test is linear in the range of 0-40 mg/L of theophylline in serum. No interference from caffeine, theobromine or uric acid at concentrations of 100 mg/L was observed.

This methodology can be easily adapted to most autoanalyzers and additionally can be utilized in electrochemical as well as dry chemistry (strip) test systems.

DETERMINATION OF THE NEW ANTIDEPRESSANT FLUoxetine AND ITS METABOLITE BY HPLC AND PRELIMINARY CLINICAL STUDIES, P.J. Ursula, J.R. Debis, C. Himmel and P. Wittman (Mental Health Research Center, Department of Psychiatry, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235) (Spon.: P.J. Ursula).

Fluoxetine, a specific neuronal inhibitor of serotonin reuptake is currently undergoing clinical trials as an antidepressant in the U.S. and is expected to be available for use in 1988. The purpose of this work was to develop a quantitative procedure to determine fluoxetine and norfluoxetine in serum from patients treated with this drug and to develop preliminary clinical data for therapeutic monitoring of this antidepressant.

The drug and its metabolite are extracted from alkalized serum (pH 10) with hexane:isopropanol 2:1. The samples are washed with NaHCO₃ (100 mL water and 1 mL trilithylamine adjusted to pH 5.0 with glacial acetic acid and 400 mL acetonitrile. A UV detector set at 226 nm is used.

The assay is linear for both fluoxetine and norfluoxetine over the range of 20-600 ng/mL. Coefficients of variation over ten days (8 assays) were 6.8% and 4.4% for fluoxetine and 6.2% and 8.8% for the metabolite at 60 and 190 ng/mL, respectively. No interference was observed from common psychoactive drugs.

In preliminary clinical studies, we found steady state serum concentrations of fluoxetine ranging from 41-673 ng/mL and norfluoxetine ranging from 66-446 ng/mL in nine patients treated with 20-60 mg of fluoxetine hydrochloride per day.


The comparative performance of several commercially available instruments and methods designed for quantitative theophylline analysis in physicians' offices was studied. Six systems, which have been designed and marketed for use by minimally trained personnel, were evaluated. The Abbott theophylline assay was utilized as the reference methodology. All methods were performed according to manufacturer's instructions. Whole blood from patient samples less than one hour old were analyzed by the Syva (Syva Acculevel) and Abbott Vision methods. Simultaneously collected serum specimens were analyzed by the Abbott Abbott Vision, 3M Diagnostics TheoFAST, Awa Seralyzer, and Kodak DT-60 methods. Hospitalized patients on theophylline therapy (n=100) comprised the subject group (conc range: 0 to 30 ug/mL). The following data were obtained:

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean OD</th>
<th>Linear Regression y = 0.99x + 0.003</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>VISION-ER</td>
<td>13.5</td>
<td>0.995</td>
<td>0.99</td>
</tr>
<tr>
<td>VISSION-WB</td>
<td>11.3</td>
<td>0.996</td>
<td>0.99</td>
</tr>
<tr>
<td>KODAK-DT60</td>
<td>12.3</td>
<td>0.978</td>
<td>0.97</td>
</tr>
<tr>
<td>SYVA-ACULV</td>
<td>12.6</td>
<td>0.974</td>
<td>0.97</td>
</tr>
<tr>
<td>3M-REFAST</td>
<td>12.6</td>
<td>0.974</td>
<td>0.97</td>
</tr>
<tr>
<td>AMES-REERAL</td>
<td>13.2</td>
<td>0.953</td>
<td>0.95</td>
</tr>
</tbody>
</table>

The Kodak DT-60 and Abbott Vision were simple to use, required minimal maintenance and were the most practical. The more manual methods (Syva-Acculevel, 3M-TheoFAST, and Awa-Seralyzer), as certified by the standard deviation/standard error statistic, were cumbersome to perform and difficult to control.


In order to correlate cyclosporine's pharmacologic function with its binding to a cellular protein, we measured the relative affinity of cyclosporine to different analogs and to cyclosporine. The analogs are based on the immunosuppressive activity of mixed lymphocyte cultures (MLC). The relative potencies in the MLC and in binding to the protein were as follows (normalized to CsA):

<table>
<thead>
<tr>
<th>Analog</th>
<th>MLC</th>
<th>Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsA</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>CaA</td>
<td>0.91</td>
<td>0.97</td>
</tr>
<tr>
<td>DHCaA</td>
<td>0.91</td>
<td>0.62</td>
</tr>
<tr>
<td>CAB</td>
<td>0.51</td>
<td>0.42</td>
</tr>
<tr>
<td>CsG</td>
<td>0.16</td>
<td>0.06</td>
</tr>
<tr>
<td>CsD</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Structural differences in amino acid 2 of cyclosporin probably account for the subtle variations in immunosuppression with Cic, Cic, and Cic-C. This site is in the region of the Cic molecule to which the erythrocyte binding protein attaches (Agarwal, et al., Transplantation 42,877, 1986). These findings suggest that the major intracellular binding protein for cyclosporin may also mediate its immunosuppressive action.


Initial identification of drugs of abuse in our laboratory utilizes EMIT (Syva) or fluorescent polarization immunoassay (Abbott) techniques whereas the general drug screens rely on thin layer chromatography (TLC) using the Toxi A/B system (Marion Labs). Confirmation of drugs/toxins is via high performance liquid chromatography (HPLC) enhanced with the use of a linear photo diode array detector (LDA) (Varian's Polychrom 9060).

When such confirmation is needed, the unknown drug is first isolated by TLC and removed from the toxigram using a Toxilab Chromatogram Strip Punch (Marion Labs). The unknown is then eluted from the chromatogram, concentrated, and analyzed by TLC using gradient elution in which the acetic acid content, beginning at 0%, reaches 50% of the aqueous phase (pH 2.5) over a twenty minute period. Chromatographic information relating to substances resolved by the TLC is then transferred from the LDA to an IBM-AT computer. The chromographic results are compared against a library of information on known drugs of abuse in our laboratory containing over one hundred species of each, a) relative retention time of unknown, based on the retention time of a p-nitrophenol internal standard; b) mathematical analysis of spectral data via the purity parameter algorithm (Alfredson T. and T. Sheehan, J. Chrom. Sci.: 24:473-482, 1986) and c) analysis of spectral overlay of unknown to library standards using the spectral profile comparison algorithm (Hill D.W. et al., J. Chrom. 590:355-353, 1987). We present data illustrating positive identification of some common drugs of abuse including amphetamines and barbiturates.


We evaluated the Genetic Diagnostic Corporation (GDC) (Great Neck, New York 11021) Cannabinoid Assay and compared it to enzyme immunoassay (EIA) (Syva, Palo Alto, CA) and Abbott (Chicago, IL) and to GC/MS. The GDC assay is a competitive ELISA for the qualitative determination of cannabinoids in urine. In this microtiter plate assay, cannabinoid molecules in a test urine and those immobilized on a solid phase compete for limited antibody binding sites. The reaction is visualized, after addition of an enzyme-labeled second antibody, with a color producing substrate.

One hundred urine samples were assayed by each of the four methodologies. The results are presented in the Table.

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC/MS</td>
<td>74</td>
</tr>
<tr>
<td>GDC ELISA</td>
<td>73</td>
</tr>
<tr>
<td>Syva EMIT</td>
<td>72</td>
</tr>
<tr>
<td>Abbott TOX</td>
<td>70</td>
</tr>
</tbody>
</table>

A 20 ng/mL cut-off was used in the EIT and TOX assays and a 50 ng/mL cut-off was used in the GDC assay following manufacturers' recommendations. The one discrepant sample analysed by GDC ELISA was found upon GC/MS analysis to contain an 11-nor-9-THC-9-carboxylic acid concentration of 15 ng/mL; this is below the 20 ng/mL sensitivity claimed by the manufacturer. The data indicate that the GDC assay compares favorably to the Syva EMIT and Abbott TOX assays and correlates well with the reference GC/MS technique. This solid-phase ELISA method should prove to be a reliable, convenient and inexpensive screening technique for the toxicology laboratory.


We compared the whole blood cyclosporin concentrations from

565 CAPILLARY GC/MS DETECTION OF THE DESIGNER DRUG 3,4-METHYLeN- DIOXYPHENETAMINE (MDMA) IN ELICIT PILL FRAGMENTS AND URINE SAMPLES. James R. Shipk, Jr. and Michael T. Kinter (Dept. of Path., Univ. of Calif. Med. Ctr., Charlottesville, VA 22908) (Spon.: J. Shipk, Jr.)

MDMA, known as "Ecstasy" on the street, is an analog of the hallucinogenic amphetamine MDA. Unknown capsules, submitted to our toxicology laboratory, were found positive for amphetamines by EIT. GC/MS analysis of the compound was inconclusive (lack of knowledge, few fragments other than the base peak of m/z 58 and no match with the NBS mass spectral library). High resolution GC/MS suggested an inconsistent with the spectral fragment (m/z 115 H8N2O2) of MDMA. Since a spectrum for this compound could not be found in the literature, we developed the following procedure to detect and quantify MDMA in urine.

Samples positive for MDMA were used for confirmatory analysis by GC/MS. Five ml aliquots of calibrator, control and patient urine samples are combined with the internal standard (deuterated analogs of amphetamine and methamphetamine) made basic using 200 ul of 5 N NaOH, and extracted for 30 minutes into 7 ml of chloroform. The organic layer is acidified, shaken with 1.5 g anhydrous sodium sulfate and taken to dryness. The amine are acetylated using 100 ul of trifluoroacetic anhydride for 20 minutes at 65°C. Excess reagent is removed prior to analysis by GC/MS using on-column injection and a fused silica capillary column (DB-1701, 15 m X 0.25 mm i.d., J&W Scientific Inc.). From data acquired scanning ions for MDMA (m/z 140,123,118), methamphetamine (m/z 154,118,110) and the two deuterated internal standards (m/z 158,144,112) are used for confirmation. Using ion mass (d0 / d3) vs concentration to generate the standard curve, the method is linear from 25 to 2000 ng/ml (r = 0.9994). MDMA's with a run precision of 8.4% (480.1 + 140.0 / N = 10) is similar to the between-run precision for the other drugs which use deuterated analogs as internal standards.

566 A SOLID PHASE IMMUNOENZYMATIC ASSAY FOR MORPHINE METABOLITE IN URINE. David Woodrum, Vina Speilhier, Alene Herman and Alan Fraser. (Sensor Diagnostics Inc., Irvine, CA 92718, VTech, Inc. Pomona, CA 91787) (Spon.: Adam Zipp)

A five minute urine drug screen has been developed which employs a solid phase immunoenzymatic assay. The anti-drug antibody is bound to a membrane. As urine sample is passed through the membrane, opiate molecules are bound to the active antibody sites. The extent of drug occupation of the antibody sites is determined by following the reaction with a solution of horseradish peroxidase (HRP)-labeled morphine derivative which occupies the remaining opiate binding sites. The binding of the HRP-labeled morphine metabolite is determined by addition of a development containing hydrogen peroxide and tetramethylbenzidine (TMB). The development of a blue color indicates a negative test result; inhibition of color development indicates the presence of morphine, codeine or
morpheine-3-glucuronide (equally reactive) in the urine specimen. The antibody does not react with oxycodone, dextromethorphan, diphenoxylate, apomorphine, methadone, merperidine, nalorphine, naltraxone, d-propoxyphene, pentozacine, or naloxone.

The extent of inhibition of color development is proportional to the concentration of opiate drug present in the urine sample. The inhibition of color development was measured using a colorimetric reflectance reader. The QUANTITSCREEN TM / TARGET TM system (solid phase assay and reflectance reader) was compared to the Spin-EMIT at EMIT for screening for opiates in 46 human urine specimens positive for opiates and 25 urine specimens negative for opiates. A cutoff of 300 ng/ml morphine-3-glucuronide was used. The two assays agreed in 69 cases, disagreed in two.

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ENZYMIC ANALYSIS OF SERUM SALICYLATE (SAL) ON A DISCRETE ANALYZER. Rolly Opperman and George F. Johnson (Dept. of Path., Univ. of Iowa, Iowa City, IA 52242). (Spon.: J. Frecich)

We have evaluated an enzymatic assay for SAL (Diagnostic Chemicals Ltd.) and adapted it to a discrete microcentrifugal analyzer (MCA) (Multitest III, Instrumentation Laboratory ). Serum (3 ul) is added to 50 ul of MAAB reagent (2x conc.) into the sample well, and 100 ul of salicylate hydrazide reagent with 50 ul of water is added to the cuvette well. Upon mixing the reaction is followed at 340 nm, 30 C, and the A change between 30 sec and 6 sec is taken. SAL concentration is read from a least-squares straight line using 6 standards (0, 50, 100, 250, 500, and 1,000 ng/ml). Response was linear with conversion (Y, 0.9999) and a non-zero intercept. A change of 1.000 ng/ml standard was always greater than 350 nm.

This method (Y) was compared to the Abbott TDx method (X) for Y = 0 (X = 312); mean Y = 144 ng/ml and mean Y = 139 ng/ml (r = 0.997) with a regression line of Y = 1.013 - 9.19 X. When enzymatic SAL (Y) was compared to a colorimetric MCA method on these samples (Z) (Clin. Chem. 29, 839 (1983)), mean Z = 162 ng/ml (r = 0.997) with a regression line of Y = 1.012 - 25.32 X. Within run CV of Y at 338.6 ng/ml was 3.1% (n = 12). Run-to-run CV at 55 ng/ml was 3.5% (n = 21). Genitalic acid (1,000 ng/ml) gave an apparent SAL concentration of 6.7 ng/ml with Y, 239 ng/ml with Z, and 229 ng/ml with Z. Salicylic acid gave an apparent SAL concentration of 6.7 ng/ml with Y, 17 ng/ml with X, and 550 ng/ml with X.

We conclude that this enzymatic assay for SAL as adapted by us to the MCA is useful for monitoring SAL in patients, with good precision and correlation to colorimetric and immunoassays.

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SOLID-PHASE EXTRACTION OF PCR FROM URINE AND SERUM. Samuel F. Mathews (Johns Hopkins Med Inst, Baltimore, MD 21205). (Spon: S.E. Mathews)

A method has been developed using solid phase extraction for the determination of urine and serum specimens for quantitative determinations of phenocyclidine (PCP).

A 20 ml stock solution was made by dissolving 11.5 mg of PCP hydrochloride (Alltech) in 5 ml methanol. This stock solution was further diluted to 500 ml methanol. The internal standard solution diluted by a factor of 10 to make a working internal standard solution.

Briefly, 1 ml of sample (urine or serum) is added to 2 ml of 1 M pH 10.0 tris buffer (pHatkat & K-1X76). 50 ul of working internal standard (50 mg/ml) is added and the specimens are vortexed for 5 seconds twice. They are then added to Bond Elut C-18 (3 ml columns, Analytic International, catalog #60752), which have been prepared with washing 2 column volumes of methanol, 2 column volumes of water, and 1 volume of Trits buffer. The samples are drawn through the columns and the columns washed with 2 ml water. The columns are next washed with 250 ul aliquots of acetonitrile (Burdick and Jackson) followed by one 300 ul aliquot of hexane (Nellmeth, Nanograde). A vacuum is maintained on the columns for 5 minutes to dry the columns. The vacuum box is then opened and dried, collected tubes added, and the PCP eluted with 50 ul of ethyl acetate (Nellmeth, Nanograde). The vacuum during elution should not exceed 5 mmHg. Eluates are then dried and reconstituted with 25 ul of hexane.

The method was compared to at least 300 ng/ml stock-run CV at 30 ng/ml 4.4% and between-day is 7.5%. Sensitivity is on the order of 10 ng/ml. Analytical recovery is 55% from urine and 40% from serum. Correlation with the TDx method extraction procedure of Lin et al (Biomed Mass Spec 2, 206(1976)) gave, for urine, a slope of 0.94 and an intercept of 17.0 and for serum a slope of 1.03 and an intercept of -4.6.

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Abbott Laboratories developed a new fluorescent polarization immunoassay for whole blood cyclosporine and metabolites. We compared the precision and accuracy of this new TDx procedure with Sandos and INCASTAR CYCLO-Trac immunoassays using 210 patient specimens from transplant recipients. All three methods use polyclonal antisera but employ different tracers for detection (TDx- fluorescein; Sandos- Rh, INCASTAR-154). As immunosuppressive drugs, especially cyclosporins, grow in use, so has the need for reliable monitoring of blood levels. Assay precision (N=40) for cyclosporine concentrations of 360, 1266, and 1770 ng/ml gave CV's of 2.8, 3.9, and 6.8%, respectively. Sandos and INCASTAR precision was determined using two blood controls for whole blood (INCASTAR precision was 9.0 and 9.6% at cyclosporine levels of 162 and 905 ng/ml, respectively. Sandos precision (N=20) was 35 and 13.4% for the two samples.

Regression analysis with the three methods gave the following results:

\[
\begin{array}{cccc}
\text{Method} & Y & X & \text{Slope} & \text{Intercept} & R^2 & \text{Sn/r} \\
\text{TDx vs Sandos} & 210 & 1.094 & 34.37 & 0.901 & 115.4 \\
\text{TDx vs INCASTAR} & 210 & 1.310 & -28.22 & 0.934 & 94.4 \\
\text{INCASTAR vs Sandos} & 210 & 0.796 & 66.36 & 0.877 & 94.8 \\
\end{array}
\]

All methods gave excellent correlation with patient specimens. There are significant a and intercept differences correlated between all methods. The Abbott TDx whole blood cyclosporine assay has excellent precision, a significant advantage over both the Sandos and INC STAR methods.

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Cross reactivity of cyclosporine (CaA) metabolites with polyclonal CaA antisera supplied in commercial test kits is not well documented. To determine the extent of cross reactivity, we supplemented drug-free whole blood with nine CaA metabolites (conc. 50-1000 ng/ml/L) and assayed specimens with the INC STAR CYCLO-Trac (1-125), Sandos (H-3), and the Abbott TDx immunoassay procedures. Sample pretreatment was necessary for the Sandos method to minimize matrix interferences. Overall, the Abbott antisera exhibited lesser affinity for CaA metabolites; the Sandos antisaera exhibited the least. Specificity of the three sources of antisera was diverse as determined by the order of highest to lowest affinity with metabolites. This information is consistent with the antisera being generated towards different epitopes of the parent CaA molecule.

CaA metabolites are listed in decreasing order of cross reactivity, normalized to parent CaA.

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>Slope</th>
<th>Intercept</th>
<th>R²</th>
<th>Sn/r</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDx</td>
<td>21 &gt; 18 &gt; 21 &gt; 203-218 &gt; 388 &gt; 8 &gt; 26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INCASTAR CYCLO-Trac</td>
<td>75 &gt; 84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>17 &gt; 1 &gt; 26 &gt; 203-218 &gt; 8 &gt; 21 &gt; MUNDP1 &gt; 25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandos</td>
<td>49 &gt; 0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>17 &gt; 1 &gt; MUNDP1 &gt; 8 &gt; 18 &gt; 203-218 &gt; 26 &gt; 21 &gt; 25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Our study verifies the diverseselectivity of these three immunosassays for CaA and underscores the anomalies associated with comparison of patient results determined by the three assays. Least understood is the clinical significance of these differences and the importance of CaA metabolites contribution to the interpretation of the CaA result.

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SERUM AMPHOTERICIN B ASSAY BY SCANNING SPECTROPHOTOMETRY. Z.R. Shihabi, B.L. Wastlaukas and J.E. Peacock, Jr. (Depts. of Pathology and Medicine, Wake Forest University, Bowman Gray Sch. of Med., Winston-Salem, NC 27103) (Spon.: Z.R. Shihabi)

Introduction:

Amphotericin B is a polyene antifungal agent. Due to its inherent nephrotoxicity, serum levels of this drug should be monitored. In the past, cyclosporin immunoassays recently NPLC have been used to determine the serum levels.

Procedure:

A 0.2 ml aliquot of serum, amphotericin B standard (5 mg/L) or control is mixed with 0.4 ml acetoqirile for 1 min; centrifuged
at 15,000 g for 30 s and the supernatant is scanned from 450 nm to 350 nm at an absorbance of 0.2 to 0.2 full scale in a 1 mL narrow cuvette. Samples with bilirubin above 25 mg/L require solid-phase extraction before spectral scanning.

**Results:**

The method correlated well with the high-performance liquid chromatography (y = 1.01x + 0.5; r = 0.96; g = 19). The assay was linear between 0.3-5.0 mg/L. The average recovery of 5 laboratory samples spiked with 5 mg/L theophylline was 98% by HPLC. The method is faster (about 3 min) compared to the HPLC or to the bioassay.

Analysis of theophylline is necessary for maximum beneficial bronchodilator and respiratory stimulant effect for asthma and to prevent its toxic effects such as vomiting or seizures. The need for rapid results for Outpatient Clinics resulted in the Outpatient Laboratory performing these assays for possible dose changes on primarily pediatric patients. The cost of instrumentation used to assay theophylline such as the duplex ACA® prevents outpatient clinics as well as physicians offices from obtaining this service. The use of the Seralyzer® affords us the ability to perform rapid accurate theophylline assay for these clinic situations.

The theophylline Seralyzer® reagent strip utilizes a monoclonal immunoassay technique by measuring reflectance at 740 nm and then comparing this response to a previously generated calibration curve. A diluted sample is added to a new reagent strip, timed, and inserted into the instrument for measurement in 80 seconds.

Manufacturing linearity limit claims for the Seralyzer® theophylline assay is from 3 to 30 mg/L. The calibration curve is stable for 2 weeks. Repeated assays for 5 and 15 mg/L produced day-to-day CV’s of 8.8% (n=43) and 5.8% (n=35) respectively.

After an initial evaluation of the Seralyzer® we found it necessary to perform duplicate assays because of erratic results. The cause of this imprecision was later identified to be a dilution error. Subsequent studies gave a correlation coeff, r = 0.9670.

In conclusion, we found the Seralyzer® easy to run but does require some technique-dependent skills. Caution should be exercised with all results and control samples must be run often.

**REFERENCES**

1. **A RAPID RELIABLE MICROCOLUMN FOR CYCLOSPORIN**
   Gehrke KL, Gilmour G, J. Solidin, Dept. of Biochemistry, Hospital for Sick Children, 555 University Avenue, and Dept. of Clinical Biochemistry, Univ. of Toronto, Toronto, Canada (Spon: Steven J. Solidin)
   The initiation of a liver transplant program in a pediatric hospital prompted the development of an HPLC method for monitoring cyclosporin, which requires minimal amounts of blood and produces fast reliable results. A protein free filtrate is prepared by mixing 250 ul of whole blood with 750 ul of organic solvent (acetonitrile/methanol in the ratio of 9/1) containing the internal standard cyclosporin D. After centrifugation the supernatant is subjected to a quick cleanup using two types of disposable cartridges (C18 and silica bonded) the eluate is dried, reconstituted in equal parts of acetonitrile and water and subjected to a final HPLC step.
   Chromatography is performed at 75°C using a Supelcosil C18 column (15 cm x 4.6mm) with 3 u packing (Supelco Inc.) and a mobile phase of 77/23 of acetonitrile/phosphate buffer 2/5. Flow rate is 0.6 ml/min providing a chromatography time of 10 minutes. Absorbance is measured at 214.4 nm as a sensitivity setting of 0.01 AUFS. Recovery for cyclosporin A is between 92-104% and the lower level of sensitivity is 15 ug/mL. Between day precision provided CV’s of < 8% for a control serum of 150 ug/mL. The method is linear to 500 ug/mL. No interfering substances have been found. The method has proven to be consistent and reliable and simple enough to be performed by a standard life for a system used daily is approximately 3 months.

2. **QUALITATIVE AND QUANTITATIVE ANALYSIS OF COCAINE AND BENOXYLOLABINICINE IN URINE BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY**
   M. W. Miller, and B. Boleyn (Keverry Labs, Inc., Bloomfield, CT 06002)
   Identification of cocaine in urine can only be accomplished shortly after drug ingestion. Subsequently, benzoylecgonine, the major metabolite of cocaine, can be used as an indication of recent cocaine use. Gas chromatography/mass spectrometry has become the method for confirmation of drug abuse screening methods required by many employers and some states.
   We have developed a rapid qualitative and quantitative method for the analysis of cocaine and benzoylecgonine using the Perkin-Elmer ion trap detector mass spectrometer. By using 0.5 ml of urine is accomplished by saturating the specimen with sodium bisulfate and extracting into a chloroform-isopropanol solvent (3:1 v/v) by mixing for 10 min. Squalane is used as an internal standard. The organic solvent is then evaporated to dryness under a nitrogen stream at 80°C. For qualitative analysis the residue is reconstituted in 50 ml of 0.0-bis(Trisdimethylsilyl)-trifluorocetamide (BESFTA) and 1 ul is analyzed. Quantiative analysis requires heating the residue to 1 hr at 100°C with BESFTA before analysis.
   Analysis is performed under the following conditions:
   Column: Perkin-Elmer bonded methyl 5% film silicone silica 0.25m X 250.0, 0.25 film thickness Carrier Gas: He, 30 cm/sec linear velocity Oven Temp: 100°C for 1 min; 100°C to 300°C at 20°C/min; hold 10 min Injector Temp: 300°C Transfer line temp: 275°C ITD Scan Mode: full scan 40 to 600 amu; 1 scan per sec Injection: 45 sec. splitless program
   The recovery of the method is 94.1%, it is sensitive to 10 ng/ml and is linear from 10 to 5,000 ng/ml.

3. **ANALYSIS OF TOTAL MORPHINE, FREE MORPHINE AND CODEINE BY URINE GAS CHROMATOGRAPHY MASS SPECTROMETRY**
   M. W. Miller, and B. Boleyn (Keverry Labs, Inc., Bloomfield, CT 06062)
   Analysis of morphine is difficult by gas chromatography due to the hydroxy functional group(s) on these drugs. Hydrolysis is required to measure total morphine because most morphine in urine is in the glucuronide form.
   We have developed a quantitative method for the analysis of free morphine, total morphine and codeine using the Perkin-Elmer ion trap detector mass spectrometer.
   Hydrolysis of 5 ml of urine is accomplished by adding 1 ml of beta-glucuronidase (500,000 units/ml) 1 ml of 3 N acetic acid and letting the sample stand at room temp for 24 hr. Measurement of free morphine and codeine does not require this step. Tetrosene is used as an internal standard. The specimen is then saturated with sodium bisulfate and extracted into a chloroform-isopropanol solvent (3:1 v/v). The organic solvent is then evaporated to dryness under a nitrogen stream at 80°C. The residue is reconstituted in 100 ml of acetic acid and heated at 100°C for 1 hr, 2 ml of methanol is added, and the sample is again evaporated to dryness under the above conditions.
   Analysis is performed by reconstituting the residue with 25 ml of methanol and analyzing 1 ul under the following conditions:
   Column: Perkin-Elmer bonded methyl 5% phenyl silicone
The recovery of the method is 96%, it is sensitive to 10 ng/mL and is linear from 10 to 5,000 ng/mL.

**EVALUATION OF THE ABBOTT FPIA BENZODIAZEPINE ASSAY USING THE ADX ANALYZER, Yale H. Caplan and Barry Lee, University of Maryland, Department of Pathology, Division of Forensic Pathology, Baltimore, MD 21201. (Spon.: Y. H. Caplan)

This study evaluated the capability of the Abbott ADX Analyzer to test for benzodiazepines (BDPs) in urine specimens. Within run and between run precision were determined using 3 control samples (200, 300, and 1,000 ng/mL). The control samples were tested for a 2-week period. Controls were analyzed in both single assay (sample batch) and multiple assay (sample flex) modes. Mean concentrations were 187.7, 293.9, and 1,078.0 ng/mL (batch mode), respectively, and 201.6, 295.1, and 1,108.1 ng/mL (flex mode), respectively. Overall within run coefficients of variation were 2.3, 1.8, and 1.5%, respectively, and overall between run coefficients of variation were 2.8, 3.2, and 1.0%, respectively. The multiassay control containing 300 ng/mL nordiazepam was used to compare instrument performance in the single flex mode and the panel flex mode (multiple specimen sampling from a single cartridge). The mean concentrations were 294.7 ng/mL (sample flex mode) and 292.7 ng/mL (panel flex mode). Fifty specimens not containing BDPs and 50 specimens containing BDPs were tested by ADX, TDX, EMIT, and GC/MS. The methods compared favorably and no false positive or false negative results were obtained by each immunoassay method.


The clinical laboratory in conjunction with government and industrial laboratories, may play a vital role in identifying potential adulteration or tampering of both pharmaceutical and food products. Taking into account the nature of the matrices encountered in such a testing program, a variety of extraction and isolation schemes were chosen followed by analyses of unknowns by various chromatographic methods including TLC, GC, GC/MS and HPLC as well as by conventional spectral techniques. In twenty five cases involving possible food (primarily beverage) adulterations, as noted by either abnormal physical observations of the product, or by the manifestation of clinical symptoms, eight were found to contain unexpected materials, five of which were identified. In the investigation of twenty eight pharmaceutical products submitted by hospitals, pharmacies, or nursing homes, and in which the presence of tampering, sixteen were found to be void of anticipated drug or contained a substitute medication.

**PHARMACOKINETICS OF CYCLOSPORINE ESTIMATED BY RADIOIMMUNOASSAY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, M. Pleban, M. Masiero, E. Del Favero, P. Bonvidini, A. Burlina. (Department of Clinical Chemistry and Clinical Microscopy, University of Padova, Italy) (Spon.: P. Bonvidini)

Since Cyclosporine A (CsA) elimination and bioavailability exhibit large interpatient variability, therapeutic efficacy might be improved by measuring pharmacokinetic parameters in individual patients. Radioimmunoassay (RIA) is the most widely utilized method to estimate serum levels of CsA but it measures CsA in a cross-reactive metabolite (cone-A). Using a selective HPLC technique specifically determines the parent drug (CsA). We have studied the pharmacokinetics of CsA in 12 children before kidney transplantation (8 x 16 years) and as bolus injections of 120 mcg/kg. Blood samples were drawn at trough (0 h), then at 0.5/1/3/5/7/12 and 24 hours. RIA determinations of CsA were done with minor modifications according to the manufacturer's (Sandoid, Ltd, Basel, Switzerland) HPLC determination of CsA was done according to Swinouwski et al by employing a solid phase extraction and a reverse phase cartridge. The area under the drug concentration-versus-time curve (AUC) was calculated according to the trapezoidal method and clearance was determined by the formula Cl = dose/AUC. The apparent volume of distribution was calculated according to the formula Vd = CsA X K is the slope of the elimination phase, calculated with linear regression according to the method of least squares. The elimination half-life (1/t) was calculated according to the equation: 1/t = 0.693/K. A significant agreement between RIA and HPLC results was observed (RIA = 0.861 HPLC + 145.9; r = 0.992). More interestingly the pharmacokinetic parameters estimated by RIA and HPLC are not significantly different (AUC RIA = 11.21 + 2.96; AUC HPLC = 11.12 + 3.28 mcg/ml/h; 1/t RIA = 9.43 + 2.42, 1/t HPLC 9.3 + 3.1 h; Vd RIA = 3.75 + 1.05; Vd HPLC = 3.41 + 1.28 L/kg; Cl RIA = 0.42 + 0.1 L/hr/kg, Cl HPLC = 0.43 + 0.14 L/hr/kg, r = 0.05). Further studies are in progress in transplant patients following oral administration of CsA in order to evaluate the possible difference in pharmacokinetic parameters obtained by RIA and HPLC due to cross-reactive metabolites.

**CALIBRATION OF THE EMIT 700 CANNABINOIDS 100 NG ASSAY WITH 11-HC: D-THC-9-CARBOXYLIC ACID, S. Massove, B. Chung, M. Crompton, J. Valdez (Spon.: Jay E. Gorsky)

The EMIT 700 Cannabinoids 100 ng Assay is a high volume initial test for the presence of cannabinoid metabolites in urine and uses a calibrator containing 100 ng/mL 11-nor-9 delta-THC-9-carboxylic acid to distinguish positive from negative samples. The calibrator and positive control have been reformulated to contain 11-nor-9 delta-THC-9-carboxylic acid at (0) 100 and 400 ng/mL in a urine matrix containing stabilizers and preservatives. This was done in order to calibrate with the compound which occurs as the major metabolite following marijuana use.

Using the standard EMIT 700 protocol on the Hitachi 705 analyzer, within-run precision of the calibrator, negative and positive controls produced standard deviations for <2.0. Twenty negative samples were spiked at various concentrations between 0 and 200 ng/mL by the Hitachi 705 Analyzer. Samples containing drug were spiked above the level of the calibrator (100 ng/mL) resulted in positive assays, while those containing drug spiked below the level of the calibrator resulted in negative assays.

Patient samples (N=20) were run with both the delta and delta calibrator formulations on the Hitachi 705 system. Comparison of results demonstrated 98% agreement of the 9 delta samples that had results less than the 9 delta calibrator but greater than the 9 delta calibrator, six were analyzed by GC/MS and found to contain concentrations of 9 delta between 8 and 80 ng/mL.
The results show the new $\delta^9$ calibrator and control provide accurate and reliable results equivalent to the $\delta^8$ formulation for the Erit 700 Cannabinoid 100 ng Assay.

552 APPLICATION OF ELECTROCHEMICAL DETECTION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FOR INTERAVENUS (IV) CLONIPRAME (DC) IN ADULTS. EL Stiller, WM Perel, BG Pollock, L Birder (Cln. Pharmcol., Western Psychiatric Inst. and Clinic, Univ. of Pittsburgh, Pittsburgh, PA 15213). PC Lin and S. Narayanan, (Section-Dickinson Vactumation Systems, Rutherford, NJ 07070).

Although HPLC analyses of tricyclic antidepressants (TCA) such as imipramine (I) have been reported in the literature, there is need for a method to measure low levels of C and its metabolite demethyl clonipramine (DC) in plasma samples. The objective of this study was to determine the pharmacokinetics of a single bolus IV low test dose (12.5 mg) of C in an adult population. The plasma obtained in this study were from blood samples collected by venipuncture and VACUTAINER brand tubes with preserved EDTA which are plasticizer-free. Blood samples were collected at 15 min, 45 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 12 hr, and 24 hr.

The HPLC method utilized a reverse phase C-18 column and a coulometric detector. The detection system is comprised of three flow through porous carbon electrodes. The potentials for guard, 1st and 2nd detectors are 0.95, 0.25, and 0.65 V respectively. Mobile phase used was acetominethol and 0.01M phosphate buffer pH 5 (50:50) and 100 ul butylinol in 1 L mobile phase. The internal standard used for quantitative analysis is I. The retention times for C and DC are 4.5, 5.5, and 6.2 min; respectively. The D and DC is extracted with 3 ml n-butanol: n-heptane (2/1) from 1 ml plasma. Recoveries were 99% and 95% and cv = 5% and 8% respectively. The lower level of detection is 1 ng/ml in plasma. Linearity range tested being 1-50 ng/ml. In 5 patients, the TV$\delta$ = 28.7 hrs $\pm$ 9.3.

The method is direct applicability for low level C analysis and small plasma samples volumes with higher levels of C. An interesting note is that no DC was detected in the sample period up to 24 hrs indicating that minimal or no demethylation of C to DC had occurred.

553 A COMPARATIVE PERFORMANCE EVALUATION OF ABBOTT'S TDX AND SANDOZ-RIA FOR CYCLOSPORIN A AND ITS METABOLITES. Ramond Prasad, Nancy Horn, Andrew Maturer & Michael Maddux (Univ. Illinois Hosp., Chicago, IL 60612) (Sponsor: Ramond Prasad).

THE ABBOTT TDX CYCLOSPORINE AND METABOLITES SERUM ASSAY WAS COMPARED TO THE SANDOZ RIA. PRECISION OF THE TDX METHOD WAS EVALUATED FOR EACH OBTAINED SAMPLE. 20 SAMPLES FIVE OF EACH FOR TEN DAYS. THE TDX RESULTS ARE AS FOLLOWS.

<table>
<thead>
<tr>
<th>MID</th>
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<td>75</td>
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LOW

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<tr>
<th>MEAN$\delta$(50)</th>
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<td>MEAN$\delta$(50)</td>
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</table>

CV WITHIN RANK 5.64 2.36 4.28
CV BETWEEN RANK 6.89 3.91 5.01

THE PERCENT CV's DETERMINED FOR THE SANDOZ RIA METHOD USING THE RIA LOW, MIDDLE, AND HIGH CONTROLS WERE 7.00, 5.18, AND 7.78 RESPECTIVELY.

THE TDX CALIBRATION CURVE WAS FOUND TO BE STABLE FOR TWO WEEKS. TO ASSESS ACCURACY, TDX CALIBRATORS WERE ANALYZED BY RIA AND RIA CALIBRATORS BY THE TDX METHOD. TDX CALIBRATORS GAVE HIGHER VALUES BY RIA WHILE RIA CALIBRATORS GAVE PROPORTIONAL LOWER VALUES BY TDX.

PATIENT SAMPLES WERE ANALYZED BY BOTH METHODS. THE REGRESSION EQUATION FOR RIA (X) AND TDX (Y) WAS Y = 0.700 + 1.292X (R=0.45; p<0.01). FOR RESULTS BELOW 100 ng/ML THE REGRESSION EQUATION WAS Y = 18.09 + 1.308X (R=0.38; p<0.005).

BASED UPON THE ABOVE DATA, THE PRECISION OF THE TDX ASSAY IS BETTER THAN THE SANDOZ RIA METHOD. OUR LABORATORY FOUND THE TDX ASSAY METHOD FASTER AND EASIER TO USE BECAUSE THERE ARE FEWER PROCEDURAL STEPS AND THEREFORE HELPS TO MINIMIZE ERRORS.

554 AN INTRACRANIAL CYCLOSPORIN RIA AND HPLC RESULTS. WILLIAM A. HARDY (DEPARTMENT OF PATHOLOGY, HOBART UNIVERSITY HOSPITAL, WASHINGTON, D.C. 20060) (Sponsor: William A. Hardy)

Cyclosporin, an immunosuppressant agent widely used in organ transplantation, is usually analysed by HPLC or RIA. Whereas RIA measures total cyclosporin, HPLC measures cyclosporin metabolites. Sometimes transplant patients are transferred from one hospital to another one. With the transfer it may be found that the cyclosporin values accompanying the patients are differ-

2.36

ent from those at the second hospital. Many times these differences are attributable to methodology dissimilarities. We compared cyclosporine A by the BioRAD HPLC assay to cyclosporin by the INCAST RIA procedure to determine if a factor could be derived to interconvert one result to the other.

Twenty-eight patient samples were assayed by both methods. By HPLC the values ranged from 852 ng/mL to 30 mg/L. Assayed by RIA had values from 30 ng/mL to 1200 ng/mL. Calculation of the ratio of the RIA to HPLC result gave values that ranged from 1.2 to 4.4. If the RIA result was 30 ng/mL, there was no detectable peak by HPLC. The variability in the ratios did not correspond to any particular cyclosporin concentration.

We conclude that cyclosporin values by RIA and HPLC cannot be interconverted by a simple conversion factor.
ANALYSIS OF PHENCYCLIDINE IN URINE BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY. Kevin W. Miller, and H.R. Michlin (Kevavi Labs, Inc., Bloomfield, CT 06002) (Spon: Ralph Alini)

Recently, certain quality assurance programs and accreditation programs are requiring quantitative analysis for drugs of abuse. Gas chromatography/mass spectrometry has become the method for confirmation of drugs of abuse screening methods required by many employers and some states. In most cases qualitative results are all that is required for accurate confirmation. However, we have developed a quantitative method for the analysis of phencyclidine using the Perkin-Elmer ion trap detector mass spectrometer.

Extraction of 5 mL of urine is accomplished by saturating the specimen with sodium bicarbonate, adding a 50-mg aqueous internal standard solution; mixing for 1 min with 5 mL of a solution of form-isopropanol solvent (3:1 v/v). The organic solvent is then evaporated to dryness under a nitrogen stream at 80°C. The residue is then reconstituted with 25 mL of methanol and 1 mL of the sample is used for analysis.

Analysis is performed under the following conditions:
- Column: Perkin-Elmer bonded methyl 5% phenyl silicone 0.25 mm X 250.0, 0.25 film thickness
- Carrier Gas: He 30 cm/sec linear velocity
- Oven Temp.: 100°C for 1 min; 100°C to 300°C at 20°C/min; hold 10 min
- Injector Temp.: 300°C
- Transfer line temp.: 275°C
- ITD Scan Mode: full scan 40 to 600 amu; scan per sec: 45 sec.splitless program
- The response of the method was 99%, it is sensitive to 1 ng/mL and is linear from 1 to 5000 ng/mL.

PROTEINS

Specific Proteins

IMMUNOLOGICAL STUDIES FROM IMMUNOLOGICALLY ACTIVATED PERSONS SHOW GREATER TENDENCY TO AGGREGATE THAN NORMAL. S.E. Levinson, D. Late, R. Goldstein, H. J. Terblanche, and D. S. Rosenthal (Hup., Sinai Hosp., Detroit, MI 482016, and 2Depart. Pathol., U. Michigan, Ann Arbor, MI 48109) (Spon.: S. Levinson).

We previously showed that pairs of endogenous restricted bands, seen migrating in the gamma region upon high resolution electrophoresis, were not circulating immune complexes (CIC), but 71 size IgG (Clin. Chem. 32:904, 1987). These bands are usually found in patients with chronically activated immune systems. We fractionated the sera from such patients with the following cuts of polyethylene glycol (PEG): 0-2.5%, 2.5-5.0%, 5.0-7.5%, 7.5-10%, and 10.0-12.5%. In the 0.2-5.5 cut, levels of IgG >5.5 mg/100 mL indicate elevated CIC. The range of IgG concentrations in the cut, respectively, in the sera of 5 patients was 3.1-25.1, 1.5-71.3, 62-400, 21-70-4, and 4.3-8.0 mg/100 mL, while the ranges from normal sera were 2.3-3.5, 58-80, 256-349, 21.3-76.4, and 4.1-13.9 mg/100 mL. The average IgG concentration in the 2.5-5.0, and 7.5-12.5 cuts from patients was 399 and 120 mg/L, while from normals the concentrations were 68 and 288 mg/L. The endogenous bands were not associated with any specific cut, but appeared to be proportionally distributed in accord with the levels of IgG. The bands can consist of immunoglobulins with common idiotopes but varying antigen specificities.

The data is consistent with the idea that immunologically activated persons exhibit a greater tendency for immunoglobulins to aggregate than normal. On the average, 52% of the IgG showed this greater tendency. This propensity to aggregate may cause CIC to form in situ in local compartments even though CIC do not appear to be present upon analysis by biochemical techniques. The phenomenon may be due to idiotypic antibodies, and IgG and IgM rheumatoid factors. These findings support the theory of Jerne, that suggests the majority of antibodies are part of an internal immunoregulatory network with activity against other antibodies.


Elevation of Alpha-1-acid glycoprotein (AAG, orosomucoid) is known to increase under the stresses of trauma, burn and myocardial infarction, likewise, AAG's acts as a strong drug binding protein and can alter drug disposition. Previous to, the turbidimetric assay of Mantucis et al (Clin. Chem. 30:873-874, 1985) radial immunodiffusion (RID) which is costly, not real time and is highly operator variable served as the reference method. In order to obtain AAG levels in trauma patients to evaluate drug interactions, we adapted this turbidimetric assay to a Microcentrifugal analyzer (MCA) (Instrumentation Laboratory) using anti-human AAG and calibrators (Atlantic Antibodies, Scarborough, ME).

Our values were compared to RID and the following results were obtained: (ng) MCA standard linearity (22-178) was y = 0.924 x + 10.94, r = 0.98 & standard linearity for MCA vs RID y = 1.109 + 0.840, r = 0.99

Precision analysis:

Within run (N=36) X±SD 6.0±1.2 CV 1.48% Between run (N=36/mt x 9 runs) X±SD 6.4±0.5 CV 1.47±0.8 2.09%

Correlation studies on plasma obtained from 60 trauma/surgical patients using RID vs MCA yields: y=39.21 + 5.8x, R=0.84

Cost analysis given the following results: MCA $320.00 RID $270.00

Totaal Cost/100 tests $422.00 $270.00
We conclude that this immunoturbidimetric method adapted to the IL MCA has excellent linearity, precision, and correlation with RID, while rendering real time, cost efficient, clinically useful results.

Measurement of Haptoglobin on the Abbott TDx® System

M.E. Coffee and R.P. DeOrell (Abbott Laboratories, Irving, TX 75015) (Spon: M.J. Hints)

A fully automated haptoglobin assay has been developed for the Abbott TDx® System. An accessory carousel with an auxiliary light source (TDx Turbo™ Carousel) is used in conjunction with the TDx to perform this nephelometric assay. Unit dose format reagents allow for fully automated batch testing or random access testing when used with other TDx Turbo specific protein assays.

Results of the performance evaluations are presented. Precision data obtained by assaying low and high controls five times once a day over a 4 day period yielded within run and total CVs of less than 4%. Sensitivity was less than 2 mg/dL Bilirubin at 20mg/dL, and moderate linearity showed less than 5% interference. Serum samples should not be run because of interference from the irreversibly binding of hemoglobin to haptoglobin.

Linear regressions of 50 patient sample comparison with the Beckman ICS II® and Behring RID over a range of 65 to 355 mg/dL yielded correlation coefficients of greater than 0.95 with minimal constant and proportional bias.

The TDx Turbo Haptoglobin assay is a precise, accurate and sensitive method suitable for clinical use.

Quantitation of Albumin in Cerebrospinal Fluid on the Abbott TDx® System

P. Waldenbach, R.P. Coffee and R.P. DeOrell (Abbott Laboratories, Irving, TX 75015) (Sponsor: R.P. DeOrell)

A nephelometric, fully automated assay for the quantitation of albumin in cerebrospinal fluid (CSF) has been developed for the Abbott TDx® System. The nephelometric assay utilizes an accessory carousel (TDx Turbo™ Carousel) which has an auxiliary light source. These format reagents allow for fully automated batch testing or random access testing when used with other TDx Turbo specific protein assays.

Results from the performance evaluation are presented. Precision data was obtained by assaying low and high controls in replicates of ten, six times, over a two day period and yielded within run and total CVs of less than 4%. Sensitivity was less than 0.4 mg/dL. Linear regression of 20 patient sample comparison with the Beckman ICS II® and Behring RID over a range of 0.4 to 84 mg/dL yielded correlation coefficients of greater than 0.95 with minimal constant and proportional bias.

We conclude that the TDx Turbo™ Albumin assay is a precise, accurate and sensitive method suitable for quantitating albumin in CSF.

A Quantitative Measurement of Rheumatoid Factor (RF) by Turbidimetric Immunodessay (TIA), Terugiti Hasagawa, H. Shibata, and T. Honda (Misui Pharmaceutical Co., Ltd., Yuki, Japan) (Spon: T. Amakawa)

We describe the measurement of rheumatoid factor (RF) in human serum by turbidimetric immunodessay (TIA). This assay measures the increase in turbidity by the antigen-antibody complex formed within five minutes at 340nm. We used the Hitachi 736 automatic analyzer (Hitachi, Ltd., Tokyo, Japan). It is an automatic analyzer that can measure turbidimetrically at 340nm can be used.

We found that the Cohn fraction II heated at 61°C for 30 minutes was the most appropriate antigen preparation. The standard value was expressed in arbitrary unit (u/mL) according to World Health Organization reference RF serum. The standard curve was linear up to 170 u/mL. Bilirubin, hemoglobin, and trypolypeptides did not interfere with the TIA.

The within-run precision studies (10 replicates) were shown in the following table:

<table>
<thead>
<tr>
<th>X</th>
<th>CV</th>
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<tbody>
<tr>
<td>35</td>
<td>2.28</td>
</tr>
<tr>
<td>57</td>
<td>2.0</td>
</tr>
<tr>
<td>142</td>
<td>3.9</td>
</tr>
<tr>
<td>167</td>
<td>2.0</td>
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In summary, this assay provides a simple and rapid way of quantitation of RF on the automatic analyzer. The determination of RF by TIA can be used to provide not only diagnostic but also prognostic and therapeutic information on RA.

A COMPARISON BETWEEN IMMUNOFIXATION AND IMMUNOBLOTTING TECHNIQUES FOR THE DETECTION OF OLGIGOCAL IgG BANDS IN CSF, Rene Costello, and N. Papadopoulos (NIH, Clin. Path. Dept., Bethesda, MD 20892) (Spon.: R. Costello)

The detection of oligoclonal immunoglobulin bands (OIB) in cerebrospinal fluid (CSF) is a characteristic finding in patients with the clinical diagnosis of multiple sclerosis (MS). To accomplish this, agarose gel electrophoresis (AGE) followed by comassie staining is used to detect the protein bands. Immunofixation (IF) is then performed before the performance of the above procedures. Here we describe a sensitive immunoblotting (IB) technique using unconcentrated CSF to detect and identify OIB simultaneously. The method is performed by transferring the electrophoretically separated proteins by capillary attraction to a polyvinyliden difluoride membrane. Subsequently the membrane is placed in a solution of horseradish peroxidase-conjugated IgG antibody and incubated at room temperature. Following incubation the membrane is washed and transferred to a solution of 4-chloro-1-naphthol to visualize the immunostained immunoglobulin bands. A comparison with our sensitive AGE and IF techniques was made to assess the performance of the immunoblotting method. We analyzed 10 CSF specimens from patients with MS and demonstrated oligoclonal immunoglobulin bands by AGE and IF. The same CSF specimens were subjected to the alternative IB procedure. The immunoblotting technique demonstrated oligoclonal IgG bands in 9 out of 10 CSF specimens. A variation in the number and intensity of the OIB was observed between the comassie stained gels and the immunostained membranes. We conclude that the immunoblotting technique is a reliable alternative to that of agarose gel electrophoresis and immunofixation and is especially valuable when only a limited amount of CSF is available.
IgG, IgA and IgM were determined by mixing serum with a reagent containing the appropriate antibody and polyethylene glycol. The scattered light was measured at an angle of 37.5 degrees and at a wavelength of 540 nm. The immunoglobulins are determined using a special carousel which contains a light source and with the unit dose reagent delivery system.

The calibration curves are stable for at least 20 days. The linear range for IgG was from 0.5 - 34 g/L, for IgA from 0.03 - 8.1 g/L and for IgM from 0.08 - 5.0 g/L. Antigen excess is observed at IgG, IgA and IgM concentrations of 500, 200 and 140 g/L respectively. There was no cross reactivity for IgG with samples containing IgA and IgM at concentrations of 77 and 34 g/L, for IgA with samples containing IgG and IgM at concentrations of 51 and 16 g/L, and for IgM with samples containing IgG and IgA at concentrations of 64 and 69 g/L. The within-run precision was 19% for samples containing either polyclonal or monoclonal IgG, IgA and IgM proteins gave CV's of less than 6%. Linear regression analysis of the TDX immunoglobulin results compared with the Beckman IGS system gave:

<table>
<thead>
<tr>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
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<tbody>
<tr>
<td>102</td>
<td>0.94</td>
<td>1.10</td>
</tr>
<tr>
<td>103</td>
<td>0.97</td>
<td>0.62</td>
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</table>

The TDX IgG, IgA and IgM assays are automated, accurate and precise.

**Critical variables in the detection of IgG paraproteins by immunoeflectrophoresis.** BM Kahn, N Bina, DS Monos (Univ. Penna, Philadelphia PA 19104) (Spon: SN Kahn)

Immunoelectrophoresis (IEP) is demonstrably superior to immunoefectroelectrophoresis (IEP) for the detection of paraproteins, but unlike IEP, is not self-fertigrating. In our case, the point of interpretation and maximum sensitivity, antigen concentration (serum dilution) and antibody avidity must therefore be carefully matched to avoid pronouncing large paraprotein bands on antigen excess or failure to detect small bands present on a normal background of polyclonal immunoglobulin. However, for convenience, some of our kits measures recommend single serum dilution for all paraproteins. We investigated the effects of using different antisera and varying dilutions of paraproteinemomic serum on the sensitivity of IEP for detection of the heavy and light chains of IgG paraproteins. Three different antisera against gamma heavy chain and each light chain isotype were tested. The anti-gamma heavy chain serum showed only minor differences in reactivity, which did not affect interpretation of the results. However, the light chain antisera showed marked differences in reactivity; the same paraprotein with the three different antisera, and the same antisera reacted unequally with different paraproteins. Light chain detection was markedly affected by paraprotein concentration. Two IgG kappa paraproteins were tested at different concentrations with a single antisera against kappa light chains and an IgG lambda paraprotein was tested at different concentrations with two anti-lambda chain antisera. Detection limits for the light chains were both paraprotein and antisera dependent. We conclude that adoption of a single dilution for all sera could result in failure to detect low-concentration or poorly immunoreactive paraproteins. In addition, no single antisera can be relied on to provide unequivocal characterisation of all paraproteins of the relevant isotype.

**FREE HEMOGLOBIN INTERFERENCE WITH HEMOGLOBIN MEASUREMENT BY SNT HEMOLYTOMETRY.** R. Kyashe-Bechar, E. Chen, and B. G. Oudry (Dept. Laboratory Medicine, Sch. Medicine, Univ. Calif., Clinical Labs., San Francisco General Hosp., San Francisco, CA 94110) (Sponsor: Klaus Bayer)

Serum haptoglobin (HPT) measurements are useful for the diagnosis of intravascular hemolysis. The influence of hemolysis in the serum on HPT measurement by rate nephelometry has not been well-characterised. Therefore, we examined the effect of added HGB on the apparent HPT concentration (HPT) measured by rate nephelometry using Beckman reagents. Sera without any visible hemolysis were used to make 13 pools containing 0.4-7.6 g/L HPT. A millipore-filtered stock serum solution prepared from a red cell hemoysate was diluted into each HPT pool over the range of 0.5 g/L (light pink) to 8.0 g/L (dark red). Samples were allowed to incubate for 1-5 hours at room temperature after HGB addition. The apparent (HPT) decreased with increasing HGB, with a minimum value of 40-55% of the initial (HPT). In general, the higher the initial (HPT), the higher the (HGB) at which the apparent (HPT) leveled off. This reflects the increased HGB binding capacity of increasing (HPT). Protein electrophoresis and immunofluorescence using beckman anti-HPT antibody confirmed decreased antibody reactivity with the HPT-HGB complex compared to the free HPT band, based on Comassie Blue staining.

Active intravascular hemolysis typically results in (HPT) 0.25 g/L, levels well below those seen in our studies even with gross hemolysis. Therefore, we believe that nephelometry may not be helpful for the evaluation of intravascular hemolysis, although we have not obtained confirmatory in vivo data.

A SENSITIVE TIME-RESOLVED IMMUNOFLUOROMETRIC ASSAY OF FERRITIN IN SERUM WITH MONOCLONAL ANTIBODIES. M. J. Khoury, M. A. Chan, A. C. Bellm, and E. P. Diamandis (CyberFluor Inc., 179 John Street, Toronto, Ontario, Canada M5T 1K4) (Spon: Barry Hoffman).

Analytical methods for quantitation of ferritin in serum most commonly include radioimmunoassays and immunoradiometric procedures. Recently, a number of alternative immunoassays involving various non-radioactive detection systems have been developed. We describe here a new non-isotopic immunofluorometric assay for ferritin in serum using ferritin sandwiched immuno-extracted with a monoclonal antibody immobilized in a white microtiter well. A second biotinylated monoclonal antibody is then added to detect captured ferritin molecules. The degree of binding of the biotinylated antibody is quantified by the addition of the indicator solution containing (a) streptavidin labeled with BCPDA, and (b) excess Eu³⁺. BCPDA [4, 7-bis(chlorosulfonyl)-9, 10-phenanthrolinato- 2, 9-dicarboxylic acid] is a europium chelate. The fluorescence of the complex on the dry microtiter well consisting of monoclonal antibody-ferritin-monoclonal antibody-biotin-streptavidin-BCPDA-Eu³⁺ is then measured with a time resolved fluorometer. The assay has a detection limit of 0.5 μg/L and a linear working range of 0.0 to 500 μg/L. It is insensitive to the high dose "hook-effect" at ferritin concentrations up to 15,000 μg/L. Within-run CV's were 6.8, 7.0, and 3.0% for ferritin levels of 40, 140, and 244 μg/L respectively. Recovery of exogenous ferritin from pooled sera ranged from 97 to 109%, with an average of 102%. Results compare well (r = 0.99) with those of a widely used radioimmunoassay (RIA) procedure in an assay of 104 clinical samples.

GLYCATED ALBUMIN (GA) BY FLUOROMETRY IN THE MANAGEMENT OF DIABETIC PREGNANCY. J. P. Grimsen, R. Alter, and T. A. Biondo (Department of Pathology, Division of Clinical Pathology) J.P. Grimsen, C. Ozark, S. Sunderji (Department of OB/GYN), SUNY Health Science Center, Syracuse, NY 13210 (Spon: Jamie Wool)

Maintenance of alyogeoemia in diabetic pregnancy to optimise fetal outcome requires accurate monitoring of maternal blood glucose control. GA (tₕ=14-20 days) is an improved alternative over glycated hemoglobin (tₕ=90-120 days) (HGB). We report the evaluation of a simple, sensitive and specific fluorometric assay for GA (Clin Chir Acta 1985), which requires no prior separation since the assay is not affected by unbound glucose, albumin or T-globulin. Thirty samples can be completed in 2 h.

Two aliquots of 20 μL diluted (1/5) serum are incubated with 2 mL each of reagent A (50 mol/L H₂O-2-hydroxyethylpiperoxazine- H₂O-2-aminoethanesulfonic acid, pH 8.5) and reagent B (reagent A + 50 mol/L H₂O, pH 8.5) for 3-5 min at 20°C. Emission intensity is recorded at 490 nm with excitation set at 360 nm. The difference in fluorescence intensities between the two reaction mixtures is proportional to GA in serum. The assay correlates well with a reference radioimmunoassay procedure (Endoicine Sciences, Tarsana, CA) (r=0.905). It is linear up to 12.9% GA with r=0.994. Assay sensitivity is 0.25% of total albumin. Assay precision is 6.0% (range 0.3-11.54, n=40).

Our reference interval for GA is up to 1.3% of total albumin (n=9). GA correlates positively with glucose in serum from 60 pregnant women 40 of whom were diabetic (r=0.709). Twenty-six pregnant diabetics under perinatal care were followed with glucose, GA and HGB during the 1st, 2nd, 3rd trimester, during labor or prior to elective cesarean section, and 6 weeks post partum. GA was much more sensitive than HGB in reflecting short-term changes in glycemic control.

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988
EVALUATION OF DAE STRATUS FOR MEASUREMENT OF SERUM FERRITIN

Because of DRG and financial constraints there is only limited work force to do the lab work. This necessitates that we look for procedures which reduce manpower needs. A fully automated method (Dade STRATUS) is now available for ferritin. It requires only one man or one operator to start the test. Our objective was to determine ferritin by STRATUS and compare its accuracy and precision with ferritin assay by RIA.

Ferritin method on STRATUS is a fluorometric enzyme immunoassay utilizing double antibody technique. Ferritin by RIA uses the HYBITECH tandem-R solid phase two-site immunoradiometric method.

Thirty-five serum samples were assayed for ferritin by the two methods. Comparison data showed: slope=1.00, intercept=0.0, r=0.988; STRATUS mean=270 ng/mL and RIA mean=245 ng/mL. Ten replicates of a given sample were assayed by each method at the same time. STRATUS had mean=207 ng/mL S.D. (Standard Deviation)=3.7, and C.V.(Coefficient of Variation)=1.8%; RIA had mean=200 ng/mL S.D.=5.4, and C.V.=7.2%. To determine day-to-day variability, a given sample was assayed every day for ten days by each method. The sample assayed on STRATUS at ferritin level of 134 ng/mL showed: S.D.=4.4 and C.V.=5.3%. RIA at 200 ng/mL showed S.D.=8.3 and C.V.=4.1.

The results suggest that the two methods are essentially equal in precision.

Conclusion is that ferritin by STRATUS is at least as good as by RIA. But since RIA is a time-consuming manual method, it is not the method of choice if there is scarcity of manpower. In these conditions, STRATUS is the preferred method.


Recently, we reported some of the biochemical properties of fluorescent substances, Ex 380 nm/Em 440 nm and Ex 400 nm/Em 460 nm, found in the sera of chronic renal patients (Clin. Chem. 21: 1152, 1975). Additional separation of the 380/440 fluorescence achieved on Sephadex G-10 gel (Pharmacia, Piscataway, NJ) buffered with 0.05M triethylenetetramine (TEA) pH 8.0. The fluorescent compound eluted in the chromatographic fraction corresponding to a Mr = 600 Da. Purification of the compound was accomplished by high performance liquid chromatography (IBI Gradient Chromatograph) using fluorescence detection at Ex 370/Em 410-700. Gradient elution was obtained on a C18 reverse phase column (IBI, 4.5 x 250 mm) using a mobile phase of 0.025 M TEA in water and 0.025 TEA in acetonitrile, pH 7.75. The purified compound was isolated from patient sera (n = 10) and their respective hemofilters and eluted in a characteristic excitation/emission maxima of 386/444.

Fluorescence intensity could be enhanced by alkalization to pH 10.5. Lyophilized residues of the HPLC fraction subjected to amino acid analysis (Beckman - Spinco Amino Acid Analyzer - Model 119 CL) revealed that the fluorescent compound is bound to a neutral peptide containing predominately glycine, serine, aspartic acid, and glutamic acid in approximate molar ratios of 4:4:1:1.

Our studies indicate that the unique fluorescence is due to a specific peptide-bound fluorophore. We are presently trying to determine structure by GC-Mass Spectrometry. We hope to elucidate the mechanism of its presence in chronic renal failure.


Mauk et al. (Clin. Chem. 33, 902 (1987)) demonstrated the feasibility of measuring total protein from human cerebrospinal fluid specimens in a multilayered thin-film assay. We have successfully developed this procedure into a slide format for the Kodak Ektachem 700 analyzer, and have evaluated its performance with CSF and urine specimens.

The assay principle is based upon the preferential binding of cupric ions to peptide bonds over an azo dye. Formation of a bluer complex is accompanied by a decrease in the concentration of the copper-azo dye complex, with consequent reduction in absorbance. The quantitative reaction is measured by the decrease in absorbance at 670 nm when the copper is removed from an azo dye complex.

The slide design involves two coated layers on a film base: a spreading layer containing L-field and an azo dye. The spreading layer consists of white plastic beads, which are sufficiently porous to permit penetration of the protein molecules which provide a white background for the reflectance measurements at 670 nm.

Calibration values were established using a biuret reference method standardized by NBS SRM 972a. Evaluation using a wide variety of patient specimens confirmed the accuracy of the slide assay. For CSF, regression statistics were: Ex slide = 0.990 x Biuret + 2.2 mg/dL; Sy.x = 1.6 mg/dL; r = 0.999. For urine, regression statistics were: Ex slide = 0.979 x Biuret + 1.03 mg/dL; Sy.x = 1.65 mg/dL; r = 0.998. It was concluded that performance of the slide assay is acceptable for clinical use with CSF and urine specimens.

DETERMINATION OF TOTAL IRON BINDING CAPACITY (TIBC)

Currently marketed TIBC kits contain an iron saturating solution and an adsorbant. The saturating solution is mixed with serum to bind all available apotransferrin binding sites with iron. The adsorbant removes excess copper from the mixture. The serum mixture is then analysed for total iron and the result is multiplied by an appropriate dilution factor to compensate for dilution of the serum by the saturating solution. ABBOTT'S A-GENT® TIBC Kit utilizes a ferric chloride saturating solution and an alumina column as the adsorbant. The serum sample (300μl) is mixed by inversion with 600 μl of the saturating solution and incubated at ambient temperature for 15 minutes. The mixture is poured into the plastic column which snap fits on to the ABBOTT Multivolume Sample Cup allowing the serum mixture to pass through the column directly into the column sample for analysis with the A-GENT Iron Reagent and ABBOTT VP system. TIBC was performed on 53 normal serum samples using the A-GENT TIBC kit with A-GENT Iron Reagent on the ABBOTT VP system and the DuPont TIBC Pretreatment with the DuPont ACR®11. The correlation coefficient was 0.10; slope was 0.87; intercept was 72. Within run precision using the ABBOTT A-GENT TIBC kit for 16 replicates of two control sera were 1.1% and 4.4% coefficient of variation for means of 200 μg/dL and 238 μg/dL respectively. Biltrubin, hemoglobin, lipemia and copper interferences were similar to the A-GENT Iron Reagent interference.

STABILITY OF HUMAN OSTEOCALCIN IN SERUM, I-Man Chen, M.I. Spreling, and H.R. Mason (Yale University Department of Radiology, Lab., Univ. Cincinnati, Cincinnati, OH 45267) (Sponsor: I-Man Chen)

Osteocalcin is a protein synthesized by the osteoblast. The serum level of osteocalcin correlates closely with the actual status of bone metabolism and thus is believed to be a specific biochemical marker for bone diseases.

Osteocalcin in circulation has been determined by radioimmunoassays. Instability of serum osteocalcin during storage has been reported. We have used an osteocalcin radioimmunoassay kit manufactured by INCSTAR Corporation, Stillwater, MN (reference range 2.3-6.3 mg/ml) determined 24 healthy adults, in order to determine the stability of serum osteocalcin under various storage conditions.

Osteocalcin in human serum was found to be stable at -20°C for at least four weeks and its stability was not affected by up to 5 freeze-thaw cycles. However, the immunological activity of osteocalcin was rapidly inactivated when stored at 4°C. The inactivation was significantly faster (p<0.01) in female sera than in male sera as shown in the following table (mean ± SD of percent immunological activity remaining; the data for fresh sera were taken as 100)

<table>
<thead>
<tr>
<th>Days at 4°C</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n = 9)</td>
<td>87.0±9.5</td>
<td>70.4±17.1</td>
<td>37.9±17.2</td>
<td>18.5±3.6</td>
<td>7.5±1.4</td>
</tr>
<tr>
<td>Female (n = 9)</td>
<td>70.2±12.5</td>
<td>35.2±17.6</td>
<td>12.9±7.4</td>
<td>8.3±2.9</td>
<td></td>
</tr>
</tbody>
</table>
Our data indicate that serum samples for osteocalcin should be frozen at -20°C as soon as practical. The reason for the gender difference in the stability of osteocalcin at 4°C requires further investigation.

We have developed a kinetic turbidimetric immunoassay for measurement of serum anti streptolysin-O(ASO) in the Hitachi-7050 automatic analyzer (Hitachi, Ltd., Tokyo Japan).

Microvolumes of calibrator or patient's sera(0.02ml) and sample diluent (HEFS-buffer saline; per liter 8.76g of NaCl, 0.15mL of HEPS, 1g of sodium azide and 40g Polyoxyethylene) 0.3ml were pipetted into the cuvette.

After a 300-s incubation at 37°C, sample-blank absorbance was measured at 340nm, and 0.05mL of diluted antigen(Streptolysin-O/SL0) was added.

Within-run precision (CV) ranged from 1.0% to 8.0% (n=10) and day-to-day precision from 1.5% to 10.0% (n=10) over the concentration range 40 to 8000U/ml.

Added hemoglobin (up to 500mg/dl) or bilirubin (up to 20mg/dl) did not interfere.

Sera from 148 subjects were assayed by the prosed method (y) and Rantz-Randall method (x). ASO measurements ranged 0 to 1067 U/ml. The linear regression equation for the data was:

\[ y = -33.8 + 1.18x \ (r=0.857) \]

There was good correlation between the different methods.

The assay requires no sample pretreatment and is performed similarly to other TIA's. Moreover, its use on other disstrect analyzers or centrifugal analyzers could considerably shorten analysis time.


(Becton Dickinson, Towson, Maryland, 21204)

A non-isotopic enzyme immunoassay utilizing a solid phase polyclonal antibody and a monoclonal antibody conjugated to horseradish peroxidase has been developed for the Becton Dickinson AFFINITY(TM) System. This instrument is a benchtop, totally automated, device with automatic scheduling capabilities which allow for both STAT and routine in-vitro diagnostic testing. Assays are packaged in a bar-coded, self-contained unit, (DMUNIT(TM), patent 4608231), which requires only the addition of 150 ul serum.

Standards are human serum matrix and have been calibrated against the WHO reference ferritin. Curve range extends to 600 ng/ml. A double conjugate incubation sequence eliminates high dose hook effects in samples greater than 20,000 ng/ml. Sensitivity of the assay is less than 1 ng/ml as determined by precision of 2 replicates of the zero standard. No carryover is observed in low samples when preceded by samples containing greater than 20,000 ng/ml ferritin. A correlation coefficient of 0.99 was obtained against an established Ferritin RIA for 65 samples (range 4.3 to greater than 600 ng/ml). Total processing time is 32 minutes. Precision data is as follows:

\[
\begin{array}{cccc}
\text{POOL} & \text{MEAN} & \text{SCV} & \text{POOL} & \text{MEAN} & \text{SCV} \\
1 & 52.4 & 2.61 & 4 & 58.3 & 11.8 \\
2 & 164.8 & 2.22 & 5 & 203.4 & 3.03 \\
3 & 541.4 & 2.42 & 6 & 647.7 & 3.3 \\
\end{array}
\]

RAPID LATEX ENHANCED TURBIDIMETRIC IMMUNOASSAY FOR SERUM BETA-2-MICROGLOBULIN

C.P. Price, Modell L, Newman DJ and Gorman HC.


A method for measuring serum beta-2-microglobulin, as a sensitive indicator of tubular damage and as a tumor marker in malignant disorders has been developed. The assay which requires no pre-treatment of samples involves latex enhancement of a turbidimetric immunoassay reaction. Affinity-purified anti-human beta-2-microglobulin is coupled to 40nm latex particles with an active chiro-methyl-erythritol shell, which covalytically binds to the free amino groups of the antibody.

The assay was developed on the II. Multilist micro-centrifugal analyzer using a 3ul sample volume and 200ul of prepared particle/antibody reagent, and requires a reaction time of 8 min.

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The standard curve is linear between 1.25 and 200ng/ml with a sensitivity (defined as 3 SD above the baseline ) of 0.4 mg/ml.

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I type serum between and/or antisera. React with measure An level influenced by treatment, interference Du

The subclass non-parallelism assay with Nesnours Hosp. M. J. 10

and 0.9% ISA particles. Newman urines 16.8 normal ranges (5.3 28.3) is

Meaning of Kappa and Lambda (L) light chains, utilizing Beckman Specific Protein Analyzers. The initial measuring range for Kappa is 400-4400 mg/dl, with an extended range of 1.85-26,400 mg/dl. The initial measuring range for Lambda is 180-1600 mg/dl, with an extended range of 5.00-50,400 mg/dl. The results are expressed in immunoglobulin equivalents. Intrar and interassay CV's were <0.5%

Serum samples, obtained from healthy donors, were assayed using single point calibration method. Reproducibility well with those by a commercially available nephelometric assay. Correlation statistics obtained for Kappa, Lambda and the K/L ratio were as follows:

Kappa 0.933 0.285 0.983 58
Lambda 0.950 23.500 0.975 58
K/L Ratio 0.934 0.003 0.901 58

The mean values of 803 mg/dl for Kappa and 439 mg/dl for Lambda agree well with the established normal range of 559-1340 and 302-748 mg/dl respectively. Forty-nine serum samples, obtained from patients with previously diagnosed monoclonal gammapathies (by IEF), were quantitated for IgG, IgA, IgM, Kappa and Lambda; each sample was also analyzed by SPE. Approximately 90% of the samples were correctly by this evaluation. The remaining 10% required an IEF to definitively characterize the monoclonal protein. In summary, we feel that the K/L ratio in conjunction with SPE and IgG, IgA, IgM quantitation will be a useful tool in the characterization of monoclonal gammapathies.

IgG MYELOMA PARAPROTEIN SUBCLUSTER USING ISOELECTRIC FOCUSING (IEF) IMMUNOBLOT ANALYSIS, Frank J. Fusullo Jr., M.A. Fritsche Jr., F. Liu, and R.G. Hamilton. (R.D. Anderson Hospital; Univ. Texas Medical Sch., Houston, TX 77030) (Spon.: H. Fritsche).

The objective of this study was the development of a method to type the subclass of human IgG paraprotein. Current methods (e.g. serum protein electrophoresis combined with immunoprecipitation) are insufficiently sensitive and specific to subclass IgG paraproteins. We have combined the resolving power of IEF with the sensitivity of immunoblotting and specificity of IUIS/WHO documented human IgG subclass 1-4 specific monoclonal antibodies to identify the subclass and pi subtype of IgG paraprotein.

Serum proteins were isoelectrically focused in a Pharmacia Phast System (1 ul of serum diluted to total immunoglobulin = 200 mg/dl, 10 min, 950 amp-hr, phosphate buffer pH 7.4). Focused proteins were negatively stained with PFS-SBA (1 hr), bound IgG was detected with peroxidase-conjugated anti-human IgG-1,4 monoclonal antibody (2 hr) and developed with substrates (4-chloro-1-naphthol).

Accuracy of the IEF-immunoblot method (e.g. ability to correctly detect paraprotein of one subclass among a mixture of purified IgG myeloma proteins of known subclass. IEF immunoblot analysis of sera from 11 of 13 uncharacterized myeloma patients produced positive IgG paraproteins consistent with the primary subclass. Subclass frequency of these IgG paraproteins was 8/11 (73%).

The remaining 2 paraproteins with IgA and IgM paraproteins were positive. The negative controls produced negative banding patterns. Normalization of the working serum dilution based on total immunoglobulin level maximized differential background staining that arose from low levels of IgG-1,4 protein found in all sera. Ability of the IEF immunoblot to define the class (IgG vs. IgA/IgM) and subclass (IgG-1,4) of paraprotein from myeloma patients has been demonstrated. This method can be used to follow in vivo changes in myeloma as reflected in serum IgG paraprotein variation (pi, subclass) that may result from immuno/chemotherapy.


We have developed a fluorometric two-site enzyme immunoassay for ferritin in serum and plasma using chromium dioxide magnetic particles as the solid phase. The assay is performed by incubating sample (10 ml) with chromium dioxide-antibody reagent (85 ml) and antibody-alkaline phosphatase conjugate (100 ml) for 30 minutes at 37 °C. After incubation, the suspension is washed three times by alternating magnet separation and resuspension. The antibody bound to the magnetic particles is measured using the substrate 4-methylumbelliferyl phosphate in diethanolamine buffer with incubation at 37 °C for 5 minutes. The supernatant is measured fluorometrically.

The assay exhibits a range of 0 to 1000 ng/ml with a sensitivity of 2 ng/ml, as determined by a non-paired Student's t-test (98% confidence). Within-run and run-to-run coefficients of variation ranged from 3 to 10% across the assay range. A 33 sample correlation study
The BL test emerges as a highly sensitive sero-test for the screening of elevated CK-MB activity, the nature of which should be subsequently clarified by electrophoresis. The ELISA technique is less precise but entirely MB-specific.

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Creatine kinase MB (CK-MB) was measured in serum samples using an immunoc hemiluminescent assay employing an acridinium-ester (AE) - labeled antibody (Magic Lite System, Ciba Corning Diagnostics Corp., MA.). Samples were incubated simultaneously with the AE labeled mouse monoclonal anti-human CK-MB antibody and mouse monoclonal anti-human CK-BB antibody covalently coupled to paramagnetic particles. Separation of the bound from the unbound AE - labeled antibody was by magnetic separation and detection of the bound fraction by chemiluminescence on addition of HRP/HRP and NaOH. The Ciba Corning assay was standardized against purified CK-MB. The sensitivity of the assay was 0.7 ng/mL and it was judged to be linear over the range 4.0 to 400 ng/mL. For intra-batch precision, 2 CVs were 4.3%, 5.5%, 6.8% and 6.4% for CK-MB values of 7.3, 29.4, 82.4 and 212 ng/mL respectively. Inter-batch precision (n=26), gave 2 CVs of 17.8%, 15.2%, 16.5% and 15.2% for the same CK-MB control levels. Mean Recovery was 108%. Triglyceride (10 g/L), bilirubin (300 mg/L) and hemoglobin (5 g/L) had no significant effect on the assay. Further, CK-MM (4500 ng/mL) and CK-BB (2000 ng/mL) had no adverse effect and interference was not observed from sera containing atypical CK variants.

Correlation with an immunoradiometric assay using 97 samples gave a correlation coeff. (r) = 0.965. In the Ciba Corning assay, CK-MB from 108 blood bank donors gave a mean of 1.3 ng/mL, range 0.1 to 7.0 ng/mL. CK-MB from 26 hospitalized patients with non-cardiac related disorders gave a mean of 31 ng/mL and CK-MM results from 33 patients with confirmed myocardial infarction gave a mean of 78 ng/mL; range 22 to 482 ng/mL at the peak of enzyme release. The Ciba Corning assay is rapid and easy to use and correlated well with an immunoradiometric assay for CK-MB although the interbatch precision was of some concern.

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Creatine kinase (CK-MM) isozyme in cardiac or skeletal muscle, which is originally MM, sub-type (isoform), is sequentially converted to MM, and then to the cardiac enzyme B (CKB). The ratio of CK-MM sub-types (MM/MM) was reported to be a good index for early diagnosis of myocardial infarction (MI).

We produced five monoclonal antibodies inhibiting porcine CK-MM subunit activity, one of which (CK-MM1) was found to inhibit more than 99% CK-MM purified from not only porcine but human muscle. CK-MM1, however, inhibited only approx. 50% CK-MM in normal serum. The chromatofocusing analysis of serum CK-MM revealed that CK-MM1 gave 100, 57, and less than 5% inhibition to CK-MM1, MM, and MM, respectively. In addition, the inhibition of human muscle CK-MM by CK-MM1 decreased according to the incubation with fresh human plasma, and consequently reaching 0% inhibition, this decrease was prevented by the addition of EDTA (inhibitor of CPB) or the heat inactivation (56°C, 1 h) of plasma. Moreover, the incubation of CK-MM with pancreas CPB showed the substantial loss of enzyme activity (40-60%). In contrast, the neutralization by the enzyme activity was not influenced by CPB to an extent of 99%.

In summary, the monoclonal antibody, CK-MM1, inhibited CK-MM, but not MM, sub-type, showing that the measurement of the inhibition by CK-MM1 will be useful for early diagnosis of MI.
In a series of patients diagnosed for acute myocardial infarction (AMI), this study was also designed to measure the degree of heterogeneity of CPK-MB activity and amount within a given AMI patient and also amongst the various lab assay methods currently available for measurement.

Twenty timed specimens from each of 26 AMI patients were analyzed and plotted for CPK-MB with three different methods of electrophoresis (EPR), immunoinhibition (IIA), and enzyme immunoassay (EIA). The activity (i.e. - U/L) assay for calculation of specific CPK-MB activity because 1) absolute CPK-MB activity by EPR was greater than that quantitated by IIA (+0.17% (0.32% of total)) IIA assay showed occasional transient CPK-BB "spikes" on the plotted enzyme curves. Two similar EIA methods, EIA-1 and EIA-2 using anti-M and anti-B as the first monoclonal antibody in the sandwich assay respectively, were used for the mass amount (i.e. - u/L) assay in specific activity calculation.

Specific CPK-MB activity showed little variability in the EIA-2 method group (mean = 1.19 u/L; st. dev. = 0.147, 1.75 u/L). In the EIA-1 method group four enzyme series showed discordantly elevated specific activity values (with matched EIA-2 values) of 11.16 (1.47), 10.16 (1.19), 8.01 (1.09) and 6.92 (1.33) u/L.

It is concluded that specific CPK-MB activity showed relatively little fluctuation amongst AMI patients and that the antibody formulation used in determining CPK-MB by EIA is extremely important in assuring acceptable assay accuracy.

PREPARATION AND EVALUATION OF AN ENZYME ACTIVITY INHIBITING MONOCLONAL ANTIBODY TO CK-MM H. Shenkin, W. Nikulka, S. Ho and F. Lucas (Coulter Diagnostics Div., Hialeah, FL 33014) (Spon.: J. Carter)

Antibodies that inhibit enzyme activity have long been prepared as polyclonal antibodies and usually occur during the latter phases of an animal's response when the affinity of the antibody has been maximized for its immunogen. Preparation of a CPK-MB antibody is dependent on finding a host animal whose native CK is dissimilar from the immunogen (human CK-MM) to provide a response. A series of rabbits were inoculated with inactive CK and given 100 ug booster shots until a titer was detected from retro-orbital blood, four days before fusion, a final series of IP boosts were given. Mouse spleen cells were fused with BALB/c A2565 myeloma using a PEG procedure. Colonies of CK-MM specific hybridomas were observed. Positive clones were re-assayed for enzymatic inhibition and these clones were soft agar recloned to remove non-producers. Assays were generated from the stable cell line in F1 hybrids (AJ x BALB/c).

The assays were tested from the eight best clones, with clone 9A12E3 demonstrating a 97% inhibition of 600 U/L CK-MM in 3 minutes at a 0.05 mg/ml Ig concentration. At the end of 30 minutes (equilibrium), 97.6% inhibition was determined. Residual activities endogenous CK activity and adenosine kinase activity was previously destroyed using 30 minute, 37°C incubations. Cross reactivity to non-CK was 52% detected. Evaluations with patient samples on DAGOIS Chemistry Analyzer shows a correlation to Corning Electrophoresis of n=166, r=0.92 and p=0.02x + 5.14/L when determining CK-MM values by inhibition of CK-MM.

In conclusion, a monoclonal antibody with the feature of enzymatic inhibition was shown to accurately predict CK-MM concentration in patient samples by inhibiting the CK-MM fraction.

EVALUATION OF DECISION LEVELS FOR SERUM LACTATE DEHYDROGENASE (LDH) IN PATIENTS WITH MYOCARDIAL INFARCTION Jane F Loughlin, Pamela M. Krijnen, Thomas G Pellar, Fred Y Leung and A Ralph Henderson (Dept Clin Biochem, Univ Hosp, London, Canada W1A 5T5) (Spon: A. Ralph Henderson)

We compared the diagnostic value of the ratios LD/1/LD-2, LD/1/LD-3, LD/1/LD-4 and LD/1/LD-5 with a documented case of myocardial infarction (MI) with 149 control (non-MI) patients. The LD isoenzymes were determined using thin-layer agarose electrophoresis and fluorescent quantification (Methods Enzym Anal 1983; 3:138-55).

The results were assessed at 6 intervals up to 108 hours after the onset of chest pain by using a computer program written in FORTRAN (Tandy, Digital Radio Shack personal computer) to calculate the receiver operating characteristics (ROC) and likelihood ratios (LR) which were used to obtain the optimal decision threshold values.

In MI with LD-1 less than 40% of the total LD activity (n=29), all ratios peaked around 36 hours after the onset of chest pain, but the greatest change was noted with LD-1/LD-5 at 1.72-fold elevation over the control (compared to 1.16-fold for LD-1/LD-2). In the group with LD-1 activity greater than 40% of total (n=40), the changes were higher at 3.44-fold for LD-1/LD-5 and 1.5-fold for LD-1/LD-2.

Based upon the ROC and LR analyses, optimum thresholds for the various ratios were obtained at 55-60 h for LD-1/LD-2, 31-36 h for LD-1/LD-3 and LD-1/LD-4, and 15-22 h for LD-1/LD-5. We conclude that, when applied to the diagnosis of MI, these LD-1 ratios can vary in diagnostic significance and reliability depending upon the time the ratio is applied following chest pain, and the selected decision threshold value. For example, the greatest increase was obtained with LD-1/LD-5 between 15-22 h at a decision level of 2.00 (for LD-1 values below 40%) and of 3.00 (for LD-1 values above 40%).

PHYSICAL AND IMMUNOCHEMICAL PROPERTIES OF A CK-BB IGA ISOENZYME, Michael A. Pence, and Selma H. Rodourist (Columbia Presbyterian Med. Ctr. NY 10032) (Spon: Michael A. Pence)

CK isoenzymes were determined in a patient with a long history of cardiovascular disease. Electrophoresis using the Bethesda system rose a single band which was determined to be CK-MM activity by EIA (1.33). The antibody showed activity with all three isoenzymes (MI, BB and MB) depending on the incubation time. The antibody was raised in rabbits and was monitored for 5 days. During this time, the total CK activity was either normal or slightly elevated, and the activity of the CK isoenzyme activity ranged between 27 and 42% of the total CK activity. No CK-MB activity was detected using the Hybridtech Enzyme Immunoassay System.

Immunoinhibition studies using anti CK-B and anti CK-MM antibodies showed that only CK-B subunits are present in this atypical CK isoenzyme. Immunoelectrophoresis of serum proteins followed by the addition of the immunogen (human CK-MM) to provide a response. A series of rabbits were inoculated with inactive CK and given 100 ug booster shots until a titer was detected from retro-orbital blood, four days before fusion, a final series of IP boosts were given. Mouse spleen cells were fused with BALB/c A2565 myeloma using a PEG procedure. Colonies of CK-MM specific hybridomas were observed. Positive clones were re-assayed for enzymatic inhibition and these clones were soft agar recloned to remove non-producers. Assays were generated from the stable cell line in F1 hybrids (AJ x BALB/c).

We have described the physicochemical and immunochemical properties of a CK-BB IGA isoenzyme. Although the CK-BB IGA isoenzyme migrated in the CK-MB position, the consistently elevated levels should alert the laboratory to the possibility that an atypical CK isoenzyme may be present.


An assay for creatine kinase MB isoenzyme in serum and plasma has been developed in which a monoclonal antibody specific for the CKMB isoenzyme is covalently attached to chromium dioxide magnetic particles. The assay is performed by incubating sample (25 µl) with the chromium dioxide particle reagent (25 µl) for 30 minutes at 37°C. After incubation, the suspension is washed three times by alternating magnetic separation and fluid aspiration with reususpension. The activity of the "captured" CKMB isoenzyme is measured fluorometrically using a modification of the enzymatic creatine kinase determination of Oliver and Roselli.

The assay exhibits a linear range of 0 to 250 IU/L of CKMB, with a sensitivity of 4 IU/L as determined by a non-nared student's t-test (95% confidence). Within-run and run-to-run coefficients of variation ranged from 1 to 3% for the range up to 20% across the entire range. A 42 day long-term stability study versus the Roche Immuno-Ck™ method exhibited excellent correlation: Y = 1.05X - 3.2. The assay shows no cross-reactivity to CKMB, CKBB, MACRO CK TYPE I, MACRO CK Type II or isoimmune from hemolytic, lipemia or icterus. Recoveries averaged 96% and parallelism was excellent when monkey heart CKMB was spiked into random human sera.

Use of this unique monoclonal antibody and magnetic particle technology allows precise and sensitive measurement of the CKMB isoenzyme.
626 A COMPLETE, RAPID, MYOCARDIAL INFARCTION PROFILE: COBAS-BIO ADAPTATION OF ABBOTT LD-1 METHOD. Walter Mastropolo & Bruce Newton (Floyd Memorial Hospital, New Albany, IN 47150 (Spon. W. Mastropolo))

We have designed a rapid, quantitative, biochemical profile with 24 hr. availability for the diagnosis of myocardial infarction (MI). In addition the profile is efficient and economical because of its utilization of automated analysis. By performing total CK, CK-MB and total LD assays on the Ektachem 747, and the LD-1 assay on the COBAS-BIO, we can report a complete profile within 15 min. of receipt of a serum specimen. Since the Ektachem CK-MB assay was non-specific, we devised to confirm certain specimens with an elevated CK-MB by the Roche Isomune method. This more lengthy procedure was required for only 8.5% of patient specimens.

The Abbott LD-1 reagent, which uses sodium chlozotate to inhibit all LD isoenzymes except LD1, was adapted to the COBAS-BIO analyzer and evaluated. Excellent correlation was obtained for patient specimens with the Roche Isomune LD-1 method (r=0.94, r=1.00, n=91). The Abbott method had a between run precision (C.V.) of 4.4% (mean=191). A major analytical advantage of the Abbott over the Roche LD-1 method is the ability of the former to completely inhibit high levels of LD-1 and LD-5 (at least 8000/L and 30000/L respectively). The Abbott method is also completely automated and thus, is much less labor intensive than the Isomune LD-1.

We conclude that these procedures provide rapid and accurate data for MI diagnosis.


We have developed a simple, rapid immunofinity procedure for the purification of creatine kinase-MB. Immunofinity gel is prepared by linking a CK-MB specific monoclonal antibody (*Conan-MB*) to cyanogen bromide activated-Sepharose 4B. Heart tissue is homogenized and ammonium sulfate (40-70%) fractionated before application to the immunofinity gel. The CK-MB activity retained on the gel is eluted with 0.1 M diethylamine, pH 10.5, containing 10 mmol B-mercaptoethanol per liter, and collected directly into glycerol and pH neutralizing buffer. The glycerol is essential to prevent dissociation of the subunits. We have used the immunofinity method to purify CK-MB from human, dog and rabbit hearts with specific activities of 527, 479 and 368 U/mg, respectively, and yields of ~50%. The preparations are pure as judged by silver nitrate protein staining after SDS-polyacrylamid gel electrophoresis (PAGE). Human and dog CK-MB are immunologically identical as recognized by *Conan-MB* in a competitive RIA but rabbit CK-MB competes with 10-fold lower efficiency. The immunofinity procedure was compared with a multi-column method in which CK-MB was purified by ion-exchange and Affi-gel® Blue affinity chromatography. Specific activities and yields were similar by the two methods, but the immunofinity purification could be completed in two days whereas the multi-column procedure required at least one week. Also CK-MB purified by the multi-column method showed significant contamination by other proteins, especially albumin, when examined by SDS-PAGE. The immunofinity method proved useful for producing CK-MB for use in research or for use as standards or quality control materials in clinical assays. (Partly supported by NIH training grant 2T32 ES07066-09.)


We have developed a rapid, one-step immunoelectroscopy assay utilizing an H-subunit specific, monoclonal antibody (8.8.3) in which serum lactate dehydrogenase-1 (LD-1) activity is measured after extraction of LD-2, LD-3, LD-4, and LD-5 isoenzymes.

In the assay, 100 µL of serum is mixed with 50 µL of a suspension of 0.8 µm latex particles coated with 30 µg of 8.8.3 and incubated at room temperature for 5 min. The latex particles to which LD-1 through LD-5 are bound are pelleted by centrifugation for 2 min at 12,000 rpm. The supernatant is measured kinetically in the supernatant. Alternatively, the LD-1 mass can be measured in the supernatant using H subunit or H and M subunit-specific monoclonal antibodies in an assay for antibody concentration, and for time and temperature of incubation. Icteric samples (serum bilirubin up to 330 µmol/L) did not interfere with the assay. The LD-1 activities were interfered solely due to LD-2 released by erythrocytes. The within-assay CV for low QC material (total LD, 50 U/L; LD-2, 33 U/L) was 3.5% (n = 9) and for high QC material (total LD, 377 U/L; LD-1, 185 U/L) was 1.9% (n = 8). The between assay CV for the two QCs were 6.1% (n = 9) and 2.5% (n = 10), respectively. The LD-1 activity was measured in 99 samples by our assay compared well with the Roche's two-step polyonal antibody-based isomune-ld® assay (r = 0.994, Y = 1.04 X - 0.88) and with the Beckman's electrophoresis method (r = 0.953, Y = 1.12 X - 0.34). (Supported in part by NIH training grant 2T32 ES07066-09.)

629 THE NATIONAL REFERENCE SYSTEM FOR CREATINE KINASE (NRS/CK): THE USE OF THE IFCC REFERENCE METHOD FOR THE UNIFICATION OF THE CK AND CK-MB RESULTS ON THREE ANALYTICAL INSTRUMENTS USED IN DAILY SERVICE. A. Valcouur, R. B. McComb, and N. H. Slowers Jr. (Clin. Chem., DuPont Hospital (h), and Hartford Hospital Laboratory (HML), Hartford, CT 06115). Creatine kinase (ATP: creatine N-phosphotransferase, EC 2.7.3.2) is one of the most critical measurements in clinical medicine. Fortunately, wide scientific agreement exists on CK standardization and a National Reference System for creatine kinase (NRS/CK) based on the revised (August, 1987) IFCC Reference Method for CK (IFCC/RMK/C) has been proposed. The recommendations of the NRS/CK have been followed to unify all CK and CK-MB results produced by three different analytical instrument systems. First, the IFCC/RMK was meticulously reproduced using a CARY model 215. Second, the U/Lppg results on patient samples (n = 90) were tested to validate a Roche Cobas-Bio analyzer using the exact IFCC/RMK/CK final reaction conditions at 37°C. Third, these Cobas-Bio results in U/Lppg were then used to calibrate three daily reference instruments for CK that included three Cobas-Bio's (30°C) and three Kodak E-700's (37°C) at H and a Technicon RA-1000 (37°C) at HML. Comparisons between daily working systems versus the IFCC/RMK at 37°C with patient sera (n = 100) that cover the clinically relevant range gave correlation coefficients of 0.99 and slopes of 1.00 ± 0.06.

CK-MB was also standardized to the NRS/CK in an analogous manner with the minor changes in reagent conditions and sample size from the IFCC/RMK required to include the inhibiting M antibody (Clin. Chem. 1982;28:1907). Comparisons of Cobas-MB results between service analyzers and our internal CK-MB reference method were performed using serum from patients with proven myocardial infarction and heart disease. This pragmatic approach unifies the service results and links them directly in U/Lppg to the IFCC/RMK and the NRS/CK.


We calculated receiver operator characteristic curves for CK-MB analysis by the Dupont AKAH procedure and by electro-photophoresis. This study included 68 patients with a diagnosis as possible myocardial infarction (MI). Samples for CK isoenzyme analysis were drawn at 3 different times on each patient. The total CK activity was measured at 37°C in the Hitachi 705 with BDM reagents which use a modification of the Rosalki procedure with N-acetyl cysteine as the reducing factor. The CK-MB isoenzyme then was determined by the Du Pont AKAH method. A combination of ion-exchange chromatography and immunoinhibition and also by a modified Beckman Parapath l.L. agarose gel electrophoreses at pH 7.0 with a Beckman gel electrophoresis system for quantitation.

The following table lists the results of the performance evaluation at single cutoffs and using a combination of absolute activity and % of total activity.

<table>
<thead>
<tr>
<th>Electrolysis</th>
<th>AKAH Electrolysis</th>
<th>AKAH Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.200</td>
<td>&gt;2.000</td>
<td>&gt;0.200</td>
</tr>
<tr>
<td>&gt;0.200</td>
<td>&gt;2.000</td>
<td>&gt;0.200</td>
</tr>
</tbody>
</table>

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988 1281
AN EFFICIENT TESTING STRATEGY USING THE EKTACHEM CK MB ASSAY. C. Grenier, C. Leendecker-Foster, M. Basara, K. Willard (Laboratory Service VA Medical Center, Univ. of Minn., Mpls, MN) (Spon: Catherine Leendecker Foster)

We investigated the accuracy and efficiency of an alternative CK MB testing strategy in the workup of suspected myocardial infarction. In this teaching hospital environment our standard double antibody inhibition-precipitation Roche CK MB assay is unavailable at night and on weekends. An alternative strategy was tested of offering the Ektachem assay on a 24 hour basis on all CK MB requests with all elevated samples retested by the Roche method (the Ektachem numbers scaled to reproduce the Roche range).

To evaluate this strategy we analyzed all CK MB requests from our CCU ward in parallel with our standard Roche double antibody assay including over 250 patients and over 500 samples.

This study revealed a false positive rate of about 7% and false negative rate of about 1.5% of the Ektachem assays as referenced by the Roche methodology. The typical "false negative" result was a Roche result of 7 IU and an Ektachem result of 5 IU. The typical "false positive" was a Roche result of 4 IU and Ektachem result of 7 IU. Since an elevated result would be analyzed by our Roche method anyway this false positive level resulted in no impact on our CCU patient management. The "false negative" samples resulted in every case from a Roche result that was just over the high end of the normal range, with the Ektachem result just below the high end cutoff.

Since cardiac enzymes are ordered in a protocol of a minimum of 1 q 12 hours X 3, these "discrepancies" in CK MB values are effectively further reduced. A review by the clinical attending of the "false negatives" in the context of their other serial values found no difference in classification of the patient's status resulting from the small discrepancy between the Ektachem and Roche numbers.

We conclude that our proposed testing strategy utilizing the Ektachem CK MB is efficient and conservative in terms of classification accuracy while supporting a rapid "rule out" capability for clinical management of suspected myocardial infarction.


The Kodak Ektachem CK-MB slide employs an antibody to human CK-MB to inhibit CK-MB activity. Remaining CK activity is then measured by a conventional CK enzymatic rate reaction, and results are reported as CK-MB activity, U/L. The assay is not CK-MB specific, however, as other CK species such as CK-BB, mitochondrial CK, "macro CK", and other variants may also show activity.

We studied 82 consecutive patients admitted to the coronary care unit of our hospital. Criteria employed to indicate a probable myocardial origin for CK-MB included: total CK>2.5 U/L and CK-MB >0.1 U/L and CK-MB %>4 but <50. We also examined LD isoenzymes, EKG reports, clinical diagnostic impressions, and hospital course and follow-up to estimate the efficacy of CK-MB slides in the setting of suspected AMI. Equivocal CK-MB studies were verified by isoenzyme electrophoresis.

A total of 28 patients had unequivocal CK-MB enzyme changes and clinical course consistent with AMI. Sixty-three patients were negative for AMI by CK-MB and other evaluations (normal criteria). Seven patients had positive or equivocal CK-MB results with no confirmatory laboratory or clinical evidence of AMI.

Two of these patients had variant CK species. The remaining causes of CK-MB elevation were: borderline false positives (2), trunca (2), and reversible myocardial ischemia (1).

Conclusions:
1. AMI is unlikely when Ektachem CK-MB studies are negative.
2. Twenty percent of positive CK-MB studies in this study were due to other causes other than AMI.
3. Equivocal CK-MB results should be verified by an alternate procedure, such as electrophoresis, to detect reacting CK species other than CK-MB.

ACTIVATED ASPARTATE AMINOTRANSFERASE REAGENT ON THE PARAMAX ANALYZER. M. Smith, M. Birla, T. Jaffron (Paramax Systems Division, Miami, FL) (Spon: M. Smith)

We are currently evaluating a modified aspartate aminotransferase (AST) test reagent for the Paramax analyzer. This method features the addition of pyridoxal phosphate (PLP) as recommended by the IFCC for the determination of aspartate aminotransferase (AST). The inclusion of PLP enhances the method's sensitivity for the determination of AST activity; its absence causes a significant negative bias in results especially for those patients with hepatic, renal and cardiac disorders.

PLP at 0.12 mmol/L is incorporated into a 30 mg tablet that contains all the necessary ingredients for the reaction. At this concentration AST is fully activated at the end of the lag phase. The reaction requires 20 ul of sample in a reaction volume of 300 ul; the test is run at 37°C and monitored bichromatically at 340/405 nm for 10 minutes.

L-Aspartate + L-Ketoglutarate + GTP → L-Glutamate + Oxalacetate + PLP

AST TESTING ON THE EKTACHEM.


A rapid method was developed for obtaining serum LD isoenzyme profiles based on the now concomitantly on the Abbott SPECTROMAX II multianalyzer, and a supporting mathematical model.

Two assays are used to obtain 'M' subunit and total LD activities; 'M' subunit act./total LD act. = 'M' subunit (2 predictors in the model). Assay #1 contains 0.1 M L-a-hydroxybutyrate as substrate; efficiencies are 74% for 'M' and 100% for 'N' subunits. Assay #2 contains 0.4 M L-Lactate; efficiencies are 47% for 'M' and 91% for 'N' subunits. By simultaneous solution, total 'M' act. = 1.267 (#1) + 0.005 (#2), and total LD act. = 0.753 (#1) + 1.942 (#2). The third assay determines LD1 act. by chotrophic removal of all other isoenzymes (CIL Chem 33, 991). LD1 act./total LD act. = % LD1 (Y predictor in the model). All assays contain 7 mM NAD and 150 U/L Glutathione buffer pH 9.0.

The model describing LD isoenzyme variability was generated using 474 electrophorograms (Beckman Paragon-LD). The total % LD1 (X) = 0.25 % (1.0 LD1 + 0.3 (1.0 LD2 + 0.75 % (1.0 LD4) + 0.15 % LD5), and % LD1 (Y) in each sample were employed as independent variables to predict the % of LD2, LD3, LD4 and LD5 (dependent variables D), utilizing the equation:

\[ D = A_1 + A_2 + A_3 + A_4 + A_5 + A_6 + A_7 + A_8 + A_9 \]

The coefficients (A, a thru A9) for each isoenzyme were determined by the method of least squares. The resulting equations accurately described the data; standard deviations (S, errors for the calculated D values were 2.0, 2.7, 1.4 and 1.5% for LD2 (data range: 11-5% of total LD), LD3 (4-35%), LD4 (1-24%) and LD5 (0-4% respectively). Regression analysis of the pooled predicted vs. real values yielded: predicted = 0.98 (real) + 0.34%, R² = 0.98, N = 1896.

CHANGES IN TOTAL CK AND THE CK-MB ISOENZYME ASSOCIATED WITH LITHIUM THERAPY. S.C. Kazmierczak and Frederick Van Lente (Cleveland CNHT Foundation, Dept. Biochemistry, Cleveland, OH 44106) (Spon: S.C. Kazmierczak, Ph.D.)

Therapeutic and toxic concentrations of lithium are associated with electrocardiographic changes and yearly cardiograms have been strongly recommended for patients on lithium therapy. We evaluated the possible toxic effects of...
among patients with CK-MB activity found in the three groups are summarized below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Toxic Activity</th>
<th>Therapeutic Activity</th>
<th>Subtherapeutic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (U/L)</td>
<td>122 ± 108**</td>
<td>79 ± 46**</td>
<td>52 ± 33</td>
</tr>
<tr>
<td>CK-MB (U/L)</td>
<td>7.8 ± 11.6*</td>
<td>1.8 ± 2.0**</td>
<td>0.5 ± 0.7</td>
</tr>
</tbody>
</table>

Statistical differences are indicated between the subtherapeutic and the other groups.

** p < 0.05

*p < 0.005

Segregating patients according to their duration of lithium therapy revealed no significant difference in total CK and CK-MB activity between those on therapy for more than one year and those for less than one year. We conclude that toxic and even therapeutic concentrations of lithium can result in increased CK and CK-MB activity in serum.


Myocardial ischemia accounted for 9.5% of B&b’s in a 1985 acute myocardial ischemia. Serum enzyme activities were stratified for complex risk and service intensity using serum enzymes.

The Abbott LD-1 method uses the resistance of LD-1 to the chelating action of NaGlu (Clin Chem 33, 991). Using the SPECTRUM®, the chelating LD-1 (chLD-1) within and between run c/V’s was 1.6 to 2.8X and 2.3 to 3.5%. The chLD-1 assay was expanded and 240 (28/32) of 755 samples that were within 10% of their duplicate value or control mean. The chLD-1 results were compared to immunoprecipitation by Roche Insim:LD-1 and electrophoresis by Beckman Peridyn. Recovery of a LR plate with the LD-1 method showed chLD-1=1.02 Roche + 0.1 IU/L; r=0.98; and chLD-1=15.15 electrophoresis + 4 IU/L; r=0.989. Consecutive CCC admissions (n=23) were studied for serum activity. Results were within 10% of their duplicate value or control mean. The chLD-1 results were compared to immunoprecipitation by Roche LDImu:LD-1 and electrophoresis by Beckman Peridyn. Recovery of a LR plate with the LD-1 method showed chLD-1=1.02 Roche + 0.1 IU/L; r=0.98; and chLD-1=15.15 electrophoresis + 4 IU/L; r=0.989. Consecutive CCC admissions (n=23) were studied for serum activity. Results were within 10% of their duplicate value or control mean.

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639 LACTATE DEHYDROGENASE ASSAY FOR THE ABOOTT VISION® SYSTEM. D. Rotimi, J. Kaminiski and S. T. Won (Abbott Laboratories, Abbott Park, IL 60066) (Spon: D. Rotimi)

A liquid stable reagent for the determination of Lactate Dehydrogenase (LDH) activity in serum and plasma has been developed for the Abbott VISION® System. The product is stable for at least 8 months at 2-8°C in the VISION® two component test pack. The LDH in the sample catalyzes the oxidation of lactate to pyruvate with the concomitant reduction of nicotinamide adenine dinucleotide (NADH) as substrate. LDH activity is determined bichromatically at 340/450 nm and is proportional to the LDH activity.

The VISION® LDH assay has a dynamic range of 15 to 1000 IU/L of activity. Between-day coefficients of variation were 4.6 and 3.2% at LDH concentrations of 125.3 and 331.4 IU/L, respectively. No significant interferences were seen for commonly prescribed drugs, 1-epinephrine and ionic samples and heparinized samples. Hemoglobin (up to 15 mg/dL) does not interfere. LDH activity comparison of the VISION® assay with the Abbott VUF and the Dupont ACA yielded the following results:

<table>
<thead>
<tr>
<th>Method</th>
<th>VISION vs. VUF</th>
<th>VISION vs. ACA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>Slope</td>
<td>0.94</td>
<td>0.92</td>
</tr>
<tr>
<td>Intercept</td>
<td>10.071</td>
<td>6.224</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.979</td>
<td>0.974</td>
</tr>
</tbody>
</table>

We conclude that the VISION® assay is an accurate and precise method for the determination of LDH.

640 EVALUATION OF BECTON DICKINSON (BD) CK MB IMMUNOASSAY: COMPARISON WITH MAGIC LITE CK MB AND ELECTROPHORESIS. Lynne Preese, Roberta Bennett, Anita Fredrickson, Fred Apple, Clinical Labs, Hemmings County Medical Center, Minneapolis, MN 55415. (Spon: Lynne Preese)

We have performed a preclinical trial of the BD semi-automated colloidal immunosassay. We compared the BD assay with a chemiluminescent assay that uses both monoclonal anti-CK-MB and
anti-CK-B antibodies (CIBA-Corning, Magiclite) and agarose electrophoresis (ELP) in acute myocardial infarction (AMI), borderline AMI and transient ischemic attack.

For the EDP assay, the within-assay precisions (n=14) were 10.1 U/L (SD 0.80), while the between-assay precisions (n=10) were 14.5 U/L (SD 1.65). The between-assay precisions for quality control material were: 59.2 (1.3) 2.25; 30.0 (1.1) 3.85; 14.5 (0.6) 4.2%. The normal range for men and women (n=50) was 0 to 10 U/L. The standard curve was linear to 100 U/L CK MB. The recovery of CK MB at 11 U/L and 33 U/L was 102% and 114%. No significant crossreactivity was detected in CK MB samples (n=24) containing CK BB, CK AE, or CK aldolase isoenzyme. In 63 samples from AMI patients, CK MB activities obtained by the BD assay correlated well with the Magiclile assay, which measures the ratio of CK-MB/[total CK], and electrophoresis: BD = 0.423 + 2.9; r = 0.97; BD = 0.467 Magiclile + 2.0, r = 0.96. The CK MB range by BD was 2.1 to 104.8 U/L. CK MB was measured by the BD and Magiclile assays in 14 slides representing 14 AMI patients with normal total CK (100 U/L). The normal range was 10 U/L. A good correlation was observed between assays: BD = 0.525 ELP + 0.4, r = 0.89. The CK MB range by BD was 0.3 to 6.8 U/L. In 18 trauma patients with total CK ranging from 204 to 4367 U/L, there was a good correlation between assays: BD = 0.381 ELP + 12.3, r = 0.92. CK MB activities for the BD assay ranged from 1.0 to 27.1 U/L. In conclusion, we found the BD assay to be simple, precise, and accurate for the determination of CK MB activity. (Supported in part by Becton Dickinson).


The Rep* (Helena Labs, Beaumont TX) is a totally automated electrophoresis system designed for high-throughput and rapid analysis. The on-board computer allows sample analysis on agarose gels, electrophoresis at various programmable voltages and currents, and precision densitometer, and precision densitometer. We analytically and clinically evaluated the Rep for creatine kinase (CK) and lactate dehydrogenase (LD) isoenzymes using the "boxed" format.

**Analytical evaluations:** The within-run precision and day-to-day precision for CK-MB were both 10% (n=10). For the LD isoforms, the precision was both 8% (LD1 = 3 SD%). The linearity of the assays was verified to 1000 U/L of total activity. The sensitivity for CK-MB was determined to be 4 U/L over a period of 1 h. The results of the correlation of LD1 to LD2 was excellent (LD1: y = 0.249x + 4.8, r = 0.929, n = 94); LD2: y = 0.94x + 6.8, r = 0.645, n = 110).

**Clinical evaluations:** A total of 34 samples were obtained from myocardial infarction (MI), and 44 samples were obtained from non-MI patients. Using the Rep's cutoffs for MB and LD, it was possible to detect MB and LD isoenzymes with good sensitivity and specificity.

**Conclusions:** The use of the Rep for CK-MB and LD isoenzymes is simple, fast, and accurate.


This study reports the analytical performance characteristics and diagnostic efficiency of the Kodak Ektachrome immunohistology slide method for the MB isoenzyme of creatine kinase in serum samples from 261 consecutive patients admitted to the coronary care unit to rule out myocardial infarction (MI). The intra-assay, inter-assay, and day to day CVs at different activity levels ranged from 1.7 to 3.1%, 2.6 to 4.1% and 2.6 to 5.1%, respectively. The reference interval of normal serum was determined from data on 194 healthy blood donors. Regression analysis of the Ektachrome MB slide in samples from patients with myocardial infarction and ischemia (without cellulosate acetate) yielded the following equation, \( y = 0.55x + 16.45 \) (r = 0.87, n = 162).

Sixty of the 261 patients were eventually diagnosed to have suffered an MI, independent of the serum MB result. At the institutional cut off rate of 4.1 and 10 IU, electrocardiography showed a 91.3% sensitivity, 94% specificity, a 97% positive predictive value, 97% negative predictive value and an efficiency of 97.9%. The optimal cut off points for the Ektachrome-MB slide method was determined at 5 and 15 IU: sensitivity 90%, specificity 93%, positive predictive value 79%, negative predictive value 96.9% and test efficiency 92.3%.

In summary, the Ektachrome CK-MB immunohistology slide method is a simple and rapid technique which demonstrated good precision. Although not as diagnostically efficient as electrophoresis, this slide method may offer analytical advantages at times, or in settings where electrophoresis is unavailable.
Enzymes in Hepatic Disease

**645** ALANINE AMINOTRANSFERASE AND OTHER SERUM CHEMISTRY TESTS AMONG BLOOD DONOR GROUPS. David A. Lacher, G. Spencer, N. Pearson and P. L., Dept. Path., MD State Coll. of Osteopathic Medicine, American Red Cross Blood Services, Northwest Ohio Region, Toledo, OH (Sponsor: Judith Saffran)

An initial observation of a discord rate over 10% due to elevated alanine aminotransferase (ALT, EC 2.6.1.2) in industrial blood donor groups led us to analyze ALT and other chemistry tests according to donor groups. Donors were analyzed for ALT and were categorized into Blood Center (I), Business/Community (II), Schools (III) and Industry (IV). Randomly selected donors were also tested for alkaline phosphatase (EC 3.1.3.1), creatine kinase (EC 2.7.3.2), aspartate aminotransferase (EC 2.6.1.1) and gamma glutamyltranspeptidase (GGT, EC 2.3.2.2). Analysis of variance with post-hoc Scheffe testing showed ALT in group IV was > I and II > III. Among the other chemistry tests, only serum GGT was different and was higher in I and IV when compared to II.

<table>
<thead>
<tr>
<th>ALT (U/L)</th>
<th>N</th>
<th>Mean</th>
<th>2SD</th>
<th>3SD</th>
<th>4SD</th>
<th>5SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7,075</td>
<td>17.635</td>
<td>2.974</td>
<td>3.562</td>
<td>4.044</td>
<td>4.525</td>
</tr>
<tr>
<td>II</td>
<td>5224</td>
<td>22.600</td>
<td>18.332</td>
<td>25.681</td>
<td>32.029</td>
<td>38.378</td>
</tr>
<tr>
<td>IV</td>
<td>4645</td>
<td>20.363</td>
<td>13.809</td>
<td>16.000</td>
<td>18.291</td>
<td>20.492</td>
</tr>
</tbody>
</table>

Our findings suggest that hepatobiliary disorders were most prevalent in the industrial donors and least in the students. Taos or decreases in ALT were found in all studies.

**646** Alamine Aminotransferase (ALT) as a surrogate test for NON-A, NON-B Hepatitis. Martin Fleisher, J. Hoffer, M.K. Schwartz, Memorial Sloan-Kettering Cancer Center, New York, NY (Sponsor: M. Fleisher)

No single test or the viral form of the hepatitis designated as NON-A, NON-B Hepatitis (NANBH), a serious complication of blood transfusion. The AAOB has recommended the use of ALT as a surrogate test for NANBH hepatitis along with hepatitis B core antibody (anti-HBc). Using donor blood from the MSKCC Blood Bank we investigated the ALT cut-off value and the relationship between ALT and the liver enzymes: aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), 5'-nucleotidase (Nuc), phosphohexose isomerase (PHI) and gamma-glutamyltransferase (GGT). The relationship between the liver enzymes ALT, AST, LDH and Nuc was also determined.

<table>
<thead>
<tr>
<th>ALT (U/L)</th>
<th>N</th>
<th>Mean</th>
<th>2SD</th>
<th>3SD</th>
<th>4SD</th>
<th>5SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7,075</td>
<td>17.635</td>
<td>2.974</td>
<td>3.562</td>
<td>4.044</td>
<td>4.525</td>
</tr>
<tr>
<td>II</td>
<td>5224</td>
<td>22.600</td>
<td>18.332</td>
<td>25.681</td>
<td>32.029</td>
<td>38.378</td>
</tr>
<tr>
<td>IV</td>
<td>4645</td>
<td>20.363</td>
<td>13.809</td>
<td>16.000</td>
<td>18.291</td>
<td>20.492</td>
</tr>
</tbody>
</table>

Donors had an ALT cut-off value of 40 U/L was obtained by taking 2 SD above the mean. The results were compared with liver enzymes: ALT, AST, and PHI.

**647** LIVER ENZYMES AND BILIRUBIN USING A 580 EXPRESS ANALYZER. B.J. Shambayazi, E. Michalski, and A.W. Seidler (Chiba Engineering Co., Ltd., Chiba, Japan)

The assessment of the 580 Express biochemical analyzer, using Chiba reagents for the determination of alkaline phosphatase (AP), ALT, AST, GGT and TPC, and direct and total bilirubin tests demonstrated that the results were determined with five serum samples using guidelines from NCICLS document EPS-P. Linearity and results from the low and high levels are below.

**648** ALANINE AMINOPREPEPTIDASE ACTIVITY IN LIVER TRANSPLANT RECIPIENTS. E. L. Ramilo, J. L. Rudy, J. C. Argyle, J. C. Rutledge, and W. S. Andrews. Children's Medical Center, UT Southwestern, Dallas, TX (Sponsor: J. C. Rutledge)

The observation that serum alanine aminopeptidase (AAP, EC 3.4.11.2) activity is elevated in patients with biliary obstruction and cholestasis suggests that AAP is a sensitive indicator of bile duct injury. To determine if AAP levels may be useful in diagnosing rejection, serum AAP was measured in pediatric liver transplant recipients by the method of Mueller, et al. (Clin Chem 1987; 33:363-6) and correlated with clinical course.

In 11 children transplanted during the 3 month study an average of 35 AAP (range 11-59) determinations per patient were made. Nine cases of clinically and/or histologically diagnosed episodes of acute rejection were associated with transient rises in serum AAP activity ranging from 360 to 5400 above the patient's base line. Two patients with massive ischemic necrosis and one patient with CMV hepatitis had AAP elevations of 750, 1400 and 745, respectively. Using a 360 rise in AAP activity as an indicator of acute rejection, the predictive value of a positive or negative results was 75% and 100%, respectively.

In the 33 children transplanted 2 to 9 months prior to the start of the study an average of 13 AAP (range 3-40) determinations per patient were obtained. In these children, 3 patients with sustained elevations (3 to 9 times the mean) had biopsies showing chronic rejection. A fourth patient had a sustained elevation but was not biopsy.

Elevations in AAP activity correlated with histologic evidence of bile duct injury and were useful in recognizing rejection.

**649** ASSOCIATION OF LD-6 WITH SERUM MITOCHONDRIAL ENZYMES. Catherine H. Ketchum and C. Andrew Robinson (Clin. Path., UAB and the VA Medical Center, Birmingham, AL 35294) (Sponsor: C. Ketchum)

We report the case of a patient in whose sera LD-6 was found concomitantly with mitochondrial creatine kinase (CK-M). The patient presented with a two week history of weakness, orthostatic dizziness, anorexia, and respiratory distress. Past medical history included hypertension, diabetes, and chronic renal failure. During the hospitalisation, the patient experienced a seizure and subsequently developed adult respiratory distress syndrome. Despite pharmacological interventions, the patient became extremely hypotensive, developed severe metabolic acidosis, and died.

The laboratory data obtained throughout the hospitalisation revealed progressive renal failure and persistent metabolic acidosis. Serum AST and LD activities were found to be increased significantly (18240 and 35240 U/L, respectively) in the last sample obtained prior to death. Electrophoretic analysis of this sample revealed the presence of CK-M and LD-6. During our studies of LD-6, we have repeatedly seen increased AST activities with the appearance of LD-6. Significant increases of AST activity have been associated with release of the mitochondrial form of the enzyme. The activities of adenosine kinase, malate dehydrogenase, and D-hydroxybutyrate dehydrogenase, enzymes of mitochondrial origin, were found to increase approximately eight fold during the hospitalisation thus also suggesting mitochondrial damage.

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We have previously shown that the mitochondria of human liver contain two forms of LD which we have designated LD-Mt and LD-NMt. LD-Mt is similar to LD-6 with respect to charge and size. These similarities and the concomitant increases of serum mitochondrial enzyme activity with the appearance of LD-6 suggest they may be the same.

Biotrol Alanine Aminotransferase and Aspartate Aminotransferase Reagents were evaluated for their reconstituted stability at room temperature (18-25°C). These reagents were checked on RA-1000 for linearity and study which was done on a series of serum dilution. The following results were observed:

<table>
<thead>
<tr>
<th>Serum Dilution</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 Hours</td>
<td>5 8</td>
<td>0-2 Hours</td>
</tr>
<tr>
<td>20%</td>
<td>127 130</td>
<td>126 130</td>
</tr>
<tr>
<td>50%</td>
<td>317 324</td>
<td>315 325</td>
</tr>
<tr>
<td>80%</td>
<td>506 499</td>
<td>506 499</td>
</tr>
<tr>
<td>100%</td>
<td>634 637</td>
<td>628 631</td>
</tr>
</tbody>
</table>

The above data indicates Biotrol ALT and AST reagents provide linearity up to 600 U/L at 37°C after reconstituted for 8 days at room temperature.

A second liver isofrom of alkaline phosphatase migrates on cell culture polyacrylamide gel has been reported in certain types of hepatic disorders. Utilizing aseptic gel with a Tris-Bicine buffer — pH 8.8 — and separation of this isofrom was seen to comigrate with the intestinal isofrom. Electrophoresis was also carried out on polyacrylamide gel (Quantimetrix, Hawthorne, Ca.) in which the atypical isofrom was observed at the interface of the stacking and loading gels totally independent of the intestinal isofrom.

Recent studies have shown five patients with this atypical isofrom of AP had increased enzyme activity. 55-100 IU/L (reference range 15-50 IU/L), with the atypical isofrom comprising 30-70% of the total enzyme activity.

In addition to electrophoretic migration the isofrom was identified based on heat stability and chemical inhibition.

<table>
<thead>
<tr>
<th>ISOFROM</th>
<th>HEAT STABILITY</th>
<th>CHEMICAL INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Stable</td>
<td>resistant inhibited</td>
</tr>
<tr>
<td>Intestine</td>
<td>Stable</td>
<td>inhibited resistant</td>
</tr>
<tr>
<td>Atypical</td>
<td>Stable</td>
<td>resistant inhibited</td>
</tr>
</tbody>
</table>

Because of the migration of the intestinal and atypical liver AP when using asparagine gel the electrophoresis media, chemical inhibition is necessary to distinguish the two isofroms.

We used the RS/ALT to unify ALT results produced by four instrument systems by assigning values using the 'corrected' IFCC/PM/ALT criterion for the four analytical instruments used in daily service. A.O. Okorududu, A. Valcuc, R. B. McComb, and G. Bowers Jr. (CIP) Div's, Hartford Hospital (H) and Hartford Hospital Medical Laboratory (HML), Hartford, CT 06115. (Spon A.O. Okorududu)

Alanine aminotransferase (ALT) L-Alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) measurements are important in efforts to identify the transmission of the hepatitis B virus and blood transfusion. Agreement exists on ALT standardization and on a National Reference System for Alanine Aminotransferase (NRS/ALT) based on the IFCC Reference Method (IFCC/PM/ALT).
than the other methods. Using comparably derived cut-off points, the RPN yielded no false negatives and 1 false positive.

We conclude that the Reflotron is precise and agrees well with other instruments; that capillary and venous plasma agree; and that plasma exhibits a slight negative bias vs serum. We think the Reflotron can reliably be used for pre-donation ALT screening in a hemapheresis center.

In humans the greatest sorbitol (L-iditol) dehydrogenase (SDH) activity, by far, is found in the liver. The enzyme, therefore, has been suggested to be a sensitive, specific marker for cell damage (Asada N, Galambos JT Gastroenterology 1963; 44:578-587). Such reports on the diagnostic utility of SDH are, however, compromised by the use of sub-optimal assays.

Two approaches can be used in the design of an optimal enzyme assay: the theoretical approach and the experimental approach. The theoretical approach involves determining the enzyme's kinetic mechanism and, using the appropriate mathematical equation that describes the mechanism, to calculate the optimal substrate concentrations.

For an assay using sorbitol and NAD\(^+\) as substrates, a plot of 1/v vs 1/[Substrate] at various fixed concentrations of the non-varied substrate, leads to a series of intersecting lines, where the enzyme exhibits a sequential enzymatic mechanism. Product inhibition studies confirmed that the reaction mechanism used by human SDH is Sequential Random Bi Bi. Using the mathematical equation which describes this mechanism, the kinetic constants for the enzyme are: 

\[
K_{m}(S), 4.9 \text{ mmol/L}; K_{i}(NAD^+), 0.3 \text{ mmol/L}; K_{i}(NAD^+), 72.8 \text{ mmol/L}
\]

Optimal substrate concentrations calculated from a theoretical approach to SDH assay optimization, therefore, 150 mmol/L for sorbitol and 2 mmol/L for NAD\(^+\).

**SERUM GUANASE AS A BIOCHEMICAL INDICATOR OF HEPATIC REJECTION IN LIVER TRANSPLANT PATIENTS.**

The early diagnosis of hepatic dysfunction in liver transplant patients is difficult. Liver biopsy is the current gold standard for the diagnosis of rejection. Standard biochemical liver function studies used to determine if hepatic damage has occurred are not precise. Guanase (guanase aminohydrolase, K. 3.5.4.3) is a purine salvage enzyme which converts guanine to xanthine and occurs mainly in the liver, kidney, and brain. Little activity is normally present in serum, but it appears after hepatocellular injury.

Five orthotopic liver transplant recipients were followed with serial determinations of guanase, AST, ALT, alkaline phosphatase, and bilirubin, as well as prospective liver biopsy. Guanase levels were determined using a UV-spectrophotometric method involving enzymatic coupling to xanthine oxidase with measurement of the rate of uric acid formation. Five episodes of rejection (positive histological and serological treatment with prednisone and/or OTC) were diagnosed in four patients. One episode of rejection was not accompanied by an elevation of AST and ALT. The increases in these parameters were the first indication of liver damage with guanase elevation noted first in the biopsies. Bilirubin and alkaline phosphatase were markedly increased in two out of the four episodes.

Bilirubin were performed either at regular time intervals (n=10) or in response to elevations in liver function studies (n=5). Positive biopsy results were obtained in three out of two of these and two out of three biopsies (68%), respectively. Two additional episodes of hepatic dysfunction were recognized by increases in guanase, AST, and ALT, but biopsies were not done and rejection was not established. In one of these instances, an episode of rejection was subsequently diagnosed. Guanase appears to be a good indicator of hepatic rejection in transplant patients. Further evaluation of its sensitivity and specificity in the detection of hepatic dysfunction and rejection is in progress.

**ACTIVATED ALANINE AMINOTRANSFerase REAGENT ON THE PARAMAX ANALYZER.**

We are currently evaluating a modified alanine aminotransferase (ALT) reagent for the Paramax Analytical System. This method follows the spectrophotometric or pyridoxal phosphate (PLP) reaction. This method is based on the International Federation of Clinical Chemistry (IFCC) for the determination of alanine aminotransferase (ALT) and aminotransferases (AST, ALT); the typical pattern at initial presentation was LD ALT AST, with peak value over 100x reference limits in 6 cases. ALT enzymes returned to normal within 5 days. ALT enzymes rapidly returned toward baseline with clearance rates approximating 24% for LD, 12% for AST, and 6% for ALT. An acute increase in prothrombin time to 15s occurred in all patients with return to baseline in 3-5d. Peak total bilirubin was 4mg/dL in all 1 patient, and was 2mg/dL in 10 cases. The pattern of markedly abnormal enzymes and prothrombin time with mildly elevated bilirubin is virtually diagnostic for this disorder. In cases without documented hypotension, these findings should still suggest ischemic hepatitis as the diagnosis.

**THEORETICAL APPROACH TO OPTIMIZING AN ENZYME ASSAY: HUMAN SORBITOL DEHYDRGENASE, DANIEL A. NEALON**

The mitochondrial enzyme, sorbitol dehydrogenase (LAD, EC 1.6.4.3) has been measured in human serum. Preliminary results using a manual assay method (Clin. Chem. 21:273, 1975) indicate that LAD is elevated in patients with hepatic or myocardial infarctions with intermediate elevations in cirrhotic and myocardial infarct patients. No elevations were found in patients with renal, pulmonary, bone, gastrointestinal, intracranial, or hemolytic disease. LAD appears to be more specific for liver disease than other transaminases.

In order to adequately examine the clinical utility of serum LAD, we have adapted the assay to the Hoffmann-La Roche & Co. COBAS FARA centrifugal analyzer. The assay requires 20 μL of specimen and 380 μL of reagents containing 50 mM Tris-HCl, pH 8.1, 50 mM L-iditol, and L-idopropionic acid. The specimen was prepared from commercial sorbitol dehydrogenase (Sigma, L-2602) diluted into normal control serum (Gillford, QCS). Recovery was 100%. Following an initial one
second delay, 4 absorbance readings were taken at 5 second intervals. All data were collected at a wavelength of 490 nm and at a temperature of 37°C.

The reaction is linear up to 35.9 µl/ml and activities above 10 µl/ml have not been measured in patient sera. Linear regression analysis over this range of activity yields a slope of 0.78, a y-intercept of 0.02, a standard error of estimate of 0.87, an intercept of 0.28, and an R squared of 0.996. The within-run CV for 8 µl/ml and 31 µl/ml were 3.89 and 2.10% respectively. The between-run CV for 8 µl/ml and 31 µl/ml were 4.32 and 4.24% respectively.

We conclude that the automated centrifugal analysis of serum lipase using dehydrogenase provides a simple, rapid, reproducible assay for its presence in serum.

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**AN IMMUNOPRECIPITATION ASSAY FOR HIGH MOLECULAR WEIGHT ALKALINE PHOSPHATASE IN HUMAN SERUM**

Gerald A. Maguire and Halima Adnan
(Dept. of Clinical biochemistry, The Medical School, University of Newcastle upon Tyne, Framlington Place, NEWCASTLE UPON TYNE, U.K., Present address Dept. of Clinical Biochemistry, John Radcliffe Hospital, OXFORD, U.K.) (Spon.: IRC Cete.)

The serum of patients with obstructive liver disease may contain an abnormal form of alkaline phosphatase. This abnormal enzyme is excluded from gel filtration columns. It is therefore referred to as high Mr, alkaline phosphatase. The objective of the present work was to develop an immunoinhibition assay for high Mr, alkaline phosphatase. It is based on the use of two mouse monoclonal antibodies: one precipitates both liver and bone alkaline phosphatases; the other precipitates placental and intestinal alkaline phosphatases. When these antibodies are mixed with human serum and subsequently precipitated with a sheep antiserum IgG coupled to colloidal gold, the only form of alkaline phosphatase left in the supernatant is high Mr, alkaline phosphatase. This was assessed using a Cobas Bio microcentrifugal analyzer (Roche Products Ltd., U.K.). Optimum conditions of antibody concentrations, incubation time and temperature, buffer and pH for maximal precipitation of non-high Mr, alkaline phosphatases were determined.

The assay was linear to greater than 375 µU/ml of high Mr, alkaline phosphatase in a sample whose total activity was 1468 µU/ml. Within batch C.V.s (n=10) were 25.6%, 17.4% and 15.3% for samples containing 8, 28, and 379 µU/ml of high Mr, alkaline phosphatase. Between batch C.V.s (n=7) were 43.8%, 27.9% and 17.1% for samples with 8, 65, and 209 µU/ml of high Mr, alkaline phosphatase. Addition of 200 µU/ml of adult intestinal alkaline phosphatase did not interfere with the assay. The results compared well with a gel filtration method (immunoprecipitation method X = 0.73 + 0.37 X; filtration method, Y = 0.87, n = 27).

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**SERUM GLUTAMATE DEHYDROGENASE (GDH) ADAPTED TO TECHNICON RA-1000 SYSTEM**

Eric Levy and Jyapanda Patil (VA Medical Center, Laboratory Service, Bronx, NY 10468) (Spon.: Eric Levy)

Serum GDH (L-Glutamate: NADP Oxido-reductase, deaminase EC 1.4.1.2) determinations has been adapted in our laboratory on the RA-1000 Technicon Analyzer.

Thirty microliter of serum was added to 350 ul of reagent (Amonium acetate, 61.5 mmol/L, Disodium EDTA 2.0 mmol/L, NADH 0.16 mmol/L, ADP 0.61 mmol/L; Tribetanolamine HCl - 40.4 mmol/L; Alpha Ketoglutarate - 143 mmol/L and LHI - 124 U/L). The calculated cal factor was 2859 using NBS glucose standard.

Fifty serum specimens were assayed manually using the optimized method of Ellis et al (Optimal Conditions for the Kinetic assay of serum glutamate dehydrogenase activity at 37°C. Clin. Chem. 1972;18:523-7) and correlated with the RA-1000 method.

Correlation coefficient was 0.9819 with a slope of 0.98192 and intercept = 1.005. R² = .9642. The method was linear up to 32 U/L.

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**SERUM CITYMAT PHOSPHATASE ISOENZYMES PATTERNS IN PATIENTS WITH LEUKEMIA**

Maai R. Manning and John A. Lott (Dept. of Pathology, The Ohio State U., Columbus, OH 43210) (Spons.: Maai R. Manning)

We assayed serum ALP isoenzymes in patients with leukemia by conventional electrophoresis (Coomassie blue agarose gels) and isoelectric focusing. Patients included those with biliary tract obstruction, liver cirrhosis, acute and chronic lymphocytic leukemia, and acute and chronic myelocytic leukemia. Serum from newborns was used to determine the location of the bone isoenzyme.

Patients with acute and chronic lymphocytic leukemia gave bands in the alpha-1 and alpha-2 regions that corresponded to the liver and biliary isoenzymes observed in those with biliary tract obstruction. Others with acute lymphocytic leukemia also showed the same pattern. Patients with chronic

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**ADAPTATION AND EVALUATION OF A a-ANLYASE ASSAY WITH P-NITROPHENYL 6'-O-BENZYL-α-MALTOPENTOSIDE, BGP AS SUBSTRATE**


A stoichiometric a-amylase assay using a p-nitrophenyl 6'-O-benzyl-α-maltopentoside (BGP) was evaluated in 3 laboratories with various automatic analyzers. Human salivary and pancreatic a-amylases hydrolyze single glucosidic bond of BGP which is resistant to glucoamylase and a-glucosidase due to the benzyl group in the nonreducing-end glucose residue. The activity of a-amylases can be assayed by measuring spectrophotometrically the amount of p-nitrophenol released from α-amylase digested product p-nitrophenyl α-maltoside.

The reagents were segregated into the coupled enzymes and the substrate solutions and were found to be stable each other for at least 2 months at 10°C and 7 weeks at 25°C. The normal range for serum samples was 22 – 77 IU/L, and the dynamic range for the reagent up to 4,000 IU/L (37°C). Within-run and between-run CV's using Hitachi Model 7150 varied from below 1.5% and 3%, respectively. No clinically significant interferences were observed from glucose, lipemia, hemolysis, and icterus. a-Amylase activity in 54 human sera ranging 36 to 464 IU/L were measured with BGP method (y) and with p-nitrophenyl α-maltoside method (Boehringer Mannheim, x). The equation of regression line is y = 0.53 x – 3.4, the correlation of coefficient is 0.997.

In summary, BGP method can be used for routine clinical use and may satisfy the essentials of recommended assay method.
lymphocytic leukemia showed bands in the alpha-2 and beta regions that correspond to liver and bone-type ALP isoenzymes. The isoenzyme fraction migrating to the beta position in chronic lymphocytic leukemia could easily be confused with that from bone. In leukemia, the "bone" isoenzymes most likely derives from leukocytes, and its presence in serum is owing to the lysis of lymphocytes. A summary of our patterns is:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Isoenzyme Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborns</td>
<td>alpha-1, beta</td>
</tr>
<tr>
<td>Biliary tract obstruction</td>
<td>alpha-1, beta</td>
</tr>
<tr>
<td>Acute myelocytic leukemia</td>
<td>alpha-1, alpha-2</td>
</tr>
<tr>
<td>Chronic myelocytic leukemia</td>
<td>alpha-1, alpha-2</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>alpha-1, alpha-2</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>alpha-2, beta</td>
</tr>
</tbody>
</table>

665 A NEW TEST IN THE REFLotron® SYSTEM: PANCREATIC \( \alpha \)-AMYLASE. A. Rothe, H.E. Will, W. Tritschler (Boehringer Mannheim GmbH, Mannheim, FDP) (Spon. W. Werner)

Reflotron® Pancreatic \( \alpha \)-Amylase is a reagent carrier for the determination of pancreatic \( \alpha \)-amylase in 30 ml of undiluted serum, plasma and venous or capillary blood.

The test is based on the known Reflotron® \( \alpha \)-Amylase with an additional reagent carrier. It contains two monoclonal antibodies specific for human salivary \( \alpha \)-amylase, one, named 667C, selectively binding to the isoenzyme with the salivary isoenzyme.

So the enzymatic activity of salivary amylase is inhibited by 98 per cent. The pancreatic amylase activity is not influenced by the combined action of the two antibodies.

Performance characteristics are similar to those of Refflotron® \( \alpha \)-Amylase: linearity up to 2000 U/L (37°C), variation coefficient \(< 5\%\), no plasma/blood differences.

Method comparison against the colorimetric test Pancreatic \( \alpha \)-Amylase PNR (Boehringer Mannheim) show identical results (y = -2.83 + 0.976 x; n = 26, r = 0.997).

In summary, Refflotron® Pancreatic \( \alpha \)-Amylase is a simple, precise and convenient test for the diagnosis of pancreatic diseases.

666 LTPASE: TOTAL AND ISOENZYME ACTIVITIES IN NORMAL HUMAN TISSUES, Irene L. Miller and John A. Lotz (Department of Pathology, The Ohio State University, Columbus, OH 43210) (Sponsor: Irene L. Miller)

Extracts of human tissue specimens were examined for lypase activity with the Radiometer pH-Stat instrument (olive oil emulsions) and Kodak Ektachem (Ekt) Lipase slides. Our results indicate U/sg soluble protein for some tissues are:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Lipase, pH Stat</th>
<th>Lipase, Ekt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>2.124 g/100 g</td>
<td>4.127 #/g</td>
</tr>
<tr>
<td>Colon</td>
<td>517</td>
<td>994</td>
</tr>
<tr>
<td>Stomach</td>
<td>310</td>
<td>167</td>
</tr>
<tr>
<td>Small bowel</td>
<td>119</td>
<td>210</td>
</tr>
<tr>
<td>Liver</td>
<td>3</td>
<td>67</td>
</tr>
</tbody>
</table>

Lipase isoenzyme assays were performed on tissues by electrophoresing an extract on a Corning Special Purpose Agarose with 50 mmol/L Heparin buffer, pH 7.8. The agarose was overlayed with XL lipase slide material (factored from the peel) and incubated for 60 min at 25°C. We observed two bands in most tissues, one cathodal and one anodal to the application point.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cathodal band</th>
<th>Anodal band</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic fluid</td>
<td>4#</td>
<td>8#</td>
</tr>
<tr>
<td>Colon</td>
<td>3#</td>
<td>2#</td>
</tr>
<tr>
<td>Stomach</td>
<td>2#</td>
<td>4#</td>
</tr>
<tr>
<td>Small bowel</td>
<td>trace</td>
<td>5#</td>
</tr>
<tr>
<td>Liver</td>
<td>0#</td>
<td>trace</td>
</tr>
</tbody>
</table>

Lipase isoenzymes warrant further examination given the simplicity of our technique with EK slide material. It may be possible to use large and small bowel lesions with lipase isoenzymes.

667 "BLOCKED" N-PITRHONPHENYLMALONEHEPTAPSIDA AS A SUBSTRATE FOR \( \alpha \)-AMYLASE ACTIVITY DETERMINATIONS ON RA SYSTEMS. J. M. Ort, K. M. N. G. (Boehringer Mannheim, Nuys, CA. 90045) (Sponsor: J. M. Ort)

A method has been developed for the determination of \( \alpha \)-Amylase activity using a new chromogenic substrate, and was adapted to TECHNICON RA SYSTEMS and ASSIST. The usefulness of the substrate was judged by its accuracy, reagent stability, and capacity to negate environmental (CO\(_2\)) and endogenous (Pyruvate) interferences.

The substrate was formulated as a single reagent, with buffer, N-pitrophenylmalononheptapside: an optimal pH 6.9 has to be maintained throughout the course of the reaction. Calibration is based on the molar extinction coefficient of N-pitrophenol at this pH, considering the matrix of the reaction components.

The method produces results which are linear to at least 1600 U/L, with a correlation of \( r = 0.99 \) to the linear segments of other substrates. Stability of reagents, after reconstitution, exceeds 1 month at 2°C. Without an increase in blank absorbance. The comparison substrate generated the following data (RA-systems):

<table>
<thead>
<tr>
<th>Method (S)</th>
<th>N SLOPE</th>
<th>INTERCEPT</th>
<th>R Syx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. pHG-5</td>
<td>83</td>
<td>0.98</td>
<td>-4.95</td>
</tr>
<tr>
<td>2. uv method</td>
<td>83</td>
<td>0.69</td>
<td>+0.34</td>
</tr>
</tbody>
</table>

We conclude that "blocked" N-pitrophenylmalononheptapside is the substrate of choice for a reliable amylase methodology on discrete chemistry analyzers to corroborate the checker system stability and is less subject to interference resulting from a pH shift or from endogenous serum pyruvate.

668 POTENTIATED INHIBITION OF LDH ISOENZYME-S BY UREA. IN FROZEN SPECIMENS, Richard B. Passay, Clayton G. Fuller, Ronald L. Gilham, and Kenneth E. Biek (Department of Pathology, University of Oklahoma, HSC, Oklahoma City, OK 73118) (Sponsor: Richard B. Passay)

Lactic dehydrogenase isoenzyme-5 (LDH-5) is a useful discriminator for the location of urinary tract infections, in that LDH-5 is excreted into the urine when the infection is in the kidney and is absent when the infection is in the bladder (Carvajal, Kidney International 8: 1-7, 1975). It is well known that LDH-5 is inhibited by urea in high concentrations (above 1 mol/L), but it has not been reported that, when frozen, this isoenzyme is very sensitive to urea in low concentration (0.15 mol/L). LDH-5 solutions in aqueous urea (0.15 mol/L) show a 94% reduction in activity when frozen for 3 hours. However, when an aliquot of the same solution is kept at room temperature, fraction-5 activity increases. The mechanism of this unusual phenomenon seems to be due to the increased urea concentration in the aqueous phase as ice crystals form along with a uric acid phenomenon associated with freezing. There was a gradual increase in activity of 20% above prefrozen activity after 0.15 mol/L urea solutions of LDH-5 were frozen for up to 8 hours, thawed, and maintained over the next 24 hours. Aliquots of the same solution were frozen for 24 hours and were permanently inhibited. It is essential that urine specimens be kept unfrozen before assay for LDH-5 isoenzyme.
We also found that macroamylase (MA) cannot always be detected by electrophoresis. The CAE failed to detect the presence of MA in 7 samples that had been shown to contain MA by the gel filtration method.

In summary, we found that (a) the identity of the isoamylase bands cannot be demonstrated accurately for some samples using CAE, (b) the WI and gel filtration are more reliable and convenient for isoamylase and macroamylase determinations, respectively.

**Poster Session 2:30pm-4:30pm**

**ENZYMES—PART B**

**Miscellaneous Other Enzymes**

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**670**  
**IMPROVED DETECTION AND IDENTIFICATION OF ACETYLCHOLINESTERASE IN ANTIMICROBIAL FLUIDS.**  
Joseph F. Cun and C. S. Chiang  
(Research & Development, SmithKline Biotech Laboratories, Van Nuys, CA 91405)  
(Sponsored by: C. S. Chiang)

We have developed an improved method for detecting acetylcholinesterase (AChE) in antimicrobial fluid featuring specific inhibition of pseudoacetylcholinesterase (PChE) by antibodies to avoid the use of highly toxic chemicals and a colored product to simplify visualization.

Duplicate samples are incubated with or without PChE antibody and electrophoresed in polyacrylamide gels to separate acetylcholinesterase (AChE) and pseudoacetylcholinesterase (PChE). Following electrophoresis, bands with cholinesterase activity are demonstrated by first incubating with acetylthiocholine iodide and copper sulfate in saturated sodium sulfate and finally incubating with 2xM potassium ferricyanide. The product of the enzymatic reaction in the first incubation, thiocholine, combines with Cu²⁺ ions to form the insoluble copper thiocyanate that is then converted to an intensely colored brown copper ferrocyanide precipitate in the second incubation. Brown precipitate forms where enzymes are located. These bands in gels are easy to see and photograph, thus making the interpretation and permanent record keeping simpler and more reproducible without using special equipment.

Acetamido from normal pregnancies contains only pseudoacetylcholinesterase and demonstrates a single slow migrating band on the gel in the absence of antibody. The band disappears with antibody inhibition. AChE positive specimens demonstrate double bands on gels in the absence of antibody; i.e., containing a fast migrating band in addition to the pseudoacetylcholinesterase slow moving band. With antibody inhibition, AChE positive specimens appear as a single fast migrating band whose intensity is not diminished compared to that in the absence of antibody.

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**671**  
**DEVELOPMENT OF A CHROMOGENIC REAGENT FOR MEASUREMENT OF ALPHA AMYLASE ACTIVITY:**  
Julie Poljakovic, C. Reasor, C. Klubak, D. Mull (Ciba Corning Diagnostics Corp., Gilford Systems, Oberlin, Ohio 44074)  
(Sponsored by: Lianne Kehlmeier)

We have developed an accurate and highly stable reagent for measuring alpha amylase activity either manually or on the Gilford SBA 800. This assay uses a blocked p-nitrophenyl-4-methoxycarbonyl-thio-β-D-galactoside (PNPG, Genzyme Corp., Boston, Mass.) as substrate. The blocking group on the PNPG renders the substrate immuno to cleavage by the two auxiliary enzymes in the reagent—maltase and glucoamylase—but not by alpha amylase. The reaction produces p-nitrophenylsulfate (PNPS), the rate of which, when measured at 405 nm, is proportional to the amylase activity in the sample. Working reagent is stable for 7 days at 25°C, and 30 days at 4°C. The working reagent is not compatible with the trichloroacetic acid phase of the Gilford SBA 800.

The assay deviates from linearity by 5% at 2500 U/L when tested with patient serum that is spiked with pure amylase (Sigma Chem. St. Louis, MO). A comparison of 35 patient serum samples (range: 45-2017 U/L) between Gilford alpha amylase and a similar, commercially available method based on the method of Blair, yields the following results: Y = 1.02X - 0.23, r = 0.90. A similar comparison of 13 urine samples (range: 1.5-6.5 mg/dL) filtered and untreated gives the following results: Y = 1.02X + 1.08, r = 0.998.

Precision studies show the following results:

<table>
<thead>
<tr>
<th>Within Run (17 samples per level, per run)</th>
<th>Low Level</th>
<th>Medical Decision Level</th>
<th>High Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>55.7 U/L</td>
<td>103.1 U/L</td>
<td>313.4 U/L</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>3.9</td>
<td>2.2</td>
<td>0.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run-to-Run (10 runs, 17 samples per run)</th>
<th>Low Level</th>
<th>Medical Decision Level</th>
<th>High Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>55.2 U/L</td>
<td>103.9 U/L</td>
<td>313.4 U/L</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.5</td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>2.8</td>
<td>1.3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

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**672**  
**EKACHEN LIPEASE LEVELS IN BLUNT ABDOMINAL TRAUMA.**  

Due to the multiple causes of elevated serum amylose levels, we examined the use of serum lipase levels as a more specific test than serum amylose for detecting pancreatic injury in our Trauma Center. Admission samples were collected consecutively on 50 patients hospitalized for blunt abdominal trauma. The lipases and amyloses were analyzed on an Ektachem 700 (Eastman Kodak Co).

The clinical staff determined by clinical course, radiographic evidence and/or surgical procedures that 49 did not have pancreatic injury. Twenty six of these had normal lipases and amyloses; 6 had elevation of amyloses only (AE); 4 had elevation of lipase and amylose; and 13 had elevations of lipase only (LE). In the LE group, 8 had lipases greater than 1.5X the upper limit of normal (ULN) and 3 were greater than 3x ULN. For the AE group, none had amyloses greater than 2x ULN.

The sera from 16 patients with an elevated lipase were reanalyzed for lipase using Boehringer-Mannheim Diagnostic's turbidimetric assay on the Multistat III (Instrumentation Laboratory) and 9 were found to be elevated.

To eliminate the possibility that the apparent lipase activity was from muscle or bone, admission samples from 30 patients with severe muscle trauma and/or bone fracture but no abdominal trauma were analyzed. The lipases were all normal.

In the lipase the Ektachem 700 lipase does not appear to be specific clinically significant pancreatic damage. It appears that this lipase method may be detecting clinically insignificant pancreatic injury. However, the possibility that the Ektachem 700 lipase method may be measuring non-pancreatic lipase or an interfering enzyme released from the GI tract during trauma can not be ruled out.

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**673**  
**SENSITIVE ALKALINE PHOSPHATASE ASSAY USING 4-METHYLLUMBELLIFERYL PHOSPHATE.**  
(Sponsored by: W. Kozurn)

The need to quantitate low activity levels of ALP can be met by using 4-methylumbelliferyl phosphate (MUP) as substrate. ALP catalyzes the hydrolysis of MUP (emission maximum at 380nm with excitation at 360 nm) to 4-methylumbellifereone (emission maximum at 440 nm). The reaction conditions examined were 0.1M Tris-Gi pH 8.0, l0mM MgCl₂, 10mM ZnCl₂, 125mM MUP, volume fraction 0.01, temperature 37°C, incubation time 15 min. The assay had a minimum sensitivity of 0.3 U/L and an linear to 6.0 U/L. The assay was applied to the measurement of activity released from E. coli during  

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**674**  
**SENSITIVE QUANTITATION OF ALKALINE PHOSPHATASE ISOZYMES BY ELECTROPHORESIS ON LECTIN-CONTAINING AGAROSE, Dennis W. Jay and W. Greg Miller (Dept. Path., Med. Coll. VA., Richmond, VA 23298-0597) (Spon.: Dennis W. Jay)

The electrophoretic separation of alkaline phosphatase (ALP) isoenzymes on agarose gel containing wheat-germ lectin has been previously proposed by Schreiber and Whitta (Clin Chem 32:1570-3, 1986). We report an improvement in the sensitivity of this method by using 4-methylumbelliferyl phosphate substrate with fluorescent densitometric detection. Complete separation of liver and bone isoenzymes was effected with the capability of detecting less than 10 U/L of bone or liver ALP activity.

The method was found to be linear for bone ALP to at least 800 U/L and to at least 200 U/L for the liver fraction. Results of preliminary precision studies performed on normal (total ALP=88 U/L) and high (total ALP=272 U/L) serum pools are shown below.

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**1280**  
**CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988**
Incubation of bone and liver isoenzymes in a liver-containing gel resulted in additional electrophoresis on agarose without lectin and quantitation of bone ALP by subtraction. The same method must be used for measurement of liver ALP in the presence of intestinal ALP. The order of electromobility on lectin-containing gel is liver > intestinal > biliary > bone while that on agarose alone is liver > bony > bone > intestinal > biliary.

<table>
<thead>
<tr>
<th>Bone</th>
<th>Liver</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>SD</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>CV</td>
<td>4.9%</td>
<td>6.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intestine</th>
<th>Bone</th>
<th>Liver</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>avg</td>
<td>38%</td>
<td>84%</td>
<td>77%</td>
</tr>
<tr>
<td>SD</td>
<td>43</td>
<td>52</td>
<td>7.6</td>
</tr>
<tr>
<td>CV</td>
<td>12%</td>
<td>6.7%</td>
<td>7.9%</td>
</tr>
</tbody>
</table>

Discrepancies between the two reported results are greatly exacerbated in the case of veterinary samples. Thus a correlation study of human serum results was performed with regression statistics: \( r(Y \text{Rachen} = 0.641 \times (\text{Beckman}) - 0.58 \)

While the correlation study for canine samples presented the following regression statistics: \( r(Y \text{Rachen} = 0.522 \times (\text{Beckman}) + 6.57 \), R = 0.830

Degradation in canine regression statistics are attributable to the occasional occurrence of endogenous enzymatic interfering pathways which artificially elevated the activity reported in the UV based method. This is confirmed by direct addition of maltase. Careful consideration of the stoichiometries of each of these assay systems allows us to make a correction in the calculation for activity:

\[
\text{U/L} = \frac{\text{Abs} \times (\text{V} \times 1000)}{\text{correction factor}}
\]

Thus the human correlation study presented the corrected regression statistics: \( r(Y \text{Rachen} = 0.667 \times (\text{Beckman}) + 6.7 \), R = 0.994

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We have developed a new type of liquid stable amylase reagent for use on the IL Monarch systems. This reagent uses a blocked pNPG-7 substrate in the presence of alpha-glucosidase and glucoamylase. Calcium chloride and sodium chloride are also present to activate the amylase in the patient sample. The reagent consists of a bottle of liquid substrate which is added to a BoaTIL container of liquid stable enzymes. The prepared reagent is stable for 2 weeks at room temperature (25°C), 4 weeks at 15°C, and 6 weeks refrigerated (2-6°C).

The following data were obtained using NCCLS guidelines:

- The new formulation (y) was compared to the existing IL TEST reagent with the following results: \( y = 0.506x + 5.50, r = 0.9715 \)
- The new formulation (y) was compared to the existing IL TEST reagent at a level of 31.6 U/L was found to be 3.2 CV within-run, and 5.0 CV Total for n=180 over 20 runs. No interference from lipemia was observed up to a sample absorbance at 405 nm of 1.4 and N=600 mm. No interference from bilirubin was observed up to a bilirubin concentration of 50.0 mg/dL. Moderately hemolyzed samples (RBC >100 mg/dL) exhibit decreased results. The assay is linear to 2,000 U/L.

We conclude that this new formulation is a significant improvement over the existing reagent.

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The Beckman Paragon™ SPP-II electrophoresis system was investigated as a possible method for routine alkaline phosphatase (ALP) fractionation. Complete separation of the five main ALP isoenzymes (liver, minor liver, placental, intestinal and bone) was accomplished by electrophoresis of 5 µl of sera for 55 min on wheat germ lectin (Sigma) soaked SPP-II gels. Quantitation of the bands was achieved by a Beckman Appraise™ densitometer, after reaction with the substrate 5-bromo-4-chloro-3-indolyl phosphate (Sigma).

Band identification was confirmed by selective inhibition and heat inactivation studies, and by comparison of band patterns obtained from extracts of fresh autopsy tissue samples. Selective retardation of the bone isoenzyme by the lectin allowed good resolution of the bone and liver bands, with only slight overlap of bone and minor liver bands seen in some patient samples.

Good qualitative correlation was obtained between a standard polyacrylamide gel electrophoresis (PAGE) technique and the modified Paragon SPP-II method (30 patients), with the exception of the biliary isoenzyme which was not identified by the lectin method. Interassay precision (CV) of the lectin method varied from 8.9 to 19.0% (depending on which isoenzyme was studied).

In summary, this method appears to be a useful alternative to established cellulose acetate or PAGE ALP fractionation techniques, having the additional advantages of being largely based on a kit-supported electrophoresis system with little technologist "hands on" time, and allowing for rapid, accurate, reproducible quantitation of the isoenzymes.

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**677** A COMPARISON OF TWO METHODS FOR SERUM AMYLASE DETERMINATION. THE INTERCONVERSION OF ENZYME ACTIVITIES BASED UPON REACTION STOICHIOMETRY. L. W. Baker (Department of Biochemistry, The University of Texas Medical Branch, Galveston, TX) and T. McCaffrey, (SmithKline Diagnostics, Sunnyvale, CA 94086) (Spons.: Josefina T. Baker)

We have observed discrepancies in serum amylase activity reported for clinical samples in a paired comparison of two methods: I. Beckman Dri-Stat™, which is based upon NADPH formation. (Absorbance at 340 nm)

II. Ratchen, which is based upon p-Nitrophenol formation. (Absorbance at 405 nm)

Discrepancies between the two reported results are greatly exacerbated in the case of veterinary samples. Thus a correlation study of human serum results was performed with regression statistics: \( r(Y \text{Rachen} = 0.641 \times (\text{Beckman}) - 0.58 \), R = 0.994

While the correlation study for canine samples presented the following regression statistics: \( r(Y \text{Rachen} = 0.522 \times (\text{Beckman}) + 6.57 \), R = 0.830

Degradation in canine regression statistics are attributable to the occasional occurrence of endogenous enzymatic interfering pathways which artificially elevated the activity reported in the UV based method. This is confirmed by direct addition of maltase. Careful consideration of the stoichiometries of each of these assay systems allows us to make a correction in the calculation for activity:

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Thus the human correlation study presented the corrected regression statistics: \( r(Y \text{Rachen} = 0.667 \times (\text{Beckman}) + 6.7 \), R = 0.994

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**678** MEASUREMENT OF AMYLASE ON DUPTON DIMENSION CLINICAL CHEMISTRY ANALYZER, Jai Singh, S.R. Dix, A.W. Dudley, Jr., L.I. Carrera, Jr. (Beckman & E. Kuliq, Beckman Med. Ctr., 2002 Holcombe, Houston, TX 77030) (Spons.: Shirley W. Smith)

Objective was to determine serum amylase by DuPont Dimension and compare it with the amylase assay by a manual ParkinElmar (P/E) analyzer.

The procedure used for amylase (=<1.4-glucan, 4-glucanohydrolase; E.C. 3.2.1.1) on DuPont Dimension involves hydrolysis by amylase at 37°C of substrate p-nitrophosphoryl-

\[
\text{p-NP} \text{(Beckman)}
\]

resulting in formation of p-nitrophenol, which is determined bichromatically at 405/510 nm. Units are U/mL. P/E analyzer uses amylase as substrate and decrease in its turbidity by decrease of amylase action at 37°C is determined nephelometrically. P/E units are nephel units, equivalent to saccharogenic Somogyi units. Reference range for normal serum by Dimension = 25-115 and by P/E = 40-200.
Fifty human sera were analyzed by two methods and showed slopes 0.89, intercept -4.389, and correlation coefficient (Y) = 0.990; avg. by Dimension=107 MIU/mL, and by P/E=126 units. Ten replicates of a serum were assayed by each method at each time. Dimension showed Mat = 852 MIU/mL, S.D.(Standard Deviation)=0.67, C.V.(Coefficient of Variation)=0.83X. P/E had: Mean=93 units, S.D.<3.91 and C.V.=4.22. Data suggests a five-fold increase in precision if Dimension is used. To determine day-to-day variability, a sample was assayed on 10 different days by each method. Dimension results (at 74 MIU/mL) had S.D.=2.17, C.V.=13.34%. Again, Dimension had five-fold increased precision over P/E. Data showed that 12% of the samples had elevated amylase by both methods. However, an additional 12% of the samples which had normal amylase by P/E, had elevated levels by Dimension.

We conclude that for amylase assay, Dimension method is at least five times more precise, and because Dimension is fully automated, it is simpler than the manual P/E method.

680 STUDIES OF THE KINETIC ASPECTS OF BILIRUBIN OXIDASE J. S. Wei1, M. E. Chai1, J. Y. Tsai1, and J. W. Wang2 1. Department of Biochemistry, Chang Gung Medical College 2. Department of Pediatrics, Chang Gung Memorial Hospital at Taipei, Taiwan, Republic of China (Spon: IRC Corp.)

Bilirubin oxidase (EC 1.3.5.5), isolated from the fungus Myrothecium verrucaria, catalyzes the oxidation of bilirubin to biliverdin and the oxidation of biliverdin to biliverdin tax. The enzyme was found to be unstable in human and animal serum and stored bilirubin in both serum and urine. The Km's of the above mentioned substrates were found ranging from 60 to 95 µM/L. Also, double reciprocal plots (1/v vs. 1/S) of the various substrates were atypical with increased 1/v values when 1/S values are smaller than 10 to 30 µM/L.

In summary, we conclude that bilirubin oxidase has Ka values at the same order of magnitude for both bilirubin in different preparations and bilirubin as well. In addition, substrate inhibition is the distinct kinetic character of the enzyme of interest.

This investigation was supported by grant NR 189 to J. S. Wei from Chang Gung Memorial Hospital.

681 COLD STABILIZATION OF ARGININOSECUVIC ACID LYSASE, Reba, K. Wright, Mary Banerji (Ciba Corning Diagnostic Corp., Gilford Systems, Dublin, Ohio 44620) (Sponsor: Reba K. Wright, Ph.D.)

Argininosuccinic acid lysase (ASAL, EC 4.3.2.1), is a cold-labile liver enzyme that we have successfully preserved by the addition of one or more stabilizers.

ASAL activity was assayed by a modification of the methodology of Takahara and Nakane, optimized for substrate concentration. After 5 hours storage at 6°C (unprotected), 68% of ASAL activity in human serum is recovered, and 50% is recovered after 24 hours. Of the 50 compounds screened for increased stability of ASAL at 6°C, five preserved more than 80% of the enzyme activity for 24 hours: ethanol, glycerol, d,l-dihydroxyethanamine, and cortisol.

The optimal levels of the three most effective stabilizers were determined by assaying recovery of bovine liver ASAL from pooled human serum stored 24 hours at 6°C. The addition of the combination of ethanol, glycerol, d,l-dihydroxyethanamine, and d,l-dihydroxyethanamine preserved greater than 95% of ASAL activity in serum stored at several temperatures:

- Stability at 25°C was extended from 6 hours to 48 hours.
- Stability at 6°C was extended from 3 hours to 24 hours.
- Stability at -30°C was extended from 2 days to 28 days.

ASAL was equally stable at -20°C and -15°C with all stabilizers.

We have received 96% of ASAL activity (74.3 ml/ml/hour) in human serum stored at -30°C for 6 months with these stabilizers added.

682 INHIBITION OF N-ACETYL-D-D-GLOUCOSAMINIDASE BY UREA, Patricia V. Mclellan, Mary Louise McCall, Karen E. Steinberg (U.S. Department of Environment Health and Injury Control, Center for Environmental Health and Injury Control, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia 30333 (Spon.: P.M. Mclellan)

The effects of urea on the assay of N-acetyl-D-D-glucosaminidase (MAG) in urine depend on a variety of factors including the substrate used, substrate concentration, and whether or not the urea has been preincubated with the enzyme. We have examined the kinetics of the inhibition of MAG by urea preincubated with enzyme using both n-acetylresilmonophosphatidylin N-acetyl-D-D-glucosaminidase (MCP) and methylumbelliferyl N-acetyl-D-D-glucosaminide (MU) as substrates. Effects on the total activity in urine were assayed as well as separate effects on the two forms of MAG (A and B) found in urine. For the coliesterase substrate, MCP, at 2.28 mmol/L no inhibition was observed at normal levels of enzyme activity up to 532 mmol urea/L (our highest observed urine urea concentration), but 7% inhibition was seen at higher enzymatic activity levels. At the same enzyme concentration range, 5% and 8% inhibition was observed with the fluorimetric substrate, MU, at 0.95 mmol/L. The inhibition increased at lower substrate concentrations but with the respective enzymes observed over the same urea concentration range, 9% and 8% inhibition was observed with the fluorimetric substrate, MU, at 0.95 mmol/L. Inhibition was observed at these respective enzyme levels by both methods when assayed for equal enzyme content, however, it is approximately 40% higher for MU (HCP=0.56±0.03 mmol/L; MU B=0.56±0.03 mmol/L; HCP B=0.56±0.03 mmol/L; MU B=0.56±0.03 mmol/L; HCP B=0.56±0.03 mmol/L).

683 ENZYMES CHANGES IN HEMODIALYSIS S. H. M. F., Ben aver M.A., Ben Dina N., El May M., Zougha H., Hospital Universitaires, Monastir - TUNISIA, (Spon. Zougha H.)

We determined some enzyme activities in the serum of 11 CRF non treated patients (8 males, 3 females), of 15 CRF patients (6 males, 9 females) treated (6-8 months) with hemodialysis (HD) on cuprophane membranes and of 20 healthy subjects controls (9 males, 11 females). All these persons were aged between 14 and 71 years.

The activities of ASAT (EC: 2.6.1.1), ALT (EC: 2.6.1.2), LDH, γ-HBDH (EC: 1.1.1.27), CPK (EC: 2.7.3.2) were assayed using NAD+ NADH and in UV system, the Alkaline phosphatase (EC: 3.1.3.1), the γ-glutamyl-chinolase (EC: 3.1.1.8) and the Alkaline phosphatase AP (EC: 3.1.3.1) with kinetic and/or colorimetric techniques, the triglyceride lipase (EC: 3.1.1.3) activities were determined using turbidimetric method in UV.

In CRF group, data obtained revealed higher levels of LDH, γ-HBDH, Amylase, lipase, alkaline phosphatase and significant lower levels of cholinesterase, ASAT, ALT as compared to controls.

In HD patients and before treatment, we observed a significant increase of CPK, Amylase, lipase and AP and a real decrease of γ-HBDH and ASAT. At the end of the dialysis period and in the beginning we evidenced very high data of LDH, of AP and lower activities of CPK and of Amylase. However our results showed that this treatment involved a total normalization of γ-HBDH, ASAT and ALT.

Thus, both two groups of patients were characterized by high levels of AP, Amylase and lipase (p<0.01) as compared to controls. It is known that the renal failure patients showed hypercalcinemia and hyperphosphorhemia, we continue this research in order to study the mechanism of the AP change.

684 ASAY OF ESTRADIOL AND PROGESTERONE RECEPTORS IN BREAST CANCER USING MONOCLONAL ANTIBODIES, M. J. Duffy, (Nuclear Medicine Dept., St. Vincent's Hospital, Dublin 4, Ireland (Spon.: IRC Cen.)

Estriol (ER) and progesterone receptors (PR) are used in breast cancer for both selecting hormone dependent tumors and as prognostic markers. Until recently, both these receptors have been assayed using radio-labeled ligands. Here, we describe the use of new ELISAs using monoclonal antibodies to assay both ER and PR (Abbott Labs).
Good correlations were found between levels of receptor as measured by ELISA and steroid binding assay; for ER, r=0.945, p<0.001, n=100; for PR, r=0.916, p<0.001, n=50. Two samples from patients receiving tamoxifen contained immunoreactive ER but lacked ER as measured by the binding assay.

Patients with breast carcinomas containing immunoreactive ER had significantly longer survival time than patients without ER (p<0.0026). ER-positive patients also tended to have a longer disease-free interval than ER-negative patients (p=0.0913).

We conclude that results from the present ELISAs for both ER and PR correlate well with standard binding assays and gives clinically significant results, at least for ER. Follow-up data is not yet available on patients who had PR assay carried out.

Compared with steroid binding assays, immunohistochemical assay technique is simpler and can be carried out on smaller amounts of tissue. Immunohistoassay may also be more useful in measuring receptors from patients who have high levels of endogenous steroids or who are receiving anti-steroids/synthetic steroids.

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**EVALUATION OF CA 125 AS A MARKER IN OVARIAN CANCER**

M.J. Duffy, M.A. Davis, J.Sheehan and J.J. Fennelly

(Department of Nuclear Medicine and Medical Oncology, St Vincent's Hospital, Dublin 4, Ireland)

CA 125 is a high molecular weight glycoprotein which reacts with the monoclonal antibody designated OC 125. The OC 125 antibody was originally produced by immunizing mice with the ovarian carcinoma cell line OVCA 433. Here, we describe our results on the evaluation of CA 125 in benign and malignant ovarian cancer. CA 125 was assay by immunoradiometric assay (Centorcor/CIB). The following table shows the distribution of CA 125 in different groups of patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>&gt;35</th>
<th>&gt;65</th>
<th>&gt;100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ovarian Cancer</td>
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<td>48</td>
<td>39</td>
<td>38</td>
</tr>
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<td>Ovarian Ca in Remission</td>
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<tr>
<td>Other Gynecological Ca.</td>
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<td>1</td>
<td></td>
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<tr>
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<td>2</td>
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</tr>
</tbody>
</table>

CA 125 levels were significantly higher in serous than in non-serous ovarian cancers (p<0.025) and tended to increase with increasing stage of malignancy. In all of 19 patients with ovarian cancer who responded to treatment, CA 125 levels fell while 17/20 with progressive disease showed a rise. In 7/8 patients, serial determination of CA 125 showed a rise before the clinical detection of recurrence, the median lead-time being 3.5 months. We conclude that CA 125 is an excellent marker in the management of patients with epithelial ovarian cancers.

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**QUANTITATION OF SERUM PROSTATE SPECIFIC ANTIGEN (PA) BY SANDWICH IMMUNOENZYMIC ASSAY**

Shigil Matsuura, and N. Kobayashi (Wako Pure Chemical Industries, Ltd., Osaka, Japan) (Spons: S. Matsuura)

We have developed a two site sandwich immunoenzymetric assay for the measurement of PA in human serum. The assay employs polyclonal and monoclonal antibodies, each having unique specificities for different sites on the PA molecule. Monoclonal antibody is immobilized on a plastic bead and polyclonal antibody is conjugated to horse-radish peroxidase. For this assay, 100 µL of serum is incubated simultaneously with enzyme conjugated anti-body and antibody coated bead. Following a two hour 37°C incubation, the bead is washed to remove unbound enzyme conjugated antibody. Peroxidase activity bound to the bead is quantified by measuring the oxidation of 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) by H2O2 substrate following a 30 minutes incubation.

The assay has a dynamic range of 0-120 ng/ml with the minimal detectable concentration of 5 ng/ml. Serial dilutions of sera containing elevated levels of PA were assayed and found to be linear over the assay range. Recovery of various quantities of PA added to serum having elevated PA was in the range of 97 to 100%. Intra and inter assay precision controls give CV's of 7.2% or less. Fifty serum specimens from patients with suspected or known prostatic carcinoma were analyzed by both our ELISA method and Tandem R immunoradio-

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**A NEW SERUM MARKER FOR BREAST CANCER**

D. M. Chan, R.A. Beveridge, D.J. Bruzek, D.J. Dammion, R.C. Rock, and D. Etinger (Johns Hopkins Med Inst, Belto, Md 21205). (Spons D. Chan)

CA 549 is a new breast cancer (BR Ca) associated glycoprotein. The CA 549 immunoradiometric assay uses two monoclonal antibodies developed against human breast tumor cell line and milk fat globule membranes. The inter-assay precision using two quality control samples are CV's of 6% and 4.5%, mean CA 549 = 11.9 and 47.3 kU/L, n=53 and 39. Parallelism is shown over the assay range, 0-100 kU/L. A reference range (0-11) kU/L was established using mean +2SD on 100 healthy women. The distribution of CA 549 values is shown:

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>% patients, kU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy 100</td>
<td>95</td>
</tr>
<tr>
<td>Breast 69</td>
<td>99</td>
</tr>
<tr>
<td>Breast 42</td>
<td>74</td>
</tr>
<tr>
<td>Breast 37</td>
<td>38</td>
</tr>
<tr>
<td>Breast 33</td>
<td>40</td>
</tr>
<tr>
<td>Breast 28</td>
<td>60</td>
</tr>
<tr>
<td>Breast 25</td>
<td>50</td>
</tr>
<tr>
<td>Breast 24</td>
<td>50</td>
</tr>
<tr>
<td>Breast 23</td>
<td>60</td>
</tr>
<tr>
<td>Breast 22</td>
<td>40</td>
</tr>
<tr>
<td>Breast 19</td>
<td>80</td>
</tr>
<tr>
<td>Breast 17</td>
<td>90</td>
</tr>
</tbody>
</table>

---

**COMPETITIVE INHIBITION IMMUNOASSAY FOR THE DETERMINATION OF DIALOSYLL LEWIS**

C.J. Turner, Diane Jette, Annelise van Heel and M.R. Seneau, Biometrics, Inc. (Spons: H. Chantry)

We have developed a new radioimmunoassay for the quantitative determination of CA 19-9 in human serum: the TRUQUANT™ RIA. This assay is a solid-phase competitive inhibition radioimmunoassay utilizing immobilized CA 19-9 antigen and 125I-labeled monoclonal antibody specific for Lewis. A series of monoclonal antibodies has been produced from mice immunized with purified human mucins containing Lewis. Antibodies were produced for their ability to react with the human Lewis antigen, while showing no reactivity with the neoglycoproteins used to terminate the immobilized Lewis antigen. Antibodies which passed this initial screen were further characterized for fine specificity based on inhibition studies with a series of related carbohydrate hapten.

The assay protocol comprises a single, three hour, 37°C incubation of 25 µL serum or standard and 200 µL of radio labeled TRUQUANT B25.10™ antibody together in a polystyrene tube coated with CA 19-9-bearing antigens. The contents are aspirated, the tube is washed twice with 2mL water and counted in a gamma scintillation spectrometer. The assay is calibrated in inhibition Units (IU) and has demonstrated improved clinical utility over assays utilizing "sandwich" technology. The improved sensitivity may be due to the fact that the TRUQUANT™ assay is capable of showing a positive response in samples containing molecules carrying only a single CA 19-9 epitope or molecules whose epitope density is not sufficient for the formation of the immunocomplex required in "sandwich" assays. In addition, the competitive inhibition approach does not show the high dose hook effect commonly seen in "sandwich" assays and continues to respond positively up to extremely high doses. The assay has a dynamic range of 10 to 3000 IU/mL CA 19-9 with analytical sensitivity of 5 IU/mL. Inter- and intra-assay precision is less than 10%. Dilution linearity studies showed correlation coefficients of greater than 0.95. Data is shown to demonstrate the correlation of CA 19-9 levels and disease progression.

We conclude that the TRUQUANT™ assay for the determination of CA 19-9 is a simple, fast and accurate alternative to the currently available technology.
Quantitative determination of interleukin-2 receptor (IL-2R) in the serum of patients with hairy cell leukemia (HCL) using a two-site enzyme immunoassay. Seagard, L. M., Bhikhu, S. H., R. P. Kung, E. M. Remsman, S. Etiah, B. R. Grove (T Cell Sciences, Inc., Cambridge, MA 02139); J. R. Quevedo, D. Imperial, and J. M. Reuben (MD Anderson Hosp. and Tumor Inst., Houston, TX) (Spon.: P. C. Kung)

IL-2R is the membrane receptor for interleukin-2, a lymphokine regulating the proliferation of activated T and B lymphocytes. Recent reports have cited the presence of elevated IL-2R in the serum of patients with hairy cell leukemia. The CELLFREE IL-2R Test Kit was used to quantify IL-2R in serial serum samples from 81 HCL patients before, during, and after alpha interferon therapy. This assay uses two monoclonal antibodies to two distinct epitopes of the IL-2R: one on a polystyrene bead and the second conjugated to horseradish peroxidase.

Serum IL-2R level observed in HCL patients in response to alpha interferon

**Table 1**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Response to alpha interferon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>Partial</td>
</tr>
<tr>
<td>Mean</td>
<td>33.058</td>
</tr>
<tr>
<td>N</td>
<td>43</td>
</tr>
</tbody>
</table>

The mean value for 82 age and sex matched healthy, normal blood donors was 421 U/ml. No interference was observed with elevated levels of hemoglobin, triglycerides, bilirubin, and protein.

Serum IL-2R levels may be of use to clinical researchers evaluating response to therapy in HCL patients.

A comparison of CA-19-9 with CA15-3 in patients with pancreatic cancer. Laurence M. D. Demers (Spon. Laurence M. Demers)

CA-19-9 and CA15-3 are circulating tumor associated antigens recently identified in high concentrations in the serum of patients with colo-rectal cancer. Several recent reports suggest the use of these two colo-rectal tumor markers in patients with pancreatic cancer. We studied 40 patients with pancreatic cancer and 10 patients with gastric carcinoma using IRMA reagents for both CA-19-9 (Hytratech) and CA15-3 (Cenotec). CA-19-9 was significantly raised (p<0.01) in 34/40 (85%) patients with pancreatic CA. CA19-9 was significantly elevated in 33/40 (83%) pancreatic cancer patients. In contrast, CA15-3 was noted in only 30% of the pancreatic cancer patients. A comparison of absolute values between CA-19-9 and CA15-3 yielded a significant correlation (r = 0.9999) between the two antigens with a correlation coefficient of 0.79. Only 22% of the gastric cancer patients showed minor elevations in either CA-19-9 or CA15-3.

These findings support the clinical utility of CA-19-9 and CA15-3 as tumor markers for patients with pancreatic cancer.
An enzyme immunoassay has been developed to quantitate CA 15-3 antigen in serum and plasma. This assay utilizes a solid-phase coated with monoclonal antibody 11508 which was raised against normal human breast membranes and a horse radish peroxidase conjugated 0F3 monoclonal antibody raised against a membrane enriched fraction of a human breast carcinoma. Using this assay normal sera or plasma had a mean of 15 k u/m). The minimal detectable dose of the assay (25 pg above 0 u/ml) was determined to 2.3 u/ml. Antibody reaction in different sera (=10) ranged from 0.1 u/ml to 0.8 u/ml. Sensitivity of the assay was observed with plasma samples collected in EDTA, citrate, or heparin. Dilutional linearity of the assay was acceptable with actual values after dilution having a slope of 1.02 and a correlation coefficient of 0.998 when compared to expected values after dilution. Inter assay reproducibility, which included lot to lot and person to person variability, ranged from a CV of 12.3 at 14.3 u/ml to 8.1 at 166 u/ml. Studies have been undertaken to determine the correlation of values between the CA 15-3 ELISA and the previously developed RIA. For samples in the normal range (30 u/ml) the correlation was 0.96 with a slope of 1.05. For elevated sample (30 u/ml) the correlation was 0.998 with a slope of 1.02. These results indicate that the CA 15-3 ELISA is an acceptable alternative assay to the CA 15-3 RIA.
The purpose of this study was to evaluate the diagnostic usefulness of Carcinomembrin antigen (CEA - IMMA Serin - Italy) in bronchioalveolar lavage fluid (BALF) regarding peripheral lung tumors (as defined by normal bronchoscopy and radiological appearance).

We analyzed CEA levels in BALF (30 ml of saline solution) of 42 peripheral lung tumors (diagnosis 1.5-5 cm) with the following histological diagnosis: 37 lung malignancies and 5 non-malignant diseases. The control group is represented by 19 healthy volunteers (11 non-smokers (NS) and 8 smokers (S)). The CEA level has been determined also in sera (CEA-S). The CEA level in BALF has been referred to total protein content. We obtained significant differences in the CEA serum levels of CEA in BALF of NS and S and 16.7 ml ng/ml vs. 55.6-74.5 ng/ml (P c 0.0005); between NS and lung cancer (18.7 ± 11 vs. 303 ± 726 ± 0.0005); between S and lung cancer (P c 0.0005); and between non-malignant diseases and lung cancer (38.4 ± 24.6 vs. 303 ± 726 ± 0.0005). The mean levels of CEA in serum among different groups didn't show significant differences also because of scattering of values in malignancies. As cut off we have taken upon the mean values of CEA in BALF and serum of 19 healthy volunteers plus 2 standard deviations (CEA in BALF > 85 etc. ng/ml; CEA in serum > 7.3 ml ng/ml). The results have been the following: (CEA in BALF vs. (CEA/S primarily) abnormally (ab) normal

- lung cancer 13/37 (35.1) 34/37 (91.8)
- non-malignancy 1/5 (20) 0/5 (0)

Our results show a very elevated sensitivity and specificity of CEA in BALF among peripheral lung cancer (very superior to the sensitivity of CEA in serum).

**Correlation of CA195 levels to disease status in post-surgical serial serum samples from colon cancer patients**

Lauren E. Little, Karen M. Suchocki, and Pramod K. Gaur (Hybritech Incorporated, 11095 Torreyana Road, San Diego, CA 92121-1104)

CA195 is a circulating tumor associated antigen defined by its reactivity with monoclonal antibody B4E4. CA195 has previously been shown to be elevated (10-150 U/ml) in the sera of 58% of Dukes C and D patients and 25% of Dukes A and B patients (n=85). This is in contrast to 10% of benign colon patients (n=50) and 5% of healthy individuals (n=100).

The purpose of this study was to evaluate CA195 as a monitor of colon cancer disease status. Serial serum specimens (n=206) from a total of 60 colon cancer patients were collected over 12 months and tested for CA195 levels by immunoradiometric assay. Thirty patients with disease progression (PRG) were monitored. Forty-three of these 30 patients had negative CA195 values at the start of the study, five of which were CA195 positive at the end of the study and the remaining 9 patients were CA195 negative throughout the study. Of the 30 patients with PRG, positive CA195 levels were obtained in 15 patients at the start of the study. Twelve of these 16 patients had increasing CA195 values (1.6 to 36.5 fold change) during the study and four maintained constant positive CA195 values (0.96 to 1.1 fold change). Twenty-three percent of patients with a stable form of the disease were monitored for CA195 levels. Seven of these 10 patients maintained constant or slightly lower CA195 levels throughout the study (0.6 to 1.1 fold change). One patient maintained stable CA195 levels for 9 months and demonstrated a 2 fold increase in CA195 levels on the last draw of the 12th month. Twenty-seven patients had no evidence of disease (NED) were monitored. Twenty-two of these 27 patients were CA195 negative throughout the study, and four had low positive CA195 values which remained constant. Only one of the 27 patients with NED demonstrated a clear increase of CA195 levels during the study. This patient is being monitored for possible recurrence of the disease.

This study indicates that changes in CA195 levels are correlated with the clinical course of colon cancer. This test may therefore be a useful tool in the management of colon cancer patients.

**Evaluation of sandwich enzymeimmunoassay for galactosyltransferase isoenzyme II (GT-II)**

Morito Umemura, Y. Yamasaki, Y. Yoshida, K. Okawami, M. Matsui, (Konica Corporation, Tokyo, Japan) (Spons., M. Matsui)

There are few studies relating to glycosyltransferases that may be responsible for cancer-associated antigens caused by the altered glycosylation of Galactosyltransferase isoyme II (GT-II) was reported as a cancer-associated antigen but its characteristics and clinical status have been unclear.

We developed sandwich enzymeimmunoassay using the monoclonal antibody MAB 3872 which had been established and characterized to be specific to GT-II, previously. The procedure of the GT-II EIA is as follows. Plastics beads coated with MAB 3872 are incubated with 50ul serum samples and 200ul buffer at 21°C overnight. The beads are washed 3 times and are incubated with 200ul MAB 3872-HRP conjugate at 21°C 2 hours. After the beads are washed 4 times, HRP activity remained on the beads is measured by color development of OPD.

The assay standard shows a straight line ranging 0U/ml to 2000/ml. Reproducibility of inter- and intra-assay are less than 9% and 7%, respectively.
GT-II values in 421 normal controls are 12.0±4.4 U/ml (mean±SD). There are no significant difference among sex, smoking, and blood types. On the other hand, GT-II values in the limited number of patients with cancer are significantly elevated. This assay can detect GT-II molecule with unique plural epitopes, which are recovered as larger molecular weight by gel filtration.

**Tumor Marker CA 15-3 and Estrogen-Progesterin Receptors in Breast Cancer.** S.S. Sundaram, D.Manimakal, S.Unni, G. Thilakavathi and P.J.Goldstein (Ob & Gyn Res., Divs.) and B.S.Bhagavan (Dept.of Pathology, Sinai Hospital of Baltimore, MD 21215) (Spon: J.Lustgarten)

We have reported elsewhere in this conference that the radioimmunoassay (RIA) kit from Centocor, Inc., PA for the tumor antigen CA 15-3 in serum is the most suitable of all presently available kits for monitoring breast cancer (BC) status. Estrogen-Progesterin receptor (ER-PgR) levels in BC tissue are measured to determine the optimum mode of therapy. We carried out this study to evaluate whether tumor marker levels might be used in the place of ER-PgR levels to choose the mode of therapy with equal success.

Biopsy specimens from patients (n = 61) with stages I to IV BC were analyzed for ER-PgR levels (femto moles per milliliter of cytosol protein) using a combination receptor assay kit from NEN (Dupont), MA. sera from these patients were analyzed for CA 15-3 levels (units/ml) using a RIA kit from Centocor, Inc., PA.

The patients had mean ± SD and (range) values of 47.1 ± 12.9 (0-530), 129 ± 26.6 (0-899) and 51.9 ± 6.2 (14-220) for ER, PgR and CA 15-3, respectively. ER, PgR and CA 15-3 were positive in 38 (23/61), 62% (38/61) and 92% (56/61) over the cut-off levels of 20, 20 and 20, respectively, and ER and PgR were negative and under 3 for 33% (20/61) and 26% (16/61) patients, respectively, although the corresponding CA 15-3 values were all positive. Overall, the values of CA 15-3 correlated poorly with those of ER (R = 0.280) and PgR (R = 0.135).

The results did not show any significant association between the levels of tumor antigen in the sera and receptor protein levels in breast cancer tissue. It is suggested that the ER-PgR measurements are made to determine the appropriate form of treatment and CA 15-3 measurement is made to monitor the therapeutic efficiency.

**Magnetic Enzyme Immunoassay for Prostate Acid Phosphatase (PAP) using microtiter wells as solid phase.** Joseph, C. Fu, B.R.S. Hsu, W. Lu, G. Lee, and K.W. Lam (United Biotech Inc. 1300 C Spacepark Way, Mountain View, CA 94043) (Spon: Joseph Lu)

Two site enzyme immunoassay using monospecific antibodies coated on microtiter well strips as solid phase has been developed for the determination of PAP in human serum. The procedure involves 1) simultaneous incubation of samples and enzyme conjugate (HRPO) for 30 min. at room temperature. 2) washing, color development with hydrogen peroxide and tetramethylbenzidine for 10 min. 3) read O.D 450nm absorbance after reaction is stopped with 2N HCl.

The assay has a sensitivity of 0.4 ng/ml PAP and a reproducible results of dynamic range 0 to 30 ng/ml. Precision data using three levels of lymphocyte 3000 series were shown below:

<table>
<thead>
<tr>
<th>Lymphocyte</th>
<th>intra-assay (n=12)</th>
<th>Inter-assay (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/ml)</td>
<td>9.2</td>
<td>9.3</td>
</tr>
<tr>
<td>S.D. (ng/ml)</td>
<td>0.23</td>
<td>0.56</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>4.5</td>
<td>11.34</td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td>11.7</td>
<td>10.37</td>
</tr>
<tr>
<td>S.D. (ng/ml)</td>
<td>0.62</td>
<td>1.11</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>5.7</td>
<td>11.42</td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td>22.75</td>
<td>20.75</td>
</tr>
<tr>
<td>S.D. (ng/ml)</td>
<td>0.94</td>
<td>1.97</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>4.14</td>
<td>9.46</td>
</tr>
</tbody>
</table>

When a comparison study was made with Tandem-E PAP, the correlation coefficient of 0.998, slope of 0.87 (n=48). The assay uses purified PAP band 2 as standard antigen and has a cross-reactivity of 60% to purified PAP band 4.

This design provides a simple and rapid method with high sensitivity and specificity for determining PAP levels in human serum.

**Multiple Markers of Malignancy in Serum of Patients with Breast Carcinoma.** Donald W. Herczog, Call Barry, Frank Pathology, Monarch Hospital, Pittsburgh and Pittsburgh Cancer Institute, Pittsburgh, PA 15213; Joseph P. Brown (Oncoenc, Seattle, WA 98121) (Spon: Donald W. Herczog)

A promising approach to overcoming the insensitivity of single markers is the simultaneous assay of several markers based on the premise that cancer cells are biochemically heterogeneous and may synthesize a broad spectrum of possible tumor markers.

**Correlation of Prostate Specific Antigen (PSA-RIA) and Prostatic Acid Phosphatase (PAP-RIA) to Clinical Outcome in Males Less Than 50 and Greater Than 40 Years of Age.** Deborah M. Crean, Frank F. Pari, Beth Wolfson, Pittsburgh Ctr. Med. Ctr., Pittsburgh, PA 15213 (Spon: Deborah M. Crean)

Randomly selected specimens from male patients were analyzed for PSA and PAP. Both assays utilize an enzyme specific monoclonal antibodies; occurrence of increased values has been used to indicate only prostatic cancer. Results were correlated to clinical outcome and further separated into two age categories: less than and greater than 40 years of age. Results were as follows, excluding two outliers:

<table>
<thead>
<tr>
<th>PSA (ng/ml)</th>
<th>PAP (U/ml)</th>
<th>Clinical Diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40</td>
<td>&gt;40</td>
<td>Anemia/Fever</td>
</tr>
<tr>
<td>2-3</td>
<td>10-40</td>
<td>Cholesterol/diabetic fistula</td>
</tr>
<tr>
<td>3-10</td>
<td>10-40</td>
<td>Electrical Burns</td>
</tr>
<tr>
<td>4-10</td>
<td>10-40</td>
<td>Bone marrow transplant for leukemia</td>
</tr>
<tr>
<td>4-10</td>
<td>10-40</td>
<td>Gastrointestinal bleeding</td>
</tr>
<tr>
<td>&gt;40</td>
<td>&gt;40</td>
<td>Congestive heart failure, pancreatitis</td>
</tr>
</tbody>
</table>

A review of data and clinical diagnoses indicates a difference between the two age groups for each analyte and increased results with diseases other than prostatic cancer.


Our earlier studies have shown the clinical significance of CA195 in colorectal cancer. CA195 test (sequential IMMA developed by HYBRITECT) measures two epitope, sialylated Lea and Lea while CA19-9 test (sequential IMMA developed by CENTOCOR) measures only sialylated Lea epitope in serum.

This investigation reports the simultaneous measurement of CA195 and CA19-9 in colorectal (N=69) and upper GI (N=95) cancer patients. The results were as follows:

<table>
<thead>
<tr>
<th>Clinical Status</th>
<th>CA195</th>
<th>CA19-9</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>31</td>
<td>14(45%)</td>
<td>22(71%)</td>
</tr>
<tr>
<td>NED</td>
<td>38</td>
<td>2(6%)</td>
<td>2(6%)</td>
</tr>
</tbody>
</table>

**Clinical Chemistry, Vol. 34, No. 8, 1988 1287**
The concentration (unit/ml) of tumor markers RIA-CA 15-3 and ELISA-CA 15-3 were assayed in sera of women with stages I to IV breast cancer (BC) (n = 95) and normal women (n = 100) by the investigational assay kits from Centocor, Inc., PA (Distributor: American Biopharma, Inc., (C) (Distributor: Polymedco, Inc., NY) (ORI). The mean ± SD. of values for the normal sera using the CEN and ORI kits were 13.0 ± 7.9 and 13.3 ± 7.2 respectively. The mean ± SD (%) and (ng) of values for the cancer sera using the CEN and ORI kits were 66.0 ± 6.8 (14-200) and 35.3 ± 3.7 (0-150) respectively. At mean ± S.D. of normal controls, 125 kit CEN showed positive values above the cut-off level for 21 (21%) for 33 kits (88/95) patients showed above the cut-off (19) for 68% (64/94). Although all the tested sera were from confirmed BC patients, ORI kit detected zero (0) levels in two (2) patients. Seven and 32% of patients were positive for CEN and ORI kits, respectively, using each kit’s own cut-off level at mean ± 1.5 SD.

Stage IV patients (n = 20) had uniformly high values, often more than the upper limits of the standard curve of either kit. Stage III patients (n = 18) had clear positive values, except for two (2) patients by CEN and one (1) by ORI. Pearson Correlation Coefficient for CEN vs ORI values was 0.657.

Results suggest that while CEN kit is the more suitable one for monitoring the success of therapy in BC patients, and may be useful for detecting BC. ORI may be used only for monitoring therapeutic efficacy. It also appears that the RIA protocol is more sensitive than the immunoradiometric assay for the 15-3 antigen common to both kits.

The ADVANTAGES OF MEASURING SERUM CA 125 TO CEA RATIO FOR PATIENTS WITH OVARIAN CARCINOMAS, James T. Wu, Terry Mays (Dept. Pathol., Univ. UT. Sch. Med. and ARUP, SCUL, UT 84132) (Spon.: Terry Mays).

Ovarian cancer is the leading cause of death from gynecologic malignancy in the United States and it lacks a specific and sensitive test for early detection and for monitoring patients' response during therapy. Recently, a CA 125 test has shown great promise for managing patients with ovarian carcinomas. However, it still lacks the needed specificity for non-ovarian malignancies.

We found by measuring the serum ratio of CA 125 to CEA that the most 125 value detected in non-

ovarian patients could be identified. In non-ovarian patients with elevated CA 125 values detected in non-

ovarian carcinomas, the ratio of CA 125 was the most sensitive and specific for non-malignant ovarian carcinomas. Of the various CEA kits tested, ratios obtained from monoclonal CEA kits provided the best distinction between ovarian and non-

ovarian patients with elevated serum levels of CA 125. Our average ratios for the two groups of patients were established using Hybrideta CEA-EIA. However, similar ratios were found when Abbott CEA-EIA (one step) kit was used. The ratio also appears to be superior to CEA or CA 125 alone in terms of sensitivity and specificity when monitoring patients for ovarian carcinoma or for detecting recurrence. We believe that this approach would eventually provide needed specificity for the early diagnosis and screening of ovarian carcinoma.

**TUMOR MARKERS FOR BREAST CANCER, G.S. Sundaram, S.Unni, S.Maharana and P.J.Goldstein (Ob & Gyn Res.Divs.)and R.Wenk (Dept.of Pathology, Sinai Hospital of Baltimore, Baltimore, MD 21215) (Spon.: R.W.Enk).**

The concentration (unit/ml) of tumor markers RIA-CA 15-3 and ELISA-CA 15-3 were assayed in sera of women with stages I to IV breast cancer (BC) (n = 95) and normal women (n = 100) by the investigational assay kits from Centocor, Inc., PA (Distributor: American Biopharma, Inc., (C) (Distributor: Polymedco, Inc., NY) (ORI). The mean ± SD. of values for the normal sera using the CEN and ORI kits were 13.0 ± 7.9 and 13.3 ± 7.2 respectively. At mean ± SD (%) and (ng) of values for the cancer sera using the CEN and ORI kits were 66.0 ± 6.8 (14-200) and 35.3 ± 3.7 (0-150) respectively. At mean ± S.D. of normal controls, 125 kit CEN showed positive values above the cut-off level for 21 (21%) for 33 kits (88/95) patients showed above the cut-off (19) for 68% (64/94). Although all the tested sera were from confirmed BC patients, ORI kit detected zero (0) levels in two (2) patients. Seven and 32% of patients were positive for CEN and ORI kits, respectively, using each kit’s own cut-off level at mean ± 1.5 SD.

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Results suggest that while CEN kit is the more suitable one for monitoring the success of therapy in BC patients, and may be useful for detecting BC. ORI may be used only for monitoring therapeutic efficacy. It also appears that the RIA protocol is more sensitive than the immunoradiometric assay for the 15-3 antigen common to both kits.

**SIMULTANEOUS C-erbB-2 ONCOCENE AND HORMONE RECEPTOR ANALYSIS FOR THE EVALUATION OF HUMAN BREAST CARCINOMA, Dennis Todaro, A. John, T. Merker, F. Bannati, H. Fidalgo (College of Biomedical Research Corporation, Mountainside, NJ 07092), J. Wiltliff (Hormone Receptor Laboratory, University of Louisville, Louisville, KY 40292). (Spon.: Ronald Gambardella)**

Recent studies have demonstrated the usefulness of c-erbB-2 oncoene expression as an indicator of malignancy and as an indication of hormone receptor by patients with breast cancer. These studies involved either retrospective analysis of frozen tissue or separate sampling of the tumor originally used for receptor analysis. Correlations drawn between receptor status in c-erbB-2 expression could be questioned when one considers sampling error, due to tumor heterogeneity, or the possibility of DNA degradation. To answer these questions, breast tumors submitted for estrogen and progesterin receptor analysis were analyzed by a multipoint dexam-coated charcoal method designed to measure the receptor binding capacity of cytosol protein. High molecular weight DNA was extracted from the remaining cell and nuclear debris pelleted during the cytosol preparation. The extracted DNA was purified by standard techniques and subjected to slot blot hybridization analysis with anti-human c-erbB-2 probe specific for the c-erbB-2 oncogene. Results of the slot blot analysis and additional isolation techniques indicate that no significant degradation of the DNA occurred during sample processing for hormone receptor analysis. The results suggest that nuclear pellets remaining after cytosol preparation are adequate for extraction of high molecular weight DNA and subsequent hybridization with DNA probes. This technique should prove particularly useful when limited tissue is available and for the simultaneous analysis of three analytes on the same sample, therefore eliminating the possibility of errors due to sampling heterogeneous tissue.

**TUMOR MARKERS FOR BREAST CANCER, G.S. Sundaram, S.Unni, S.Maharana and P.J.Goldstein (Ob & Gyn Res.Divs.)and R.Wenk (Dept.of Pathology, Sinai Hospital of Baltimore, Baltimore, MD 21215) (Spon.: R.W.Enk).**

The concentration (unit/ml) of tumor markers RIA-CA 15-3 and ELISA-CA 15-3 were assayed in sera of women with stages I to IV breast cancer (BC) (n = 95) and normal women (n = 100) by the investigational assay kits from Centocor, Inc., PA (Distributor: American Biopharma, Inc., (C) (Distributor: Polymedco, Inc., NY) (ORI). The mean ± SD. of values for the normal sera using the CEN and ORI kits were 13.0 ± 7.9 and 13.3 ± 7.2 respectively. At mean ± SD (%) and (ng) of values for the cancer sera using the CEN and ORI kits were 66.0 ± 6.8 (14-200) and 35.3 ± 3.7 (0-150) respectively. At mean ± S.D. of normal controls, 125 kit CEN showed positive values above the cut-off level for 21 (21%) for 33 kits (88/95) patients showed above the cut-off (19) for 68% (64/94). Although all the tested sera were from confirmed BC patients, ORI kit detected zero (0) levels in two (2) patients. Seven and 32% of patients were positive for CEN and ORI kits, respectively, using each kit’s own cut-off level at mean ± 1.5 SD.

Stage IV patients (n = 20) had uniformly high values, often more than the upper limits of the standard curve of either kit. Stage III patients (n = 18) had clear positive values, except for two (2) patients by CEN and one (1) by ORI. Pearson Correlation Coefficient for CEN vs ORI values was 0.657.

Results suggest that while CEN kit is the more suitable one for monitoring the success of therapy in BC patients, and may be useful for detecting BC. ORI may be used only for monitoring therapeutic efficacy. It also appears that the RIA protocol is more sensitive than the immunoradiometric assay for the 15-3 antigen common to both kits.
These control values indicate that the agreement of CA 19-9 and CA 15-3 is comparable with that observed for CEA assay (CV: 29.3%). In the national quality control program carried out by current laboratories, CEA values were abnormally high variabilities, mainly due to large within-kit variations; in fact, four kits with variabilities ranging from 25% to 30% were used for CA 125, while all determinations of TPA were produced by the same kit.

The plasma and tumor homologues of human cancer patients have been shown to contain a unique 60-kilodalton oncofetal protein with the ability to stimulate transport of monoclonal ribonucleic acid (mRNA) from isolated nuclei in a cell-free system. Prior to this study, the biossay for this protein involved titration of small 60-kilodalton polypeptide transport protein by chromatography of large volumes of pooled patient plasma on a Sepharose CL-6B gel filtration column after ammonium sulfate fractionation and dialysis, then bioassay of the collected fractions. In order to apply the analysis of this tumor marker to individual sera, we submitted high-performance gel permeation chromatography (HPGC) for the Sepharose CL-6B step. With this procedure we were able to eliminate the dialysis step and chromatographically separate samples as small as 0.5 mL. The column used was TSK-250 (7.5 x 600 mm) with an isocratic mobile phase of PME buffer (50 mM Pipes, 25 mM KCl, 2.5 mM MgCl₂, pH 6.8) and Superose-12 (10 x 300 mm) with TMA buffer (50 mM Tris, 25 mM KCl, 2.5 mM MgCl₂, pH 7.5). Both columns and mobile phases, running at 1 mL/min and collecting 1-mL fractions, adequately separated the 60- and 25-kilodalton proteins.

Substantial activity of the 60-kilodalton protein was found in the plasma of all cancer patients tested, regardless of tumor type. The protein was absent or present with very low activity in noncancer patients, including many with non-cancerous disorders. The tumor marker was also found in the homologues of a colon tumor, but not in normal colon homologs from the same patient.

Analysis of this tumor marker may be a significant factor in the diagnosis, prognosis, and monitoring of cancer patients.

A solid phase immunoradiometric assay (IRMA) for carcinoembryonic antigen (CEA). James Peterson, Caroline Lee, and Mary Stawicki, (Becton Dickenson Immunodigastic Center, Mountain View Avenue, Orangeburg, New York 10962)

A solid phase IRMA for the measurement of CEA in either serum or plasma has been developed. The solid phase consists of monoclonal antibody adsorbed to reaction tubes. The label is 125I-anti CEA, 100 uL of specimen and 100 uL of assay buffer are incubated for 1 hour. The tubes are washed and 200 uL of label is added to each tube. After a 1 hour, 37°C incubation, the tubes are washed and counted. The amount of CEA present in the sample is proportional to the counts bound to the tube.

The assay has a range of 0 - 1000 ng/mL and the sensitivity is calculated to be 0.2 ng/mL. Linearity studies comparing the calculated CEA concentration to volume of sample added gave an average correlation coefficient of 0.9971. CEA added to normal patient sera gave an average of 99.3% (range 92.2 to 106.7%). Five assays of a three level control gave intersay CV's of 4.1, 8.3, and 7.1% respectively for the medium and high levels.

Comparison of this assay to the Abbott CEA-RIA Monoclonal One-Step (Abbott Labs., N. Chicago, IL) gave the following data: BD = 97; Abbott +1.3, r = .96, n = 30.

Comparison of this assay to the Behring RIA-ghost CEA (Behringwerke AG, Marburg, Germany) gave the following data: BD = 0.94 Behring +1, r = .97, n = 63.

We conclude that this method is a simple, rapid system for the quantitation of CEA in serum or plasma.

Usefulness of squamous cell carcinoma antigen (SCC) in carcinoma of the esophagus.

R. M. Donlon (Division of Laboratory Medicine, Tata Memorial Hospital, Parel, Bombay 400012, India)

The clinical utility of SCC, a subfraction of T-A-4 antigen, and Carcinoembryonic antigen (CEA) was investigated in oral and esophageal cancers. Both cancers are common in India. The study group included 210 patients with head and neck cancers, 101 surgically treated and 109 conservatively treated.

The respective correlation coefficients and slopes were 0.972 and 1.04 vs CEA-RIA Monoclonal One-Step.

ADAPTATION OF A CA 15-3 ENZYME IMMUNOASSAY TO ENHANCED LUMINESCENCE, Peyton S. Metzkel (Ames Research Center, NASA, Moffett Field, CA 94035) (Spons.: S. Clark)

The purpose of this experiments was to determine the feasibility of adapting a pre-existing horseradish peroxidase-based immunoresoan for the Ames group enhanced luminoscence system. The Centocor CA 15-3 enzyme immunoassay (EIA), which had been successfully developed on a 1/4" polystyrene bead assay, served as the control system for study. Opaque white wells were coated overnight with anti-CA 15-3 (Centocor, mouse monoclonal, 11508) at various concentrations. The conjugate, mouse anti-CA 15-3: peroxidase (horseradish), (Centocor, DF3) was diluted to various concentrations in a modified EIA conjugate diluent and the S/W optimized. The two-step assay protocol consisted of a one hour incubation of diluted sample in the microwell followed by a wash, then one hour incubation with reagents. Using these reagents and protocols, but different signal development systems (o-phenylenediamine vs. luminol enhancer), parallel assays demonstrated that enhanced luminoscence extended the linear range of the standard curve to greater than 700 U/mL compared to a linear limit of 200 U/mL attained by the EIA.

Furthermore, the enhanced luminosence signal-to-noise ratio of the standards were increased five-fold compared to the EIA; this would directly translate into increased sensitivity with enhanced luminoscence.
96 with esophageal cancer, 100 normal healthy subjects and 28 with benign diseases. SCC and CEA were assayed in the sera of these subjects by radioimmunoassay (Abbot, U.S.A.). 130/210 (62%) of the oral cancer patients had elevated SCC levels, while only 10% of these patients showed slight to moderate elevations in CEA levels. Increased SCC activity was seen in all oral cancer patients, irrespective of the cell types. In esophageal carcinomas SCC levels were increased only in patients with squamous cell carcinoma. While raised levels of SCC correlated well with progressive disease, CEA did not reflect the clinical course. The sensitivity of SCC levels in the cancer and control groups was highly significant statistically. Determination of SCC may provide a valuable tool in the management of esophageal cancer.

A mouse monoclonal antibody (Mab) 47D10 was produced against a human colon carcinoma cell line, A549. Immunohistochemical studies showed that Mab 47D10 was reactive with 75-95% of carcinomas derived from lung, breast, colon, and pancreas. Staining was most intense with tissues from lung and lung metastases. The antigen of A549 cells defined by Mab 47D10 is a cell surface glycoprotein which by radioimmunoprecipitation and Western blotting appears as a diffuse band of 67-97kDa. The antigen of Mab 47D10 showed a lower molecular weight of 60-80KD (Ref. #1). To determine whether the 47D10 antigen is shed into the circulation by tumors in vivo, a competition ELISA was used to detect levels of 7260 serum samples. Circulating 47D10 antigens were found in sera of patients bearing breast, colon, pancreatic, ovarian and lung tumors. Even though immunohistochemical studies indicated strong reactivity of 47D10 Mab with lung carcinomas, relatively low levels of circulating antigen were detected in sera of patients with lung tumors. In contrast, patients with gastrointestinal cancer with pancreatic and ovarian tumors showed elevated levels of 47D10 antigens as compared to normal controls. Sera from patients with breast carcinomas could be differentiated from those with breast fibroadenoma by a sensitivity of 70% and specificity of 86%. Furthermore, colorectal cancers with hepatic metastasis showed significantly (p<0.0001) higher levels of 47D10 antigens than those with no hepatic involvement. Therefore, quantitation of circulating 47D10 antigens may be useful for monitoring of patients with pancreatic, ovarian, breast and colorectal cancers.

**972** USE OF PROTON NMR FOR THE STUDY OF PLASMA LIPOPROTEINS IN MALIGNANCY, Peter Wilding, Marilyn Senior, Maria Ludwick and Toshio Inubushi. (Dept. of Pathology, Philadelphia College of Osteopathic Medicine, and Biomechanics, Hosp. of University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104)(Spn.: L. Shaw) A series of blood plasma specimens were assayed using Proton NMR Spectroscopy at 30 MHz and 366 MHz. The results demonstrated that patients with untreated malignancy and/or hyperlipemia had reduced average line-widths at 0.9 and 1.3 ppm. However, the results obtained do not confirm published studies (New Eng.J. Med. 1986,315:1369) which showed clear differentiation of patients with malignancies. In our study the line-width data showed marked overlap, which eliminated clinical utility of the test.

In each case studied, plasma was also assayed for cholesterol, triglyceride and a tumor marker (e.g. CEA, AFP, CA-125, SGU). The selection of tumor marker was determined by the nature of the malignancy. The value of Proton NMR versus tumor marker was then assessed. In addition, plasma samples were fractionated by gradient gel centrifugation in NMR prior to assay for lipids, apo-lipoproteins, analysis by NMR and thin layer chromatography (TLC).

The results show that the key constituents influencing line-width measurement are VLDL and LDL (especially triglyceride) but that patients with established malignancy cannot be differentiated between the LDL and HDL regions which does not markedly affect line-width. The data also confirms published evidence with value of NMR assay for detecting malignancy if the plasma triglyceride is significantly elevated.
carcinomas (n=4, 356.9 ng/ml), squamous cell carcinomas (n=8, 152.9 ng/ml) and large cell carcinomas (n=2, 74.9 ng/ml). Sixteen cancer cases were studied by immunohistochemistry using an avidin-biotin immunoperoxidase technique, and in the five cases that showed high immuno-reactivity of the tumor cells, the mean CEA level was 967 ng/ml. In contrast, the mean CEA level was 59.4 ng/ml in all cases (S.D. 24.1 ng/ml). We conclude that measurement of CEA in BAL fluids may be useful in the diagnosis of lung carcinoma. There is a strong tendency for cancer cases to have higher CEA levels, but the values may be dependent upon the tumor type, and whether CEA can be demonstrated immunohistochemically within the tumor.

**Hemoglobin and Coagulation**

**Hemoglobin and Glycosylated Hemoglobin**

Fructosamine could be utilized for medium-term control of glucose metabolism in diabetic subjects while glycosylated hemoglobin (HbA1C) is useful in a long term monitoring. To clarify the interaction between the two proteins, we have investigated the trend of total daily glycosuria for a period of 8 weeks in 30 diabetic patients (16 of type I and 12 of type II); after this period, fructosamine, HbA1C and glycemia per hour have been determined. The patients have been divided into three groups the first of which included diabetics with constant decompensation (glycosuria 5 g/l); the second included compensated and subjects and, finally, the third group patients have been compensated in the last two weeks from the dietician and/or pharmacologic point of view.

The first group points out a mean value of fructosamine (3.45 mmol/l) and HbA1C (9.8%) significantly higher (p 0.01) in comparison with the control group (2.51 mmol/l and 5.8% respectively). In the second group we have not noted any significant variation either for fructosamine (2.74 mmol/l) or for HbA1C (6.2%); the subjects belonging to the third group show no important change in fructosamine levels (2.05 mmol/l) while HbA1C (8.0%) results meaningfully higher (p 0.01) compared with controls.

Our results suggest that fructosamine determination cannot replace glycosylated hemoglobin dosage but fits in the control of glucose metabolism between the last one and daily glycemic profile.

**Evaluation of the Bio-Rad Diamat Analyzer for Hemoglobin A1c Determination.** Joan A. David, Judy Goodman, Herbert H. Haito (Dept. of Biochemistry, The Cleveland Clinic Foundation, Cleveland, Ohio, 44195) (Sponsor: Joan A. David)

The increased use of Hemoglobin (Hb)A1c to evaluate and treat diabetic patients requires methods which are reliable, accurate and precise. We evaluated the performance of the Bio-Rad Diamat, a rapid method for the measurement of HbA1c by high pressure liquid chromatography (HPLC).

Within-assay precision was measured at three levels.

<table>
<thead>
<tr>
<th>Pool</th>
<th>N</th>
<th>Mean ± S.D. C.V.</th>
<th>N</th>
<th>Mean ± S.D. C.V.</th>
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<tr>
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To evaluate the accuracy of the analyzer, we compared samples to those run on a Diamat at the University of Miami (U of M). We compared our results with the HPLC reference method. The U of M laboratory is in the process of standardizing HbA1c measurements for all NIH Diabetic Clinical Trials. The mean value from the U of M Diamat was 6.46% compared to 6.49% on the U of M Diamat with n=20, Y=0.999X + 0.038 and r = 0.999.

**Liquid Chromatographic Analysis of Hemoglobin Variants.** Harry BS, and HSS. Capillary Blood Collection and Storage System, Steve Tanaka and David K. Hanley, California, CA

We evaluated a capillary blood collection system especially applicable for diabetic patients to send a sample of blood to the mail for measurement of hemoglobin A1C (HbA1C) on the Diamat™ Analyzer. Also evaluated was the quantification of hemoglobin variants; HbF, HbS and HbC with the system. Precisely 17 ml of
whole blood is forced into a vial containing 6 ml of sodium citrate solution (Bio-Rad Labs) via the spectrophotometer equipped with data reduction software. We further automated the procedure using a robotic arm to place samples in a spectrophotometer equipped with data reduction software. This study demonstrates the feasibility of complete automation of the boronate affinity chromatography for GHB using a combination of a programmable pipetting station and a robotic arm.

Fructosamine, HbA1c, Glycerated Hemoglobin: Correlation with average daily glucose in diabetic outpatients. Robert N. Wheaton, Garth E. Austin, Richard E. Mullins, Suzanne S. P. Gebhart, Diane Kremers (Depts. of Pathology and Medicine, Emory Univ. Sch. of Med., and Atlanta VAMC, Atlanta, GA 30322) (Spon.: Robert N. Wheaton)

We measured fructosamine, HbA1c and glycerated hemoglobin every two weeks for 5 months in patients who were using recording home blood glucose meters. Patients were instructed to measure blood glucose 4 times per day. They were supplied with a Glucometer-M and all reagent strips at no cost. Fructosamine was measured using a kit from Roche Diagnostics, Nutley, NJ. HbA1c was quantitated using ion-exchange columns from Bio-Rad, Richmond, CA. Glycerated hemoglobin was quantitated using boronic acid affinity columns, (Pierce Chemical Company, Rockford, IL). Recording home glucose monitors (Glucometer-M) were provided by Ames (Miles Diagnostics Division, Elk hart, IN). Patients were Type I or Type II diabetics with initial HbA1c greater than 8.9%. To date 19 patients have entered the protocol. All three parameters proved to be reflective of average daily glucose concentration. Substantial reductions in mean glucose concentration were accompanied by corresponding reductions in fructosamine, HbA1c, and glycerated hemoglobin values. Fructosamine showed changes by two weeks after changes in glucose concentration. HbA1c and glycerated hemoglobin showed changes by four weeks. Patients who showed no decline in blood glucose concentration over time did not exhibit changes in these three values.

In some patients problems were encountered in obtaining adequate and reliable home glucose data. In this set of individual assay of glycerated hemoglobin, HbA1c or fructosamine will be helpful in determining the degree of metabolic control. Providing that standardization on a regular basis and at appropriate intervals we conclude that any one of the assays is adequate for monitoring metabolic control in outpatients with diabetes.

**Coeusol Testing**

**AN EVALUATION OF A COESOLATION ANALYZER DESIGNED FOR USE BY NON-TECHNOLOGISTS: THE DUPTON COESOLATR. Robert E. Gutkain and R.E. Belsky (Dept. of Clin. Path., Oregon Health Sciences Univ., Portland, OR 97201) (Spon.: Robert E. Gutkain)**

The Dupert Coesolatr is a hand held instrumet employing prepackaged reagents and used to determine the prothrombin time of whole blood from capillary or venous samples. This study compared the reliability of Coesol results produced by a professional laboratory (operator A, a medical technologist) with that of non-laboratory personnel (operators B, C and D: phlebotomists). Precision was assessed, following NCCLS EP-5 guidelines, by assessing normal and abnormal whole blood controls supplied by Dupert. Additionally, split patient samples were assayed by the phlebotomist operators on the Coesolatr and compared to results obtained from the OHSU clinical laboratory.

**Precision Results:**

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<th>Operator</th>
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<th>Abnormal Control</th>
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<tbody>
<tr>
<td>D Mean</td>
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<td>41.8 ± 1.08</td>
</tr>
<tr>
<td>C Mean</td>
<td>39.4 ± 0.67</td>
<td>41.8 ± 1.08</td>
</tr>
<tr>
<td>B Mean</td>
<td>39.4 ± 0.67</td>
<td>41.8 ± 1.08</td>
</tr>
<tr>
<td>A Mean</td>
<td>39.4 ± 0.67</td>
<td>41.8 ± 1.08</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Operator</th>
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<th>C Mean</th>
<th>B Mean</th>
<th>A Mean</th>
<th>Intercept</th>
<th>Corr. Coefficient</th>
<th>P Value</th>
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<td>-0.4</td>
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<tr>
<td>C</td>
<td>-0.4</td>
<td>-0.4</td>
<td>-0.4</td>
<td>-0.4</td>
<td>0.953</td>
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<tr>
<td>B</td>
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<td>A</td>
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<td>-0.4</td>
<td>0.953</td>
<td>10.04</td>
<td></td>
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</tbody>
</table>

No significant difference was noted in Coesol precision among the 4 operators. However, the Coesolatr yielded results significantly greater than the manufacturer's claims (3.5%, normal; 2.2% abnormal) and the OHSU-Laboratory, control precision limits (2.2%, normal; 2.2% abnormal). Method comparison results indicated a positive bias of approximately 10% for each operator compared to the laboratory method.
Patients receiving chronic hemodialysis require anticoagulation with heparin to prevent clotting in the dialyzer during the hemodialysis procedure. The purpose of this study was to assess the variability of heparin responses as measured by activated coagulation time (ACT) (Hemotec, Inc., Englewood, CO) and plasma heparin concentration (HC) determined by the chromogenic substrate assay (ACA, DuPont, Wilmington, DE). We also examined the degree of correlation between HC and response. Twenty-one patients with end-stage renal disease were enrolled in this study. Heparin 40 ± 28.94 units/kg was administered as a single bolus injection directly into the circulation. Both ACT and HC were obtained simultaneously, immediately prior to heparin administration, and at half-hour intervals through the duration of the dialysis. A profile of log ACT-HC was generated for each patient as well as for the mean data to assess the relationship of HC to response. An excellent correlation was observed between log ACT and log HC (r²=0.81-1.0) and mean data (r²=1.0) indicating that HC are useful predictors of response. We also found that HC in the range of 0.3-1.0 μg/μl maintained the ACT between 1.5-3.0 times baseline. Examinations of mid-dialysis ACT values and HC revealed a high degree of interpatient variability with ACT and HC values ranging from 131-285 sec and 0.21-1.54 μg/μl respectively. The HC required to maintain a patient at approximately twice baseline ACT (2000±20 sec) varied between patients from 0.26-1.39 μg/μl (0.68±0.24 μg/μl). Because of the high degree of interpatient variability and the excellent correlation between HC and response, we recommed that each patient have a log ACT-HC profile done to assist with dosage individualization during dialysis. The chromogenic substrate assay provides a method for simple, rapid determination of plasma HC allowing the log ACT-HC profile to be easily generated.

### PLASMA D-DIMER MEASUREMENT IN THE DIAGNOSTIC EVALUATION OF ACUTE PULMONARY THROMBOEMBOLISM

Michael Wilson, S.M. Aguayo, T.E. Miller, G. Frielefeld and L.M. Fink (Laboratory, Pulmonary and Radiology Services, Denver VA Med. Ctr., Denver, CO 80220)

D-Dimer is known to be elevated in a variety of thromboembolic states, including deep venous thrombosis (DVT) and pulmonary thromboembolism (PE). To determine the utility of quantitative D-Dimer measurements we independently compared the plasma D-Dimer levels and ventilation-perfusion (V/Q) scans in 10 patients suspected of having PE. The D-Dimer was quantitated using ELISA methodology (American Bioproducts Co., Parsippany, NJ). The mean for varied adults was found to be 114 ng/ml and the upper limit of normal was determined to be 205 ng/ml. The D-Dimer levels and results of the V/Q scans were as follows: Patient D-Dimer (ng/ml) V/Q Scan 1 700 Low 2 210 Normal 3 250 Normal 4 760 Normal 5 525 Normal 6 3000 Low 7 152 Normal 8 1400 Indeterminate 9 1450 Low 10 900 High

*As defined by Sostman, et al (Radiologic Clinics of N. Amer.) 21:759-774, 1983) and Spies, et al (Chest 83:122-127, 1983), a normal V/Q scan essentially excludes PE. Low probability scans correspond to the presence of thromboemboli in only 4.8% of patients. In our study, levels of D-Dimer below 800 ng/ml correlate with normal or low probability V/Q scans, thus indicating a very low probability of pulmonary thromboembolism. Two patients with V/Q scans suggestive of PE had D-Dimer levels higher than 800 ng/ml. Refinement of this methodology may result in more cost effective initial evaluation of suspected cases of pulmonary thromboembolism.

### STUDIES ON THE ALTERED BLOOD COAGULABILITY IN PATIENTS WITH GASTROINTESTINAL CARCINOMA

Evelyn S.C. Kooy, M. Fruchend and P.Han (Clinical Chemistry & Haematology Labs, National University Hospital, Singapore 0511) (Spon: E Kooy)

Thrombosis and/or bleeding is a common cause of death in hospitalized cancer patients. It has been reported that local or systemic activation of blood coagulation can be induced by tumor products, thus favoring tumor metastasis. We have studied the potential for host monocytes to activate blood coagulation and measured several coagulation and fibrinolytic parameters in 20 patients with gastrointestinal carcinomas. The patients were screened to exclude complications such as thromboembolic disease or recent intravenous administration of chemotherapy which might distort the data. Measurement of protein C (PC), protein S (PS), antithrombin III (ATIII) and e2-antiplasmin (AP) were by Laurell's immunoelectrophoretic assay; tissue plasminogen activator (tPA) and its inhibitor (PAI), von Willebrand factor (vWF), D-dimers (DD) and fibrinopeptide A (FPA), by ELISA. Monocyte procoagulant activity (PCA), expressed as tissue thromboplastin units, was measured by a recalification time assay, following endotoxin stimulation (P<0.01/29,71). Pe1122; 11.3:3; AV102.33±24.21; AP<0.01; 11.3:1.512; tPA(9).20±0.29; and vWF(11.35±2.122) levels of the patients were not significantly increased compared to those of controls with no known malignancies. In contrast, a significant increase in the monocyte PCA of patients compared to controls (200 vs 67). Coagulant studies, utilizing factor VII-D-end Xa assay, were performed on plasma and platelet-rich plasma. The procoagulant activity to be a FX activator. Patient DD, FPA and PCA levels were significantly elevated above control levels (p<0.005).

In summary, we found no correlation of coagulation control protein levels with presence of malignant tumors. However, the increased FPA levels reflected increased fibrinogen turnover rates, coupled with the increased DD and PAI levels provide further evidence of the favoring of coagulation activation and fibrin generation in malignant states.

### COMPARISON OF TWO AUTOMATED COAGULOMETERS AND THE MANUAL TECHNIQUE FOR DETERMINATION OF THE PROTHROMBIN TIME: INFLUENCE OF REAGENTS.

P.A.Bonini, M.P.Deveso, S.Vigano' D'Angelo, F. Gilardoni, A. Macagni, and A. D'Aniello (Istituto Scientifico S.Raffaele,Milano Italy) (Spon: F.Cerottii)

We have compared the ACL (Instrumentation Laboratory), a recently introduced laser nephelometric and turbidimetric analyzer, and the Koagulab A (Ortho Diagnostic), an optical automated coagulometer, with the titr-tube technique for the performance of prothrombin time (PT) in normal subjects, patients with liver disease and patients on stabilized oral anticoagulant treatment (n=97). Seven calibrated (ISI) commercial thromboplastins have been evaluated. Overall imprecision (CV) determined on a normal and an abnormal plasma pool were 2.0% and 2.6% with the ACL, 2.0% and 3.1% with the Koagulab 40 A, and 7.6% and 7.5% with the manual method.

Two reagents (Thrombotech, Nyegaard and Manchester Reagent,Baldaci) proved unsuitable for use with both coagulometers. Correlation coefficients and slopes of the clotting times observed with the different reagent/method combinations on the whole series of plasma samples ranged from 0.87 to 0.99 and from 0.61 to 1.15. The F test for the two-way interaction of PT ratios was statistically significant (0.01<p<0.0001) for the major relevant/reagent/method combinations in normals and in the patients' groups. In patients with liver disease, the frequency of abnormal PTs was similar irrespective of the reagent/method combination. In patients on oral anticoagulants, insufficient (International Normalized Ratio, INR >2.0) or excessive (INR >4.5) anticoagulant effects were observed in 18% of patients with the ACL and 7% with the Koagulab 40 A and 36% and 2% with the manual method.

Standardization of both reagents and instrumentation is required to improve monitoring of oral anticoagulant treatment.

### COMPUTER-ASSISTED DETERMINATION OF COAGULATION DEFECTS

Helen Barnhill (Dept. of Path. Community Hosp., Springfield, OH 45501) (Spon: Helen Barnhill)

Coagulation screening has been difficult to perform due to 1) the large variety of coagulation defects and 2) the rarity of many of these defects. Too often, test results are delayed because the technologist does not have adequate experience and knowledge in coagulation, and may not proceed effectively in identifying the defect.

Working with our Laboratory Information System vendor, we have developed an expert system program which guides the technologist through the coagulation studies. The study begins with a prothrombin time, partial prothrombin time and prothrombin consumption test. Based on these results taken together the program orders appropriate additional testing. When the results are entered into this database, the program evaluates all of the results and may order additional testing. When the program has obtained all of the information it deems appropriate, the indicated defect is supplied by the program.

The decision tree contained in the program encompasses all of the potential factor deficiencies after the determination of DSC, DIC, primary fibrinolysis, platelet defects and circulating antibodies. The test menu is comprised of forty six test results displayed on one screen.

**Clinical Chemistry, Vol. 34, No. 6, 1988**
NUTRITION AND TRACE METALS

Trace Metals

**738**

**METHOD OF PREPARING IRON-FREE SERUM, Katrina Bramstedt**

(Nelcos Biotechnologies, San Marcos, CA 92069) (Spon.: Katrina Bramstedt)

Iron-free serum was prepared from human delipidized serum by dissociation of Fe(III)-transferrin, chelation with CaEDTA, and dialysis against CaEDTA and NaCl. Serum iron concentration and TIBC were monitored throughout the chelation and dialysis by RI Analytical technique using the Abbott TdB and Abbott Fe/TIBC Reagent and LOW Control.

To ensure complete removal of the chelator had been achieved during the dialysis, the iron-free serum was spiked with Ferrous and assayed for iron and TIBC by an additional method, absorption spectrophotometry. Using EM Diagnostics Fe/TIBC Reagent and Abbott Fe LOW Control. The percent difference between the actual and theoretical iron values were less than +/- 2.0%, indicating that this CaEDTA chelation/dialysis method is a successful process whereby an iron-free serum may be prepared.

**IRON µg/dL**

Post-dialysis: *76.0 µg/dL (2-205.25 µg/dL)*

**TIBC µg/dL**

Post-dialysis: *80.25 µg/dL (20-205.25 µg/dL)*

FeMso4 spike: *78.13 µg/dL (20-205.25 µg/dL)*

The method of preparing iron-free serum is significant to clinical chemistry in that iron-free serum is seen as an important component in the manufacture of diagnostic reagents and controls. (NOTE: THIS METHOD IS PATENT PENDING)

**739**

**DEVELOPMENT OF A SENSITIVE ASSAY FOR SERUM COPPER, Akira Abe, S. Yamaohita, A. Nomu, (Dept. Lab. Med., Sch. Med., Univ. Gifu, Gifu 500, Japan), and T. Itou (Meijiyo Hosp., Nagoya 460, Japan) (Spon.: A. Abe)

We have developed a sensitive determination for serum copper, in which the color reagent 4-(3,3-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylanilined was used. Serum and reagent are mixed directly and after 5 min-incubation at 37°C, the resulting color is measured at a wavelength of 580 nm. Molar absorptivity is 80,000.

The method was linear to at least 5 µg/mL. Within-run precision testing on 10 samples gave CV's of 1.6% at 1.03 µg/mL and 3.3% at 0.72 µg/mL. Between-run precision (n=14) gave a CV of 2.8% at 1.22 µg/mL. Results correlated well with those determined by standard atomic absorption testing (linear regression equation: y=0.92x + 0.11, y=present method, correlation coefficient: 0.977 for 56 samples). Iron, zinc, cadmium, cobalt and lead did not interfere. Hemoglobin and gross lipemia slightly interfered. 5 µg/mL of nickel reacted as 0.6 µg/mL of copper. However, nickel does not affect the assay, because the levels of serum nickel are very low (approximately 2 µg/mL).

We conclude that the method is precise, rapid and easily performed, and will be suitable for routine procedure for a sensitive copper assay.

**740**

**PERFORMANCE OF TECHNICIAN IRON ASSAY ON THE CHEM 19 SYSTEM, R. Levy, T. Teyakli, H. Raitly, F. Fu (Department of Clinical Evaluations, Technicon Corp., Tarrytown, NY, 10591) (Spon.: R. Levy)

The iron method for the Technicon CHEM 19 SYSTEM was evaluated, using patient samples and controls. The assay was based on the iron-parenchymal reaction, total iron determination is performed at pH 4.8. A one-reflectometer sample is used for the assay making it ideal for pediatric and geriatric testing.

Calibration stability was greater than 80 days and reagents on system stability is at least 80 days.

Within run and total imprecision data shown below is derived from patient sample duplicates, and multiple replicates of controls.

<table>
<thead>
<tr>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
</tr>
<tr>
<td>Level 2</td>
</tr>
</tbody>
</table>

**741**


We examined the plasma, both before and after desferrioxamine (DFO) therapy, from patients on chronic hemodialysis for the effect of citrate on aluminum (Al) binding to plasma constituents. We have demonstrated that citrate enhances the mobilization of Al from its binding sites using gel-filtration column chromatography.

Using the Amicon Centrifree micropartition apparatus we compared the chelation effect of citrate to ethylene diamine tetra acetate (EDTA) to enhance Al release from its plasma binding sites. The concentrations of citrate and Al in the plasma and ultrafiltrates were monitored using a citric acid assay (Cat No. 139076, Boehringer Mannheu) modified for determination on the EPOs discrete analyzer (EM Diagnostics) and atomic absorption spectrometry (Clin Chem 1982; 28:2139-43, respectively).

We found that EDTA had no effect on the re-distribution of Al from its plasma binding sites. By contrast, citrate at physiological to elevated levels (100-1000 µmol/L) progressively increased the recovery of Al in the ultrafiltrate. In those patients with elevated Al who did not have DFO therapy, citrate increased the proportion of free Al in the ultrafiltrate from 28 to 72%. Even in those patients who had DFO infusions with a free Al level of 67%, citrate increased the amount of dialyzable Al to 90%.

These in vitro studies therefore suggest that (with careful monitoring of ionized calcium levels) citrate may enhance the removal of Al from dialysis patients undergoing DFO chelation therapy. Such a study requires future investigation.

**742**

**SPECTROPHOTOMETRIC/ION EXCHANGE METHOD FOR THE RAPID MEASUREMENT OF SERUM IRON, Dadee Johnson and Harold L. Williams (Department of Hematology, Walter Reed Army Institute of Research, Washington, DC 20307) (Spon.: Harold L. Williams)

Most eosinophytic cells from animal sources have a requirement for iron in order to maintain life. Knowledge of serum or plasma levels of this element can aid clinicians in evaluating the iron status of patients. In addition, a rapid method for measuring serum iron levels can provide valuable information when treating cases of accidental iron poisoning. Other tests, such as serum ferritin, may be more accurate in determining iron stores, but lack the simplicity offered by some serum iron methods. We have developed a rapid and sensitive method for quantifying serum or plasma iron in which the measurements may be made accurately.
with a spectrophotometer or may be "eyeballed" within 25 ug/ml to 200 ug/ml, by comparing the color of unknowns with that of standards which have been assayed on the surface of a cellulose ion exchanger. Protein bound iron was instantly released with an acetate, polyoxyethylene (20) sorbitan mononoleate emulsion, ascorbic acid and complexed with buffered bathophenanthroline. The complexed iron was quantitated at 535 nm. In addition, the iron complexes were removed from solution onto the surface of DEAAS-phenothiazine and the intensity of color compared with serum standards treated in a similar manner. The proposed spectrophotometric method compares favorably with the reference method, r = 0.997 and with the "eyeballed" technique, r = 0.97. Fresh hemolysis of up to 50 mg/dl hgb did not significantly alter the results. This rapid and sensitive technique can easily be adapted for diagnostic use in the field, hospital or physician's office.

ASSOCIATION OF ALUMINUM WITH PLASMA AND LIVER CONSTITUENTS, Frederik A. de Wolff, Michiel F. van Ginkel, Annelles E. Brandmaa and Gijsbert B. van der Voet (Toxicology Lab., University Hosp. of Leiden, 2300 RC Leiden, The Netherlands) (Spon.: F.A. de Wolff)

Increased body burden of aluminum (Al) in man may lead to microcytic anemia, osteomalacia, or encephalopathy. The development of other neurological diseases such as Alzheimer's dementia is possible also related to Al. The interindividual variation in Al metabolism might explain the differences in response to equivalent internal doses of Al between patients.

To study the induction and binding to liver proteins, the association of Al with constituents of rat liver cytosol was investigated. After i.p. loading (5 mg Al/kg, 48 h during 1 wk, n=3), liver were homogenized and centrifuged. The cytosol was subjected to a Sephadex G-75 column and protein (Coomassie) bands were measured in the 50 collected fractions. In comparison with positive (Cd-treated) controls, Al was shown not to induce metallothionein synthesis, but was incorporated into and binding to serum proteins. Al was associated with a high-MW protein peak (400 kD). In addition, an Al peak not coinciding with protein was found in the low-MW fractions (6-5 kD). Experiments in plasma also showed two populations of Al compounds in the high-MW and low-MW range.

To further analyze these Al compounds, plasma of rats loaded with different doses of Al (1, 5 and 10 mg/kg, 48 h during 1 wk) was eluted on a Sephacryl S-200 SF column to achieve a better separation. The high-MW peak contained both albumin and transferrin. Al was mainly bound to transferrin. In the low-MW range, Al co-eluted with citrate, as measured by an enzymatic assay.

It is concluded that Al in plasma is transported both as a high-MW protein complex and in association with citrate. No Al-metallothionein complex was detected in this study. Future studies may show the significance of the citrate complex for Al accumulation and toxicity.

DETERMINATION OF METALS AT TRACE LEVELS IN BIOLOGICAL FLUIDS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY USING A VOLATILE METAL DETECTOR, Michael Kinter, Suresh K. Aparinaw, David A. Herold, Michael R. Wittis and John Savory (Dept. of Path., Univ. of Va., Charlottesville Va 22908) (Spon.: Michael R. Wittis, M.D., Ph.D.)

Determination of metals at trace levels in biological fluids is required in view of their clinical, nutritional and toxicological effects. Metals are commonly measured by atomic absorption and neutron activation. Isotope dilution mass spectrometry is an important alternative with the recognized capability of being incorporated into diverse analytical methods. Moreover, mass spectrometry provides data on the isotopic composition of the element and thus can be used for studies in human beings employing stable isotopes as tracer agents.

With these objectives, we have developed the use of gas chromatography-mass spectrometry using volatile and thermally stable metal chelates for such determinations and have evaluated the accuracy and precision of the isotopic determinations for various metals at trace levels. Acetylaceton, trifluorooacetylaceton and sodium diethyldithiocarbamate were used as the chelating agents. Reaction conditions, e.g. pH, temperature and reaction time, were optimized for achieving nearly quantitative extraction by first adjusting pH of Cr, Mn, Fe, Pb, Cd, Cu and Pt. The metal chelates were chromatographed on a DB-1 capillary column and ionized using electron ionization. At the 10 ng level of chromium, accuracies of 99% and 95% were achieved for the Cr ions in a selected ion experiment, isotope ratios could be measured with a precision and accuracy of 2 to 5%. In the case of nickel, the high-MW peak regt the 10 ng level, good precision (2-3%) and accuracy (4-8%) was also obtained. The diethyl dithiocarbamate chelates of Zn, Pb, Cd, Cu and Pt were also found to elute under these chromatographic conditions. The present GC-MS method, therefore, offers a viable alternative for the determination of metals for laboratories equipped with general purpose organic mass spectrometers.

ANALYTICAL FACTORS INFLUENCING MEASUREMENT OF SERUM UNSATURATED IRON BINDING CAPACITY (UIBC) OR A DISCRETE ASSAY, P.A. Laiply, Pan L. Roberts, G.F. Johnson, and R.D. Feld (Dept. of Path., Univ. of Iowa, Iowa City, IA 52242) (Spon.: Pan L. Roberts)

We have adapted to Diagnostica Stan- dards Ltd. (DCL) for UIBC to the Multisatet III (Instrumenta- tion Lab) microcentrifugal discrete analyzer (MCA) and studied the effect of serum volume on UIBC results. The iron saturating reagent (R1) was prepared by equal volumes of kit binding buffer reagent (BBR) and iron standard (Fe). 50 ul of serum was added centrifugally at 37°C with 100 ul of R1 (+ 1 ul of H2O wash) and reacted for either 3, 10 or 20 min. A blank bichromatic absorbance (A540) reading was then made (590 nm-660 nm). The Fe alone reagent (R2) was then mixed-in and a final bichromatic A540 taken at 5 min. Abs change and initial Fe concentration were used to calculate UIBC. For the 10 ul serum method Fe was first diluted 1:10 with 0.01 mol/L HCl and then an equal volume of BBR added to give R1. 200 ul of R1 and 7 ul of R2 were used in this method.

The 50 ul BCL adaptation (Y) was regressed against the UIBC (Z) obtained by subtracting serum Fe from TIBC in our lab's MCA Fe method (American Monitor and 610 with BBR iron exchange reagent). The correlation was r = -0.91. A non-linear regression was used to fit a curve to the Fe vs. UIBC results. The correlation was r = 0.999. Increases in UIBC were fit with a 3rd order polynomial.

P/A. Laiply


Because most techniques for measuring red cell volume involve isotopic labels, their use in children and pregnant women is limited. We have modified the commonly-used Na232CrO4 labeling method by using the nonradioactive compound followed by assay of the total cell chromium content to further undertake atomic absorption spectroscopy (GFAAS).

Freshly-drawn, packed red cells were incubated with sterile Na232CrO4 solution (Cr/V, 0.14 mg/ml) in isotonic saline. Cells were labeled at room temperature for 30 minutes, followed by centrifugation, and two saline washes. The mean cellular chromium uptake was 9%. Before refrigeration, two 1 ml aliquots of cells were transferred to clean microtube flasks for determination of hemoglobin (heme), hemoglobin (hbg), and chromium content. Venous specimens (1 ml) were collected just before (blank) and over a period of 5 to 60 minutes after reinfusion. The chromium content of the specimens was determined using GFAAS. The high content was determined using the cyanomethemoglobin assay. Red cell volumes were calculated using the following:

Cr conc. labeled cells = blank - ug/g hbg X vol labeled (1 ml)

The assay was linear to 7.5 ug Cr/ml in the diluted specimen, precision ranged from 2% to 7% per patient. This method agreed well with results obtained using the cyanomethemoglobin associated techniques for red cell estimation, performed simultaneously in seven subjects.

CONCENTRATIONS OF CHROMIUM, COBALT, CERIUM, AND TIN IN CEREBROSPINAL FLUID OF PATIENTS WITH BRAIN NEOPLASMS, LEUKEMIA, Lymphoma, or NON-METASTATIC NEUROLOGICAL DISEASES, Alvar El-Yazigi, Cassio Matos, Cesar P. Ferreira and S. Izumi (Departments of Biological & Medical Research and Neurosciences, King Faisal Specialist Hospital & Research Centre, Riyadh 11211, Saudi Arabia) (Spon.: A. El-Yazigi)

We measured the concentrations of Co, Cr, Sn, and Se in the
cerebrospinal fluid (CSF) of 29 patients with brain tumors [21 benign (BMB) and malignant (MBM)], 14 patients with lymphoma or non-cerebral solid tumors (NLCT), and 32 control patients (15 with neurological disorders (CONDCS) and 17 with neurological disorders (CONDPI)) by use of flameless atomic absorption spectrophotometry.

We detected Co in all the patients, Cr in 94%, Sn in 79%, and Cu in 50% of the cerebrospinal fluid samples. The concentrations of these metals in the control group were 4.7±1.1 (Cr), 96.8±16.2 (Co), 3.8±1.6 (Cu), and 6.4±2.1 (Sn). Cobalt was significantly decreased (p < 0.05) decreased in patients with BMB or other neurological disorders (CONDCS). Also, we observed significant differences (p < 0.05) in the concentration of Cr in CSF between the MBM group and all other tumor groups with ratios for the mean CSF concentration of Cr in patients with BMB, NBM, or NLCT/patients with MBM of 2.6, 2.1, or 4.4, respectively. We observed no significant differences in the concentrations of Cu or Sn among the various groups investigated. Also, the age and sex of the patient had no significant influence on the CSF concentration of any of the elements.

The depletion of Cr in patients with MBM may be attributable to disturbed metabolism in the MBM cells where Cr may be involved, such that an additional supply is needed and can only be supplemented from the CSF because of the blood brain barrier. Further investigations are in progress to elucidate the mechanism and explore the clinical significance of these findings.

748 DETERMINATION OF SERUM IRON AND UIBC BY A COLOMOTRIC METHOD, Hibiki Mori, T. Samori, and K. Kawamura (Dept. of Clin. Path., Kobe Medical College, Toyko University School of Medicine, Saka, 313, Japan) (Spon.: M. Mori)

We are using an electrode method (Perrocham Model 3950) for determination of serum iron and UIBC. However, there are some demerits in this particular method such as instability and iron contamination of the electrode, iron contamination, economic disadvantage and effect of chalybeate. Therefore, we tried to evaluate colorimetric method using reagents of Diichi Chemical and apparatus of Technicon RA1000 to observe above mentioned demerits.

In electrode method within-run precision and day-to-day precision are 2.28%, 5.04% in serum iron and 3.82% respectively in serum UIBC. On the contrary in colorimetric method within-run precision and day-to-day precision are much improved to 1.36%, 1.87% in serum iron and 0.78% respectively. Effect of interfering substance in serum iron was not seen in bilirubin and infraftr, but a mild negative interference was observed in hemoglobin. Effect of interfering substance in serum UIBC was minor in bilirubin, infraftr and hemoglobin.

Serum iron measured by colorimetric method and electrode method, correlated well: y=1.05x-3.81, r=0.998, n=25. Correlation between colorimetric method and electrode method in serum UIBC was also good: y=1.29x-19.78, r=0.989, n=25.

In summary this colorimetric method can be used for routine analysis of serum iron and UIBC because of good sensitivity, economy, short analytical time and the small volume of sample.

749 COMPARISON OF TWO SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF SERUM ZINC, Milagros Cruz, Ruth Homsher, and John Koetters, Mt. Carmel Mercy Hospital, Chemistry Laboratory, Detroit, MI 48235. (Spon.: Ruth Homsher)

Two methods for the spectrophotometric determination of zinc (Zn) in serum were evaluated. Both methods employ 2-(5-bromophenylazo)-S-(N-propyl)-N-sulphopropylamino) phenol (5-BR-PAPS) as complexing agent. Different masking systems are used for achieving reaction specificity for Zn. In method I (available in kit form from Technicon Instruments, Ltd., Japan) the reaction of 5-BR-PAPS with iron and copper in a trichloroacetic acid (TCA) filtrate of serum is suppressed by salicylaldehyde, sodium citrate and dimethylglyoxime (DFO). In method II (available in kit form from MichCon, Detroit, MI), specificity is obtained using a masking-demasking system in which iron, copper and Zn are present in the TCA filtrate are complexed with cyanide. Selective demasking of the zinc cyanide complex is achieved by addition of chloral hydrate which permits reaction of the free Zn with 5-BR-PAPS.

Twenty three sera were determined by method I and method II versus atomic absorption (AA). The regression equation for comparison of method I (Y) with AA (X) was: Y=0.81X+29.3 ug/dl (r=0.94). The regression equation for comparison of method II with AA was: Y=1.05X-3.8 ug/dl (r=0.98). The regression equation for comparison of method I with method II (Y) was: Y=1.01X-24.3 ul/dl (r=0.98).

Although the same colorimetric reagent, 5-BR-PAPS, is used, the procedural sensitivity is greatest with method II. Zn values were consistently higher in method I than AA whereas method II showed only slight differences. The bias in method I does not appear to be related to standardization.

750 EVALUATION OF IMPROVED TRACER MELT BLOOD COLLECTION TUBES BY ZEEMAN ATOMIC ABSORPTION SPECTROPHOTOMETRY (ZAAS), E.C. Lam, R. W. Leider, and W. H. van der Meulen (Spon.: M. Dickinson)

VACUTAINER Systems, Rutherford, NJ 07070) An earlier study (Clin. Chem. 31, 992, 1985) had reported on the levels of trace elements obtained by both 0.1N nitric acid and deionized water extraction of the contents of an improved 7al VACUTAINER Brand trace metal tubes without antioxidants. The objective of this study was to evaluate trace element tubes using either sodium heparin or disodium EDTA as antioxidants. Tubing (N=56) of each type were filled to nominal draw with deionized water and maintained inverted for 4 hours to allow intimate contact with stopper. Analysis of extract was performed using Perkin-Elmer Zeeman-5000 AAS equipment with an HGS-100 program and PE-10 printer sequence. The following data were obtained:

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (ug/dl)</th>
<th>Concentration (lg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>&lt;0.40</td>
<td>Mg 24.36</td>
</tr>
<tr>
<td>Ca</td>
<td>&lt;300</td>
<td>Mg 22.53</td>
</tr>
<tr>
<td>Cu</td>
<td>0.16</td>
<td>Sb 0.35</td>
</tr>
<tr>
<td>Mg</td>
<td>0.29</td>
<td>Sb 0.40</td>
</tr>
<tr>
<td>Sn</td>
<td>1.27</td>
<td>Zn 14.5</td>
</tr>
<tr>
<td>Fe</td>
<td>4.44</td>
<td>Fe 24.06</td>
</tr>
</tbody>
</table>

The mean values of deionized water extractable elements of antioxidant containing trace metal tubes are comparable to those of non-antioxidant tubes significantly reduced as compared to previous 10ml trace metal tubes.

The data obtained demonstrate the efficacy of all three types (plain, heparin and EDTA) of improved VACUTAINER Brand Tubes for trace element determinations.

751 INDIRECT MEASUREMENT OF DESFERRIOXAMINE (DFO) AND ITS CHELATED COMPOUNDS ALUMINOMONAXIME (AIO) AND FERRIOXAMINE (FO) IN SERUM AND URINE BY ZEEMAN ATOMIC ABSORPTION SPECTROPHOTOMETRY (ZAAS), Patrick C. D'Hasee, L.V. Lambert, and M.E. De Broe (University of Antwerp, Dept. of Nephrology-Hypertension, Antwerp, Belgium) (Spon.: S. Scarpard)

In order to study the pharmacokinetics of DFO, a well known metal chelator used in the diagnosis and therapy of iron (Fe) and aluminium (Al) overload, an indirect high sensitive method was developed for measurement of DFO and itschelated compounds AIO and FO.

AIO and FO were extracted from the accompanying matrix with benzyl alcohol at pH 7. DFO was extracted after it was converted into its by adding trivalent iron in excess. The concentration of the compounds was then assessed by measuring the Al and Fe in the organic layer with ZAAS. Special attention was paid to the pH of extraction, Fe-Al interactions, extraction times and volumes, centrifugation rates and instrumental settings and to avoid extraneous addition of AI and Fe. The addition calibration technique was used for standardization.

With our method co-extraction of unchelated Al or Fe was not observed. DFO, AIO and FO were found to be stable for at least 7 days. Recoveries of AIO and FO after extraction were respectively 97.3±3.7% and 101±3.7%. Sensitivity was 44 pg/0.0044 Abs/sec for Al and 9 pg/0.0044 Abs/sec for Fe. Detection limits were 0.08 µg/mol injection volume (injection volume 20 µl) for AIO and FO. Injection volume 20 µl for AIO, injectability 80% (0.68 µmol/l), 4.2 (10.6 µmol/l), 4% (15 µmol/l). Those for FO were 2.6 (9.8 µmol/l), 7.4 (19.6 µmol/l) and 1.4% (36 µmol/l). Only 250 µl of urine or serum was required for measurement of the three compounds.

The proposed method was found to be simple, rapid and suited for routine determinations. Moreover, it has proven to be a valuable tool in the study of the removal of Al and Fe in patients undergoing hemodialysis.

752 VALUE OF SERUM ALUMINUM (sAl) MONITORING IN PATIENTS WITH END-STAGE RENAL FAILURE (ESRF), Patrick C. D'Hasee, J.P. Clement, L.V. Lambert, M.W. Elsevier, and M.E. De Broe (University of Antwerp, Dept. of Nephrology-Hypertension, Antwerp, Belgium) (Spon.: M.E. De Broe).
Although the problem of aluminum (Al) accumulation/toxicity in ESRF patients is now well recognized, data about the value of Al-monitoring are scarce. In a retrospective multicenter study (1984-1987, N=16) we evaluated Al concentrations obtained at semestral screenings of 1,238 ESRF patients. Al levels were determined by electrothermal atomic absorption spectrometry (Clin. Chem., 31, 24-29, 1985). Laboratory performance was assured by monthly participation in an external quality control program.

The overall mean Al value of 4.035 determinations was 43 µg/L. After the mean semestral Al value reached a maximum of 53 µg/L (N=360) (beginning of 1986), mean values steadily dropped to 25 µg/L (N=591) at the end of 1987. The percentage of patients showing Al levels above the normal range, defined from 1.0 to 3.6 µg/L, fell from 51% to 6.9% of all patients showing clinical conditions associated with a higher risk for Al-accumulation was compared with the overall mean Al.

Risk factor patients (N=360) p-value

current TIA disease 77 0.005
osteomalacia 74 0.001
encephalopathy 5 0.001
diabetes I or II 116

During the study Al levels of water and dialysis solutions of all participating dialysis centers were below 7 µg/L. It is therefore supposed that an individually assessed decrease of the Al(III)-intake is the most important determinant of the observed evolution of Al-levels. Moreover, our data indicate that, provided analyses are performed by a qualified lab, regular monitoring of Al levels decreases the risk for Al-intoxication.

Evaluation of Nutritional Status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal (N=23)</th>
<th>HIV + (N=27)</th>
<th>AIDS (N=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (g/L)</td>
<td>64.8 ± 1.0</td>
<td>72.9 ± 0.9*</td>
<td>71.7 ± 1.26+</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>43.3 ± 0.5</td>
<td>43.5 ± 0.5</td>
<td>39.5 ± 0.8Φ</td>
</tr>
<tr>
<td>PALB (mg/L)</td>
<td>252 ± 13.7</td>
<td>194 ± 9.1Φ</td>
<td>223 ± 13.7Φ</td>
</tr>
<tr>
<td>RBP (mg/L)</td>
<td>41.5 ± 2.3</td>
<td>42.6 ± 2.8</td>
<td>38.0 ± 1.9</td>
</tr>
</tbody>
</table>

* p < 0.05 between HIV and normal
+ p < 0.05 between AIDS and normal
Φ p < 0.05 between AIDS and HIV

Although age-related alterations in physiology and metabolism have been studied, there has been relatively little research to define the impact of these alterations on biochemical markers used for nutritional assessment. For example, age-related alterations in biochemistry metabolites emphasize the need to use currently established adult reference data, which is usually based on young adults. The present study was performed to establish reference levels for the biochemical markers of nutritional status in a free living elderly population (76 y of age, n=171).

Values for this healthy elderly population were compared to the currently used adult references. Two biochemical measurements commonly used for assessing protein status are albumin and prealbumin (transferrin). Albumin is used in epidemiologic surveys as an index of general health status. Prealbumin, due to its short half-life of 1-2 d, is a better indicator of visceral protein synthesis and transmission a more reliable picture of protein metabolism. Mean albumin concentrations in the healthy elderly subjects was significantly lower when compared to the normal adult reference range (mean = 4.0 ± 0.4 vs 4.4 ± 0.3 g/dL, p<0.01). Likewise, mean prealbumin concentrations in healthy elderly subjects were significantly lower compared to the normal adult range (25 ± 5 vs 31 ± 4 mg/dL, p<0.01). The 95% confidence interval for the albumin data in the healthy elderly subjects was 3.1-4.4 g/dL as compared to 3.5-5.2 g/dL for normal adults. The 95% confidence level for prealbumin in healthy elderly subjects was 15-35 mg/dL compared to 19-43 mg/dL for normal adults. These data confirm that reference ranges for albumin and prealbumin should be established separately for use in evaluation of the nutritional status.

Several additional markers of nutritional status were evaluated in this same elderly population including Ca, Mg, P04, Se, Zn, Fe, Hct, carotene, choi, trig, and vitamins A, C and E.

| REFERENCE RANGES OF BIOCHEMICAL MARKERS FOR NUTRITIONAL STATUS IN AN ELDERLY POPULATION | Robert P. Labbe, Bert Toivola (Dept. of Laboratory Medicine, University of Washington, Seattle, WA 98195) (Spon. BL Betterm) |

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (Ca)</td>
<td>9-11 mg/dl</td>
</tr>
<tr>
<td>Phosphorus (P04)</td>
<td>2-5 mg/dL</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>1.5-2.5 mg/dl</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>80-130 µg/dl</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>50-150 µg/dl</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>3000-5000 IU</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>70-110 mg/dl</td>
</tr>
</tbody>
</table>

**NUTRITIONAL STATUS OF PATIENTS WITH ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS). C.M. Huang, Mark Rudden and Ronald J. Elin (Clin. Pathol. Dept., MIR, Bethesda, MD 20892.) (Spon.: C.M. Huang)**

Nutritional status may be an important prognostic factor for hospital patients morbidity and mortality. We assessed the nutritional status of normals, human immunodeficiency virus (HIV) seropositive individuals confirmed by Western blot and patients with AIDS by determining the concentration in serum of transferrin, albumin (ALB), prealbumin (PALB), and retinol binding protein (RBP). Our findings are the following (mean ± SEM):
1.6, AA in FBR 36.8 ± 0.4, and placebo in FBR 35.2 ± 0.5. Hemo-
globin paralleled Hct; 13.5 ± 0.6 with vs 12.1 ± 0.6 FBR, 12.1 ±
0.1 FER and 12.9 ± 0.1 FER with AA. In protoporphyrin (ZnPP)
(Gm/Hgb) also was affected by AA; admission values were 1.6 ±
0.2 in FBR 2.0 ± 0.6, FBR with AA 1.8 ± 0.2, and FER with place-
b 2.7 ± 0.3. The findings indicate that ferritin is the most
sensitive, and ZnPP is the least sensitive indicator of Fe sta-
High diet iron did not enhance FER. AA seemed to be
help restimate some indicators of iron status.

757
THE EFFECT OF VITAMIN A SUPPLEMENTATION ON THE VITAMIN
A NUTRITIONAL STATUS OF MIDDLE-INCOME ALCOHOLICS.
D.D. Bankson, A.M.B. Bankson, R. Schlossman3 and B.A. Underwood1
University of North Carolina, Chapel Hill, NC 27514; Tufts Univ. Sch. of Nutr.,
Medford, MA 02155; and the National Eye Inst., Bethesda, MD 20892.
(Sponsor: D.D. Bankson)

Vitamin A nutritional status was assessed using the relative
dose response test (RRT), the change in serum retinol at 5 hr
post-dosing with 600 retinol equivalents (RE); (t weights (xT/5T) x
1000) in 38 middle-income alcoholics entering a rehabilitation
center and in 31 age-matched controls. Since its initial develop-
ment in animal models, we and others have found biochemically
that the RRT is negatively correlated with hepatic vitamin A con-
centration above a critical level, i.e., approximately 20 μg/g
and therefore, it is an indirect measure of vitamin A status.

In the present study, 113 (4/38) of the alcoholics had posi-
tive (vee) RRTs (>152) while RRTs were normal (<152) for the con-
tralaboratory subjects. Serum retinol (by high performance liquid
chromatography, HPLC) was not statistically different between the 2
groups (x2SD = 34.9 μg/dl) before or after vitamin A supplemen-
tation with 3000 RE per day for 28 days. However, oral supplemen-
tation with 9000 RE daily for 7 days normalized the RRT and in-
creasing fasting serum retinol by an average of 30% in the 3 RRT vee
alcoholics available for follow-up study. Additionally, as a group,
37% (14/38) of the alcoholics had serum α-tocopherol (by HPLC)
levels below 0.5 mg/dl compared to 8% (2/31) for controls (p<0.01).
The low α-tocopherol levels normalized without vitamin E supple-
mentation during a hospital stay of 7 to 14 days.

Subclinical vitamin A deficiency and non-optimal vitamin E
status, therefore, may affect a significant sub-population of non-
cirrhotic alcoholics seeking rehabilitation.

758
Plasma Vitamin A Levels in Upper Gastrointestinal (GI) Cancer Patients.
AK Bhargava, L. Fochtler, W. Lane, H. Douglass, C
Nicholson, JE Fitzpatrick. Roswell Park Memorial Inst., 666 Elm
St, Buffalo, NY 14263. (Sponsor: Michael N. Myreis)

Role of vitamin A in the regulation of cell proliferation
and differentiation has been known. In the human low plasma
levels of vitamin A have been found in patients with gastric,
eosophageal and lung cancer. We have investigated a group of 55
upper GI cancer patients in stable condition for serum vitamin A
levels measured at 1-2 week intervals during their hospital stay
as a part of a dual nutritional survey. Patients classified
according to site of cancer origin, clinical status of the
patient, tumor burden and metastasis with their vitamin A levels
are shown in the table below.

<table>
<thead>
<tr>
<th>PATIENT GROUP</th>
<th>MEAN (μg/L)</th>
<th>STANDARD ERROR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROLS</td>
<td>20 ± 50</td>
<td>15</td>
</tr>
<tr>
<td>PATIENT GROUP</td>
<td>55 ± 30</td>
<td>15</td>
</tr>
</tbody>
</table>

PRIMARY SITE
Upper GI 42 ± 34 15 343 15
Other 17 7 504 63

TUMOR BURDEN
Small 29 12 410 31
Large 116 46 331 17

METASTASIS
No 14 16 977 66
Liver Met 50 19 328 28
Other Met 73 28 347 23

At the time of sampling.

In stable patients vitamin A levels were approximately 3X
lower than those found in normal controls. Further vitamin A
levels were substantially affected by primary site, tumor
site and tumor burden. Significance of hyperalimentation and adequate
oral diet is suggested in upper GI cancer patients.

759
Quantitative Analysis of Vitamin B6 in Plasma by HPLC
M. Z. Storer, J. Leiper, J. Leiper. American Medical
Research Center, 1515 NW 70th St., Miami, Florida 33169.
(Sponsor: J. Leiper)

We have developed and evaluated a sensitive and reliable
procedure for the determination of vitamin B6 in plasma by
high performance liquid chromatography with electrochemical detec-
tion. This technique makes use of a novel anode, the zirconia-ox-
ide anode which is very effective at oxidizing vitamin B6. A known
amount of L-tryosine, a known marker for vitamin B6, was added
to each sample before the sample was oxidized. The subsequent de-
termination of vitamin B6 by HPLC was monitored using the z
-oxide anode. The results of the assay were determined by
analyzing the vitamin B6 plasma samples to prepare stock solutions
of vitamin B6 in ethanol. In this way the vitamin B6 levels can be
compared with the vitamin B6 levels found in normal and disease
samples by simply measuring the area under the free radical
of vitamin B6 in the plasma sample. We will present our results in
abstract form at this meeting.

760
MEASUREMENT OF TOTAL THIAMIN IN WHOLE BLOOD, RBC AND
PLASMA BY HPLC WITH FLUOROMETRIC DETECTION
E. Kamin and R. Beutler. Siemens Medical Devices, Inc.,
11019 Main Street, Fairfield, CA 94023.
(Sponsor: Nancy J. Wisniski)

We have developed a simple and sensitive procedure for the determi-
nation of total thiamin (vitamin B1) in whole blood, RBC and plasma
by the pre-column thiochrome method with HPLC. Only 250 μl
of sample was required. After deproteinization with cold perchlo-
ric acid (0.9 N (21) for blood; 1.2 N (11) for plasma), 250 μl
of the supernatant of the deproteinized sample was added to 125 μl
of 1.8 M acetic buffer and incubated overnight at 37 °C with
25 μl of freshly prepared acid phosphatase (12.5 μg/ml, Sigma)
unconvert thiamine phosphate ester to free thiamin. Standards were
prepared in the same manner as the unknown samples. Prior to
injection onto the column, the total thiamin in 125 μl of the treated
sample was oxidized to thiochrome by the addition of 25 μl of 10
K2CO3(NH4) in 3 M HAc. The formed thiochrome was stable up to 4
hours in the dark and was detected fluorometrically by the excita-
tion and emission wavelengths were 365 & 440 nm respectively. The
HPLC system consists of a BSA dual piston pump (MPN-30A), a Hit-
achi fluorescence spectrophotometer (F1000), and a Supelco Supelo-
nil LC18, 3 um column coupled with 2 cm Supelguard column.
The mobile phase consisted of methanol and 100 mM of KH2PO4
(25:75, v/v, pH 7.5). The flow rate was 1.5 ml/min, and the retentio
of thiamin was approximately 4 min. We had found no endogenous
or exogenous substances that interfered with the chromatographic
assay. Post-column enzymatic conversion of thiamine phosphate
esters to free thiamine. The within and between run C.V. were 2.5% and
6.9% respectively for thiamine, and we found a recovery of 98.8% and
100.9%. The assay was linear up to 200 μg/L, and the detection
limit was 1 μg/L. We had determined the thiamine reference range
to be 100-400 μg/L for plasma and 10-70 μg/L for whole blood
(N=40), and 75-153 μg/L for RBC (N=40). Our reference range agrees
well with other methods and published reference ranges.

761
SERUM UBQUITONE ANALYSIS OF HEALTHY MALES AND FE-
MALES BY HPLC. L. A. Kaplan, E. A. Stein, and J. Fearn.
University of Cincinnati Health Care, University and Clinici-
cal Chemistry Laboratory, Cincinnati, Ohio 45267.
(Sponsor: L. Kaplan)

Ubiquitones are a series of lipid-soluble benzoquinones
involved in mitochondrial electron transport processes. Ubiqui-
tone-10 (Q10), with the longest side chain, is the ubiquitine
present in serum at the highest concentration. The HMG-CoA
reductase class of cholesterol lowering drugs has the potential
effecting the synthesis of Q10. As a preliminary investiga-
tion, we established reference ranges for Q10. We have measured
Q10 serum levels in a healthy population by an HPLC assay
modified to contain an internal standard.

Samples (1 ml) are mixed with a fixed volume of ethanol
containing the internal standard, Vitamin K1 (2.5 μg/ml) and
extracted with 4 ml of hexane. The hexane layer is removed
and evaporated to dryness. The dry samples are reconstituted
with ethanol and analyzed by HPLC: column, 5μm ODS (Bioanalytical
Systems); mobile phase, 50% acetonitril, 38% ethylaceta-	e, 10% CH3OH: flow rate, 1 ml/min; absorbance, 0.01 AUFS at 280
The average absolute recovery of Q10 added to lyophilized serum was 90.7% (n=82); when the internal standard was used, the average recovery was 103.2%. Between-day imprecision was assessed by analyzing 3 serum pools; X = 0.529 ug/ml, C.V. = 11.9%; X = 2.69 ug/ml, C.V. = 6.5%; X = 4.75 ug/ml C.V. = 5.9% (n=21, 13, 13 respectively).

Sera from healthy males (n=40) and females (n=40) between 21 and 71 years of age were analyzed for Q10 by the HPLC procedure. The 95% range for serum Q10 was 0.418 to 2.50 ug/ml for males and 0.413 to 1.091 ug/ml for females. The median Q10 level of the males (0.74 ug/ml) was significantly higher than the median Q10 levels of the females (0.59 ug/ml) (p=0.27, Wilcoxin Rank Sum Test)

**TEST RESULTS AND OTHER**

**Factors Affecting Test Results**

**EFFECTS OF BILIRUBIN ON CREATININE ASSAYS FOR THE OLYMPUS DEMAND ANALYZER. James Baldwin and Robert Janetscheck.**

It has been previously documented that elevated concentrations of bilirubin can result in a negative bias for serum creatinine assays due to its oxidation in strong bases. An evaluation for potential interference was conducted on the Olympus creatinine procedure for use on the DEMAND random access chemistry analyzer. The Olympus procedure is based upon a modification of the Jaffe method by Heinagard and Tisdalos. The procedure is a colorimetric, fixed endpoint assay performed at 37°C utilizing a bichromatic read of 520/570 nm.

In the first study conducted, human based bilirubin standards were reconstituted with known concentrations of NBS certified creatinine standards. The treated standards were then assayed in duplicate. The resulting data is presented below:

<table>
<thead>
<tr>
<th>BILL CONC.</th>
<th>EXPECTED CREAT. (MG/DL)</th>
<th>OBTAINED CREAT. (MG/DL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>15.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>10.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

In the next study, a 7.0 mg/dl NBS certified creatinine standard was serially diluted with a 20.0 mg/dl human based bilirubin standard, traceable to a standard solution prepared using NBS bilirubin (SRM 916). The results are as follows:

<table>
<thead>
<tr>
<th>BILL CONC.</th>
<th>EXPECTED CREAT. OBTAINED CREAT.</th>
<th>INTERFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.0</td>
<td>1.4</td>
<td>0.99</td>
</tr>
<tr>
<td>12.0</td>
<td>2.8</td>
<td>2.72</td>
</tr>
<tr>
<td>10.0</td>
<td>4.2</td>
<td>4.28</td>
</tr>
<tr>
<td>4.0</td>
<td>5.6</td>
<td>5.54</td>
</tr>
</tbody>
</table>

Based upon our findings, we conclude that this creatinine method will suffer from interference only when concentrations exceed 12.0 mg/dl of bilirubin.
The location of the reference value for an analyte is frequently a distribution of the population. It affects the magnitude of the population at risk due to a bias in the method. Using the Gaussian distribution, we evaluated the effect of positive proportional and systematic biases on the population at risk for four reference values for cholesterol as shown:

<table>
<thead>
<tr>
<th>% of Population Misdiagnosed due to Bias</th>
<th>Chol(g/L)</th>
<th>Proportional Error</th>
<th>Systematic Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2.1</td>
<td>6.7</td>
<td>10.8</td>
</tr>
<tr>
<td>75</td>
<td>2.4</td>
<td>6.3</td>
<td>10.6</td>
</tr>
<tr>
<td>97.5</td>
<td>2.9</td>
<td>1.6</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Men, 50-54 years old; mean = 2.137 g/L, SD = 0.37 q/L

The 97.5th and 2.5th percentiles of a "normal" population are usually chosen as the reference interval. The NHI Consensus Development Conference chose the cholesterol reference values for coronary heart disease based on outcomes (JAMA 1985;253:2080-6).

These reference values are near the 90th, 75th and 50th percentiles of the population distribution. The effect of bias on the population at risk is as much as 3- to 4-fold greater at the 75th or 50th percentiles compared with the 97.5th percentile. Further, the degree of error differs for systematic vs. proportional biases. When using reference values in the immediate context, the tail of the distribution (some instead of statistical assessment), the bias of a method has a greater effect on the population at risk.

767  DUAL EFFECT OF MYELON PARAPROTEINS ON SERUM PHOSPHORUS DEPRENITATION BY THE ATRA IDEAL SYSTEM: II. ALEXANDRINE AND M. P. Duliott (St. Mary's Hosp., Montreal, Que H3T 1S5, Canada) (Spon: H. Axonamine)

- Phosphorus concentrations measured on the Astra Ideal System were either spuriously elevated or undetectable in the sera of two patients with multiple myelomas.

- Patient #1, a 73-year-old man was investigated because of a recent onset of pain in the lower back and the right hip. Laboratory results were: total serum protein, 89 g/L; albumin, 22 g/L; and calcium, 89 mg/L. Serum immunoelectrophoresis demonstrated Ig kappa paraprotein. Phosphorus concentrations in serum were unexpectedly high, ranging from 185 to 208 mg/L. However, when protein-free filtrates were used instead of serum, phosphorus concentrations ranged from 28 to 36 mg/L.

- Patient #2, a 60-year-old male was admitted for investigation of intermittent pain in the neck and the right shoulder. Initial results showed: serum proteins, 106 g/L; albumin, 37 g/L; and calcium, 97 mg/L. Immunoelectrophoresis demonstrated Ig lambda paraprotein. Serum phosphorus concentration was undetectable on the first day of the study but was present in protein-free filtrates of the serum giving normal values. After several weeks of treatment with Melphalan, serum phosphorus concentrations became normal with a simultaneous decrease in serum globulin. Identical phosphorus values were obtained for protein-free filtrates and whole serum.

The erroneous phosphorus results observed in these two patients appear to be caused by the interaction of paraproteins with the acidic ammonium molybdate used in the Astra system. This interference may be eliminated by the use of protein-free filtrates.

768  FALSE POSITIVE CARCINOEMBRYONIC ANTIGEN TEST DUE TO HETEROPHIL ANTI-MOUSE IGG IN HUMAN SERUM, Tokuko S. Wiedemann, B. Jeffery, and P. Sohmer (The Pathology Institute, Berkeley, CA 94705) (Spon: P. Sohmer, M.D.)

Carcinoembryonic antigen (CEA) is a tumor-associated antigen frequently used to monitor the clinical course of patients with diagnosed malignancies. The recurrence of a high CEA level by one, but not another method in an otherwise asymptomatic patient was apparently survived by curtailment of rectal cancer and not requiring any other type of therapy prompted us to assay two consecutive samples of the patient by 5 methods using commercial CEA kits: A) CEA-EIA Monoclonal One-Step (Abbott), B) CEA-EIA Monoclonal (Abbott), C) Method applied to acid and heat extracted serum (Hybritech); and E) TANDEM R CEA (Hybritech). All methods were performed strictly according to the manufacturers' guideline.

Results (ng/ml):

<table>
<thead>
<tr>
<th>Method</th>
<th>specimen 1</th>
<th>specimen 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA-EIA</td>
<td>69.9</td>
<td>96.6</td>
</tr>
<tr>
<td>CEA-EIA Monoclonal</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Hybritech</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>TANDEM R CEA</td>
<td>1.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Rheumatoid factor, high titers of which are known to interfere in some EIA systems, was negative to weakly positive. The apparent high levels of CEA by method A were reduced in a non-linear fashion by addition of mouse serum and in a linear fashion by addition of 2.5 to 5.25 mg purified mouse IgG.

Conclusion: The presence of heterophil antibodies which react with mouse IgG can cause falsely elevated CEA results with One-Step EIA method, which could lead to unnecessary costly and invasive diagnostic procedures.

769  EFFECTS OF METHODS FOR 24-HOUR URINARY CATECHOLAMINES COLLECTIONS ON ELECTROLYTICS IN CLINICAL PRACTICE. Paun R. Krasnovska, Linda S. Lindquist, Debra J. Schier, American D. Freiberg, Diane R. Mitchell, Ravindor K. Singh, John C. Li, Richard H. Mantero, Frank W. Cerns (Dept. of Surg. & Dept. of Anaesth-Path St. Paul's/Ramsey Med Center, Clinic and Univ. of Miss. St. Paul's & Mepkin, MN) (Spon: H. A. Alford). The preservatives used for urinary catecholamine preservation in 24-hour urine collections have the potential of altering urinary electrolyte determinations. In order to evaluate their effect, a split sample (and vs. room temperature) was analyzed (J. Hig. Med. 1983, 84: 9.3). EDTA-Na2-SO4 (8), post-collection HC1 (5%) and no preservative (5%) study was prospectively performed on urine collected from 11 surgical/trauma patients during the same phase of the illness. Analysis of Sodium (Na+), Potassium (K+), Calcium (Ca) and Magnesium (Mg) were performed for each patient for the eight possible combinations. The following results were obtained:

- *mean ± st dev as g/ml collection time (n=11)

<table>
<thead>
<tr>
<th>Method</th>
<th>Na+</th>
<th>Ca+</th>
<th>Mg+</th>
<th>K+</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICED</td>
<td>88.3±194.9</td>
<td>74.4±352.2</td>
<td>183.7±191.1</td>
<td>77.0±196.6</td>
</tr>
<tr>
<td>H</td>
<td>223.6±157.6</td>
<td>78.4±245.5</td>
<td>202.0±240.7</td>
<td>93.2±180.5</td>
</tr>
<tr>
<td>PH</td>
<td>83.0±200.0</td>
<td>78.4±245.5</td>
<td>202.0±240.7</td>
<td>93.2±180.5</td>
</tr>
</tbody>
</table>

When compared by matched paired t-tests no detectable differences were found between samples collected with ice and at room temperature. Using ANOVA, no statistical differences were found among the eight collection techniques for the 3 analyzed tests.

770  EFFECT OF VENIPUNCTURE VERSUS CAPILLARY FINGERSTICK SPECIMEN COLLECTION UPON CHOLESTEROL TESTING IN THE GROUP SETTING. Jay B. Jones and Mary Jaccorsi (Dept. Lab. Med., Geisinger Medical Center, Danville, PA 17322) (Spon: A.J. Shulski)

The objective of this study was to determine the suitability of a fingerstick specimen in the type of group cholesterol screening test that is projected to become clinically relevant in the near future.

Specimens were obtained from 47 normal volunteers using three collection methods as follows: 1) antecubital venipuncture using a 5 ml vacuum tube, 2) self-administered capillary fingerstick using the Benne collection system (Bennet Inc., Phil., PA), 3) phlebotomist-administered capillary fingerstick using the Benne system. Each of the three specimens was obtained from the respective volunteer within 20 minutes under conditions mimicking a group specimen collection setting.

Each blood specimen was separated, placed in a sealed analyzer tube, and assayed for total cholesterol and lactate dehydrogenase (LDH) within four hours of the collection (Roche Cobas FARA, Nutley, NJ). A separate aliquot cup was used to measure potassium (Kodak Ektachem, Rochester, NY).

<table>
<thead>
<tr>
<th>LDH</th>
<th>POTASSIUM</th>
<th>CHOLESTEROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>184</td>
<td>214</td>
<td>214</td>
</tr>
</tbody>
</table>

Potassium and LDH exhibited unacceptably high positive biases and poor correlations in comparison to fingerstick collection methods (y). Cholesterol showed acceptable correlation amongst the three collection methods in specimens that showed presumed hemolysis and tissue disruption during fingerstick collection (i.e. - elevated potassium and LDH).

In summary, capillary fingerstick collection may be an adequate specimen collection technique for the group cholesterol screen.
F. Lucas (Goulter Diagnostics DiL. Hialeah, Fl 33014 (Spon.: B. Steele)


Zweig, et al (Clin. Chem. 33:6)408-844, 1987) demonstrates that this approach does not always eliminate this interference in immunoassays. The DAKO CK-MB immunohiassay (Goulter Diagnostics DiL) was shown to be insensitive to non-immune sera and has appeared adequate in previous clinical trials. However, in one clinical study in a county hospital, 5 of 80 patients were falsely positive for CK-MB. Consequently, the 80 patients were tested for anti-species antibodies and their titer.

Using an in-house immunoelectrophoresis procedure, 264 (21) indicated the presence of antibodies for anti-got and anti-mouse antibodies. Ten demonstrated extremely high titers. Assays for rheumatoid factor (125 Scientific) and C1q (Kent Laboratories) on these patients were negative.

In conclusion, even 5x the suggested amount of blocking antibodies appears insufficient for some populations & will require a new approach to remove this interference.

INTERFERENCE IN THE KODAK EKTACHEM CREATININE METHOD BY N-ETHYLGLYCINE, A METABOLITE OF LIDOCAINE. Randall T. Hobbs, Nicholas W. Alexander, Michael J. Kaliner (Div. of Laboratory Medicine, Dept. of Pathology, U.C.S.D. Medical Center, San Diego, CA 92103). (Sponsor: R.T. Roberta)

We and others have noticed that sera from patients receiving lidocaine show positive interference when assayed for creatinine by the single-slide method on the Kodak Ektachem 700.

Consideration of the metabolism of lidocaine and the enzme cascade employed on the Kodak creatinine slide suggested that the interference was due to oxidation of a lidocaine metabolite, N-ethylglycine, by the sarcosine oxidase in the slide.

To assess this possibility, we synthesized N-ethylglycine from monochloroacetic acid and ethylamine. The identity of the N-ethylglycine was verified spectrophotometrically. Human serum was spiked with increasing amounts of N-ethylglycine and assayed for creatinine on the Ektachem 700 using first (gen01) and fourth generation (gen04) reagents.

The results showed increasing interference with increasing N-ethylglycine concentration in a linear fashion as follows:

- N-ethylglycine (mg/dl) gen01 bias (mg/dl) gen04 bias (mg/dl)
- 0.0 1.3 0.8
- 2.0 3.4 1.9
- 4.0 6.4 3.4
- 6.4 11.4 5.7
- 10.0 16.1 7.7

Interference with the fourth generation creatinine slide is less than that with the first generation slide but is still significant enough that some patients receiving lidocaine appear to have markedly elevated creatinine. We are measuring the N-ethylglycine levels in these patients and correlating them to the degree of interference with the Ektachem creatinine assay.

The results demonstrate that N-ethylglycine, a metabolite of lidocaine, can interfere with the first and fourth generation creatinine slides on the Kodak Ektachem 700.


We have recently started using a one-step sandwich immunoassay (Tandem<sup>™</sup>-PSA) for prostate specific antigen (PSA) marketed by Hybritech Inc. (San Diego, CA). This assay is linear up to 100 ng/mL. While we reviewed results of 435 consecutive samples submitted for PSA measurement over a 5 month period, we identified 3 patients with extremely high PSA levels of 650, 1400, and 3200 ng/mL. The acid phosphatase values in these samples were 3.2, 1137, and 2.8 fold, respectively. In these 3 patients, the acid phosphatase values did not correlate with the PSA values. When two serum samples (A and B) from the patient with the highest PSA level was serially diluted, we obtained the following apparent PSA values:

<table>
<thead>
<tr>
<th>Serum dilution (fold)</th>
<th>Apparent PSA (mg/ml)</th>
<th>Serum dilution (fold)</th>
<th>Apparent PSA (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>undiluted</td>
<td>106</td>
<td>102</td>
<td>1070</td>
</tr>
<tr>
<td>1.7</td>
<td>349</td>
<td>10</td>
<td>1970</td>
</tr>
<tr>
<td>2</td>
<td>328</td>
<td>20</td>
<td>3000</td>
</tr>
<tr>
<td>2.5</td>
<td>520</td>
<td>60</td>
<td>3120</td>
</tr>
</tbody>
</table>

We noticed that the apparent PSA values of the undiluted serum samples were lower than the PSA values obtained after dilution of the sera. This observation is consistent with the phenomenon of the "hook effect". These results suggest that when Tandem<sup>™</sup>-PSA assay is used for the measurement of PSA from undiluted serum with extremely high level of PSA, apparently lower values could be obtained.

SPECTROMETRIC MAGNIFICATION DETERMINATION ON THE IL MULTISTAT III CORRECTED FOR INTERCUVETTE ABSORBANCE DIFFERENCES. Mary Todd Smith, and Todd Light (Veteran Administration Medical Center and Vanderbilt Univ., Path. Dept., Nashville, TN 37212) (Spon.: Mary Carol Smith)

To improve the precision of an endpoint colorimetric magnesium determination performed on a centrifugal analyzer we investigated methods to correct for differences in absorbance readings on the IL Multistat III the manufacturer specifies an intercuvette absorbance tolerance of 0.01 units at an absorbance of 1.6, however this absolute error is constant over the range of the instrument. For the American Monitor calomelode magnetism assay, we used, the absorbance change is 0.018 Absorbance Units per mg% of Mg. As a result intercuvette absorbance differences can account for errors of as much as 0.5 mg/L, a clearly unacceptable error for clinical use of the assay on this instrument.

We describe a modification of a standard calomelode magnesium determination which applies an absorbance correction at 300 nm. The assay reagents were prepared as described by the manufacturer. The total final volume of reagents was 228 ul of which 208 ul was reagent with a wash volume of 18 ul, and 2 ul was sample with a wash of 18 ul., the reference cuvette was a reagent blank. The initial reading is obtained at 308 nm at 1 minute after mixing and a second is made at 558 nm. The delta absorbance is linearly related to magnesium concentration up to 3.5 mg/l.

The method, as modified, is linear over the range from 0.0 to 3.5 mg/L. Within run precision is 0.0,4 mg/L, and at 3.0 mg/L is 2%. Between run precision is 2% and 4% for the same concentrations. Correlation with the manual method described by the manufacturer yielded an intercept of -0.12 mg/L and a slope of 1.86 ; r=0.96. For centrifugal analyzers with disposable cuvettes a single wavelength correction compensates for intercuvette absorbance discrepancies that may be the principle source of analytical error.

EFFECT OF FINGERSTICK BLOOD COLLECTION AND FASTING STATUS UPON SERUM CHOLESTEROL. Harvey Jones and B. Jackson (Hendrick Med. Ctr., Dept. of Path. Athens, TX 78601) (Spon. H. Jones)

To better educate the public in factors contributing to increased risk of coronary artery disease "TODD", our laboratory utilizes the American Heart Association (AHA) Heart Hazard Appraisal (Risk) form and cholesterol (CHO) levels determined from fingerstick. This method allows collection and testing in remote locations.

To determine the effect of fingerstick and fasting status upon the CHO level, data from 897 fasting outpatients (OP) and 515 non-fasting blood donors (BD) collected by venipuncture was compared to 244 non-fasting participants collected by fingerstick in a local health fair. CHO levels were determined on the serum samples with an Astra Ideal Chemistry Analyzer (Beckman Instruments) in May, 1987.

The mean CHO level for age groups C20, 20-29, 30-39, and 40 years was determined for each group of patients. The correlation coefficient was 0.89, slope 0.84 for fasting OP vs. non-fasting BD. The correlation coefficient was 0.98, slope 1.00 for OP venipuncture vs. health fair fingerstick participants.

Specimens collected from non-fasting patients by fingerstick did not display significant differences from fasting patients collected by venipuncture. The mean CHO for fasting, non-fasting, and fingerstick patients >40 years was 219, 212, and 224 mg/dl, respectively.

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In summary, total cholesterol determined on samples collected by fingerprick on fasting patients is reliable in monitoring the risk of coronary artery disease.

We evaluated and compared the performance of new and washed rotors for the centrifuge analyzer. Precision and accuracy were determined by analyzing laboratory QC material for twelve chemistry tests with absorbance measurements at various wavelengths – from 380 to 600 nm. The F test was used to statistically verify the results. Only for Calcium at both levels was the F test statistically meaningful at the 95% confidence limit. Therefore for IL tests at two levels the F test indicated no difference between the new and washed rotors. To further study the Calcium test with new and washed rotors, 49 normal subjects were tested for calcium using new and washed rotors and results correlated and evaluated. The correlation coefficient was 0.95 and the regression line was y(washed)=1.00x(new)+0.18. The F test was higher than the critical value indicating that the difference between the two means (9.64 vs 9.78) is statistically significant. The diagnostic performance of the calcium test was determined and using the washed vetticle would have resulted in 4 out of 49 tests being false positives and one false negative. In only two of these five cases, however, were the Ca results more than 0.3 mg/dl different.

The actual coefficients of variation for new and washed rotors were compared with the manufacturers stated precision values. Using new rotors resulted in 8 of the 24 tests outside the manufacturers limits and the washed rotors yielded 9 tests outside the limits. We conclude that either a narrow wavelength contamination from the washing procedure or an idiosyncrasy of the calcium assay itself is causing the observed discrepancy. Nevertheless, a statistically valid discrepancy does not necessarily imply a clinically significant discrepancy and considering the substantial cost savings in using washed rotors, each lab will need to decide the issue.

We detected a significant negative interference on the Olympus AU5000 with uric acid values with elevated total bilirubin. The Olympus method for the AU5000 utilizes uricase and the oxidative coupling of 4-aminophthalein (4-AAP) and sodium hydrox-3,5-dichlorphenol. Samples with increasing total bilirubins were compared with two different uricase-dye methods on the AU5000 and with the DuPont ACA method, the latter 1% uricase method directly measures the decrease in uric acid at 293 nm. The alternative uricase-dye method used on the AU5000 was from Boehringer Mannheim (BMS) and uses an N-ethyl-N-

**THE EFFECTS OF BILIRUBIN ON TWO DIFFERENT URICASE METHODS FOR URIC ACID ON THE OLYMPUS AU5000 AND ACAs**

**METHODS**

We investigated additives claimed to invalidate EIA drug assays to learn to identify adulterated urines so they might be rejected.

Adulterants were added at several levels to more than 220 EIA positive specimens confirmed by GC/MS for illicit drugs. Specimens were reanalyzed by the EIA screening procedures using a Hitachi 704 analyzer.

In the highest levels evaluated, the adulterants (1-MeCl, 1-

```
II III IV V VI

Amphetamines X X
Barbiturates X X
Benzodiazepines X X
Cocaine X X
Oxales X X
Cannabinoids X X X X X
```

To identify adulterated urines, we monitored pH, relative density, urine color and turbidity at different levels which falsified the EIA results. Specimens contaminated with MeCl had relative densities greater than 1.035. Drug or vinegar caused non-physiological pH's. Golden Seal tea caused a dark appearance, and specimens containing liquid soap were unusually cloudy. Visine® was the only adulterant not detected.

Since EIA assays can be invalidated by specimen adulteration, drug testing should include assessment of pH, relative density and appearance; suspect specimens should be rejected. Because not all adulterants can be detected, observed collection is recommended.

**ALTITUDE AND RELATIVE HUMIDITY INFLUENCE RESULTS PRODUCED BY GLUCOSE METERS USING DRY REAGENT STRIPS, MAUDRY GREGORY, FRANK RYAN, J. CRAIG BARNETT AND TERESA TOUTZ (SAINT JOSEPH HOSPITAL, DENVER, CO, 80218).**

Four glucose reagent strip systems were evaluated for their dependence on changes in % relative humidity (R%H) and partial pressure of oxygen (pO2). These studies were prompted by observations in Denver (altitude 1600 meters) during low humidity seasons that the linearity of certain reagent strip systems did not meet manufacturers' claims.

The systems tested were the Accu-Chek II (A-II, Boehringer Mannheim Diagnostics), Glucoscan 3000 (G. Lifescan, Inc.), Glucometer II (G-II, Ames Division of Miles Laboratories) and Diascan (D. Home Diagnostics, Inc.). An isolote was sealed and perfused with varying mixtures of oxygen and nitrogen to achieve pO2 levels similar to that at sea level and 1600 meters. The gas mixtures were passed through either water or air dryers to obtain high and low RH in the chamber. Whole blood samples and, where feasible, aqueous standards were analyzed for glucose in the chamber. The table below shows the maximum % change observed when one atmospheric condition was modified while the other was held constant. The pO2 was changed from 100KPa to 20KPa. The RH was changed from 20% to 85%.

**CHANGING RH**

<table>
<thead>
<tr>
<th>Change in RH</th>
<th>A-II</th>
<th>D</th>
<th>G-II</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>+20%</td>
<td>-3%</td>
<td>+1%</td>
<td>+5%</td>
<td>+6%</td>
</tr>
</tbody>
</table>

In many cases the combined effect of changes in RH and pO2 caused variations in excess of those listed above. Variations in results were statistically significant (p<0.05). Samples having higher glucose levels were most affected by low RH and low pO2. We conclude that both humidity and pO2 changes can significantly influence the results obtained using glucose meters.

**DISTRIBUTION OF THE POTENTIALLY INFLUENCING SUBSTANCES**

**HEMOLYSIS, ICTERUS AND LIPIDEMIA IN SERUM SPECIMENS SUBMITTED FOR QUALITY TESTING, ARNOLD RYDER AND MALCOLM KILCH (DEPT. PATH., WIDN PROV. MEDICAL CENTER, INDIANAPOLIS, IN 46205).**

To determine how frequently significant concentrations of hemolysis, bilirubin and lipide mia occurred in serum specimens and whether these potentially interfering substances could be accurately detected visually. Fresh specimens from hospitalized patients were compared with a series of reference specimens supplemented with known amounts of hemoglobin, bilirubin or lipemia (intralipid). The reference specimens, identical to those we used to prepare samples of unknown concentration, were stored in truce, or 1:4 through 5:1, corresponding to increasing amounts of interfering substances. For all specimens judged to be 1+ or greater, the actual concentration of bilirubin, hemoglobin or triglyceride was determined chemically.

Significant (1+ or greater) hemolysis, icterus or lipemia was seen in 27% of 8900 (26%) specimens; in 107 these specimens, 2 interventions were present simultaneously. 88% (80.8%) of the specimens were icteric. Of 28 specimens there were icteric (1.3%) 6; 77 (3.5%) 6; 72 (8.0%) 9; 49 (1.9%) 844 (9.4%) showed icterus (1.3%) 6; 80 (0.8%) 9; 88 (1.0%) 8; 348 (3.7%) were lipidemic (4.4%) 6; 34 (0.4%) 9; acetaminophen (1.0%) 8; 12 (0.9%) 9; 7 (0.8%) 8; 6 (0.8%) 8; 2 (0.3%).
Little correlation was found between the original grade assigned and the actual concentrations of interferent. For all three interferents the highest actual concentration graded 1.0 was greater than the lowest concentration graded 0 or 4.

We conclude that the potential interferents hemoglobin, bilirubin and lipoprotein occur frequently in sera from hospitalized patients, and that it is not consistently possible to visually distinguish among the concentrations of interferents examined here. The concentrations of various interferents found in our hospital population would have caused significant errors in selected analyses, were it not for the fact that we used methods (mainly dry-film technology) which are almost completely interference-free.

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We have determined over 1500 tricyclic antidepressants by Syva EMIT system in our laboratory. The drugs assayed are amitriptyline, nortriptyline, imipramine, and desipramine. Precision studies were performed using two levels of quality control material. The results are summarized below:

### Drug Level n %CV Level n %CV

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level</th>
<th>n</th>
<th>%CV</th>
<th>Level</th>
<th>n</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMITRIPYL1NE</td>
<td>40 ng/mL</td>
<td>76</td>
<td>6.9</td>
<td>200 ng/mL</td>
<td>69</td>
<td>6.7</td>
</tr>
<tr>
<td>NORTRIPYL1NE</td>
<td>40 ng/mL</td>
<td>80</td>
<td>6.6</td>
<td>200 ng/mL</td>
<td>80</td>
<td>9.1</td>
</tr>
<tr>
<td>IMIPRIMINE</td>
<td>40 ng/mL</td>
<td>78</td>
<td>7.6</td>
<td>200 ng/mL</td>
<td>80</td>
<td>6.4</td>
</tr>
<tr>
<td>DESIPRIMINE</td>
<td>75 ng/mL</td>
<td>75</td>
<td>7.0</td>
<td>400 ng/mL</td>
<td>75</td>
<td>13.8</td>
</tr>
</tbody>
</table>

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The concentrations of aminotriptyline were determined by the Hitachi CRT method. Sixty-nine patients were studied. In the specimens considered, aminotriptyline showed no significant correlation with the calculated serum alkali value. The correlation coefficient was higher than 0.71 (r = 0.80, p < 0.05) between the aminotriptyline concentration and the base excess calculated. The correlation coefficient was higher than 0.71 (r = 0.80, p < 0.05) between the aminotriptyline concentration and the base excess calculated. The correlation coefficient was higher than 0.71 (r = 0.80, p < 0.05) between the aminotriptyline concentration and the base excess calculated. The correlation coefficient was higher than 0.71 (r = 0.80, p < 0.05) between the aminotriptyline concentration and the base excess calculated.

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 Seventy-one patients were studied. Among the 71 patients, 10 were found to have high concentrations, 25 were normal, and 36 were low. In our laboratory, the ratio of aminotriptyline to total serum calcium was found to be 1.0 ± 0.2 (mean ± standard deviation). This ratio was significantly different from that found in the literature (mean = 0.9, standard deviation = 0.2).

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The conflicting relationship of Ca++ values with respect to pH, pCO₂, and lactate in vitro and in vivo was studied by Biochemistry Laboratory, Meaux Hospital, 77104 MEAUX FRANCE (Spon: Bernard Gouyet). This was found to be 0.74 for acute changes induced by either lactate content or pCO₂ variations, both in vitro and in vivo. The tests were performed on 4 instruments (1C31, 1C23, NOVA B. CORNING 634). For pCO₂ variations, tonometered sera were used with pH ranging 7.8-7.0, and hyperventilation (HV) was monitored for 3 min. in six supine subjects (pH, Ca++, Lactate, TCA, T Protein).

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As a preliminary study in a step on the effects of gallic acid on the pH standardization of ionized calcium (Ca⁺⁺) measurements in blood, the change in Ca⁺⁺ obtained by modifications induced by pCO₂ variations was investigated on a normal serum pool from 10 different apparatus. This type of study should yield a linear relationship between the pH sensitive calcium buffer capacity of the specimen. The pCO₂ correction line should be horizontal. The results showed a high correlation coefficient (0.95), indicating that the pCO₂ correction line is horizontal. The results showed a high correlation coefficient (0.95), indicating that the pCO₂ correction line is horizontal.

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tion-dilution"; 2) with sodium heparinate to study binding; 3) with heparinate titrated "calcium-dilution". Syringes containing dry calcium titrated heparinate ("pure calcium dilution") were also tested. For each type of mixture the mean of 20 measurements have been compared to the dry syringe result taken as a reference.

"Solution-dilution" is minimal with 5 ml syringes (<1%), it increases when the syringes are not used to their nominal capacity and can be significant (5%) on small specimens. Binding by sodium heparinate is important in all cases from 15 to 50%. It must be emphasized that even in best sampling conditions (5 ml syringe) the fact that a "syringe rinse" might introduce as much as 40 IU/ml heparinate, well above the recommended maximum limit of 15 IU/ml, causes a 5% decrease in ionized calcium results. With calcium titrated heparinate modifications are minimal in dry as well as wet conditions (less than 0.5% on 1.25 mm Ca++ mixtures). Modifications remain below 7% for high Ca++ and range from 1 to 10% according to sampling conditions for low Ca++ values.

785  EFFECT OF HEMOCRIT ON 3 BLOOD GLUCOSE MONITORING SYSTEMS, Raymond Poore and Irvin Hinberg (Bureau of Radiation and Medical Devices, Health and Welfare Canada, Ottawa, KIA OZ2) (Spon. D. Pringle)

We measured the effect of hematocrit (Hct) on blood glucose readings with 3 new blood glucose monitoring systems: glucometer strip/Glucosestat/ Glucostat II (B) and Chemstrip BG/Accu-check II (C). Blood containing 14 mmoles glucose/L, adjusted to 18-75% Hct with water was used. The table below summarizes our results expressed relative to readings at 44% Hct.

<table>
<thead>
<tr>
<th>Hct</th>
<th>Relative Blood Glucose Readings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>75</td>
<td>0.55</td>
</tr>
<tr>
<td>65</td>
<td>0.67</td>
</tr>
<tr>
<td>56</td>
<td>0.81</td>
</tr>
<tr>
<td>47</td>
<td>0.97</td>
</tr>
<tr>
<td>38.5</td>
<td>1.06</td>
</tr>
<tr>
<td>29.5</td>
<td>1.16</td>
</tr>
<tr>
<td>18.1</td>
<td>1.17</td>
</tr>
</tbody>
</table>

The table shows that B is the most affected and C, the least affected by variation in Hct. The inhibitory effect of increased hematocrit is not due to increases in viscosity, since adding hyaluronic acid to increase the viscosity of blood specimens from 6.8 mpa.s (45% Hct) to 30 mpa.s (equivalent to >80% Hct) had no effect on blood glucose readings. The differences in susceptibility of the 3 reagent strips to changes in Hct appears to be due to differences in the surface properties of the reagent pads. In view of these findings the strips should not be used in clinical serum samples with extreme variations in Hct (e.g. anemic or neonates) are likely to be found.

787  SERUM PROTEIN AND ALKALINE PHOSPHATASE CONCENTRATIONS MAY EFFECT THEOPHYLLINE MEASUREMENT BY THE KODAK EKTACHEM 400, Glen Herrin, Carl H. Smith, Gal E. Stahlschmidt, Brad V. C. Lau, and Michael Landt (Depts. Pediatrics and Pathology, Washington Univ. School of Medicine, St. Louis, MO 63110) (Spon: M. Landt)

The theophylline method of the Kodak Ektachem 400 employs a unique approach—measuring theophylline's inhibition of bovine alkaline phosphatase (AP). We evaluated this assay with regard to accuracy, precision and freedom from interferences. Recovery of theophylline added to normal serum over the range of 0-40 mg was excellent (Ektachem = 1.01 ± 0.05, r = 0.999). Determination of a second alternative method indicated close agreement (Ektachem = 0.98 ± 0.08, r = 0.989, n = 20). Within-run precision was good (CV = 6.1% at 7.2 mg/L, CV = 2.6% at 16.5 mg/L). In interference studies, two effects unique to the Ektachem assay were noted: 1) SER with high levels of AP caused negative interference. Measured theophylline was decreased about 3 mg/L by AP at 1,000 U/L. Similar effects resulted from addition of bovine AP to serum. 2) At normal protein concentrations, there was close agreement between Ektachem and IRMA measurements. However, theophylline values increased about 6% for each 10 mg/dL decrease in total protein. Dilution experiments showed that the effect was consistent with high-affinity binding of theophylline by an unidentified serum protein (not albumin). Ektachem calibration and serum-based standards compensated for the binding at normal protein levels, but the interference became clinically significant when binding was reduced in hypoproteinemic sera.

788  ENHANCED SOLUBILITY OF GAMMA-GLUTAMYL-PARA-NITROANILIDE BY ALPHA-CYCLODEXTRIN, M. Shirazi, Dan Vlasteli (Technicon Instruments Corp., Tarrytown, NY 10591) (Spon. S. Yun)

L-γ-glutamyl-p-nitroanilide (GNA) has been widely used for the determination of gamma-glutamyltransferase (GGT) in serum because of its sensitivity and capability of direct measurement of reaction. The method has been recommended by the committee of the Scandinavian Soc. for Clinical Chemistry for routine usage in clinical laboratories. The subject low solubility and instability, thus negating the use of an optimal concentration in the reaction and the use of an added reagent. Its solubility and stability have been markedly improved by Cyclodextrins (Kawae & Ono, J.Clin. Chem. Clin. Biochem. 19, 727, 1981). We have investigated the possibility of improving the reaction stability by addition of alpha-cyclodextrin (α-CD) and have studied its effect on the kinetics of the GGT assay. The absorption spectra of both chromophores are shifted to a longer wavelength by cyclodextrin and form an isosbestic point, signifying a formation of inclusion complexes in a 1:1 ratio. The absorbivity of p-nitroaniline is greatly increased. The Michaelis constant (Km) for GNA is affected, 1.8 mM without and 1.4 mM with α-CD. The dissociation constant of GNA-α-CD and p-nitroaniline-α-CD determined spectrophotometrically is 14 X 10⁻³ and 1.2 X 10⁻⁷ mol/L, respectively. Thus the enhancement of GNA solubility and stability is achieved by the formation of inclusion complexes.


Cytosine arabinoside, a pyrimidine antimetabolite, is a primary drug in the treatment of acute non-lymphoblastic leukemia. High-dose Ara-C (HDAC) regimens are used to improve therapy in relapsed or refractory leukemia patients. However, there are no stated therapeutic or toxic levels for Ara-C concentrations in blood. Our objective was to develop a simple and rapid method for Ara-C and its metabolite, uracil arabinoside, in order to establish a toxic threshold.

Following Ara-C infusion, blood was collected and preserved with tert-butanol (80%/20%) and separated; and, after addition of an internal standard, 5-methoxytindine, the serum was deproteinized with acetonitrile, dried, reconstituted, and analyzed by C18 reverse-phase HPLC (Waters). The mobile phase was 0.5 M ammonium acetate, pH 6.5 at 1.5 ml/min. Detection was at 290 nm, and the peak area was quantified by peak-height ratio. A linear standard curve (r = .999) was constructed from 0-100 µg/ml Ara-C. The CV for within-run and day-to-day precision were 2.0 and 4.3% for a 20 µg/ml control and 2.7 and 2.7% for an 80 µg/ml control, respectively. Recovery of Ara-C from leukemia serum showed that the concentration of TNW used prevented its degradation to Ara-U. A matrix study was done, as well as an interassay study, using 29 drugs and 11 endogenous compounds. Low levels of endogenous material impinged on the sensitivity of detection of Ara-C. The method of patient decay indicates the feasibility of the method for this study.


We have developed an enzyme immunoassay for the specific testing of hepatitis B surface antigen (HBsAg) in serum and plasma using chromium dioxide magnetic particles (DPM-FCAs) in enzyme immunoassay (EIA) and enzyme linked immunosorbent assay (ELISA). The method (total time 1.5 hr) and meets the definition of a "third generation" HBsAg test.
Briefly, the particles were coated with a murine monoclonal anti-HBsAg antibody. Serum or plasma samples (200 μL) and a citrate buffer (100 μL) were incubated with the particles for 30 min at 37 °C. The particles were washed twice with distilled water and then washed three times. Conjugate reagent (50 μL) consisting of a second anti-HBsAg monoclonal antibody linked to alkaline phosphatase, was added to the particles and incubated at 37 °C for 20 min. A fluoroetric substrate, 4-methylumbelliferyl phosphate in diethanolamine buffer, was added and incubated for 30 min. At 37 °C, EDTA was added to quench the enzymatic reaction. The fluorescence was measured in a fluoroscent microtitr plate reader.

When tested against Paul Ehrlich Institute HBsAg reference serum, the prototype essay exhibited a sensitivity of 0.2 mg/mL for IgG subtype and 0.3 mg/mL for IgM subtype. It can be used for testing of both serum and plasma. The assay correctly identified all positive and negative samples in FDA HBsAg Panel No. 8. No "hook-effect" was observed when up to 5 μg/mL of the antigen was tested. The assay can be adapted to either manual or automated forms.

**EVALUATION OF THE MILLI-Q® PLUS WATER SYSTEM TO PROVIDE REAGENT GRADE WATER FOR USE ON THE DIMENSION® CLINICAL CHEMISTRY SYSTEM AND THE ace® DISCRETE CLINICAL ANALYZER.** David Miller and M. Church (Brendlywine Hospital, Costa Mesa, CA 92628) and S. Malhotra (E. I. du Pont de Nemours & Co. [Inc.], Medical Products Department, Wilmington, DE 19899) (Sponsored by W. R. Grace)

We have undertaken this study to determine if water purified through Milli-Q® Plus water systems (Millipore Corporation) yields equivalent chemistry performance to water purified through MILLI-Q® (Type I as defined by NCCCLS) when used with the DIMENSION® clinical chemistry system and the ace® discrete clinical analyzer.

Water quality was constantly monitored by an on-line resistivity meter. Water quality testing for all test samples was performed by the DIMENSION® chemistries. Their results were selected for testing, based on their reaction mechanisms and sensitivity to water quality. Studies were performed using Du Pont purified water (control) and MILLI-Q® Plus purified water (test).

With run-to-run reproducibility is comparable. Representative data are shown below. Patient results, as well as water blank values, are unaffected by the type of water used. Bacterial content was always negative at the endpoint.

**Recalibratability (Day-to-day, Mean [SD])**

<table>
<thead>
<tr>
<th>Method</th>
<th>Control Test</th>
<th>Control Test</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHOS mg/dL</td>
<td>5.5 (0.08)</td>
<td>3.8 (0.20)</td>
<td>3.9 (0.11)</td>
</tr>
<tr>
<td>IRN μg/dL</td>
<td>128.5 (12.4)</td>
<td>127.0 (3.44)</td>
<td>122.9 (3.05)</td>
</tr>
<tr>
<td>AMOCl μmol/L</td>
<td>30.4 (1.42)</td>
<td>29.7 (1.22)</td>
<td>30.9 (1.10)</td>
</tr>
<tr>
<td>Water Blank Extinction (Mean [SD])</td>
<td>19.5 (1.00)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In summary, the MILLI-Q® Plus water system provides a constant supply of high quality water for use on the DIMENSION® and the ace® presents a viable alternative to the storage of bottled water.

**A NOVEL MONOCLONAL ANTIBODY TO RECOMBINANT HEPATITIS B CORE ANTIGEN IMMUNOASSAY FOR ANTI-HBc TOTAL Ig USING CHROMIUM DIOXIDE MAGNETIC PARTICLES AS SOLID SUPPORT.** Martin Adnich, T. Hatfield and D. Mihm. (E. I. Du Pont de Nemours Co. [Inc.]), Medical Products Department, Wilmington, DE 19899) (Sponsored by J. W. Freytag).

Commercial immunoassays use polyvalent antibodies to native Hepatitis B core antigen which has been purified from blood or plasmapheresed material from human donors. In this study, we describe a novel immunoassay for anti-HB core total Ig using monoclonal antibodies to recombinant HB core antigen that compete effectively with human polyvalent antibodies for native HB core antigen.

The fluorometric enzyme immunoassay combines chromium dioxide particle containing covalently linked anti-HB core total Ig and monoclonal antibody to recombinant HB core antigen with serum or plasma (100 μL) samples for 15 minutes simultaneous incubation. Magnetic separation and aspiration are followed by the addition of monoclonal anti-HB core antibody conjugated to alkaline phosphatase (250 μL) and incubation continued for 15 minutes. The reaction mixture is separated followed by washing and 200 μL of 2.5 mmol 4-methylumbelliferyl phosphate is added. After 5 minutes, the reaction is quenched with EDTA. All incubations are at 37 °C.

The fluorescent response is inversely proportional to anti-HB core total Ig.

This assay displays a 250-fold dilution range in sensitivity, a with-run precision for positive control of 99% CV (n=19) and for negative control of 8.3% CV (n=19) and a correlation coefficient of 0.96 (n=72) when compared with the three hour Abbott COREZYMEX® EIA assay. The research prototype anti-HBc total Ig assay is fast, sensitive and yields results that are comparable to the Abbott assay.

**A FLUOROMETRIC ENZYME IMMUNOASSAY FOR THE DETECTION OF HEPATITIS A ANTIBODY (IgM) USING CHROMIUM DIOXIDE MAGNETIC PARTICLES AS SOLID SUPPORT.** Walter W. Russian and Carol J. Luttonson. (E. I. du Pont de Nemours & Co., [Inc.], Medical Products Department, Wilmington, DE 19899) (Sponsored by J. E. Davis).

An assay has been developed for the detection of human antibodies to Hepatitis A Virus (HAV) in sera. Sample (10 μL), diluted 1:100, is first incubated with biotinylated anti-human IgM (100 μL) to form the complex. Next, streptavidin-horseradish peroxidase (100 μL) are added to partition the complex. The particles are washed and then incubated with HAV (250 μL). Unbound HAV is washed away and the remaining antibody is incubated with anti-horseradish peroxidase conjugate (250 μL). Finally, unbound conjugate is washed away and 4-methylumbelliferyl phosphate in diethanolamine buffer (450 μL) is added to develop fluorescence.

The sensitivity of the assay has been shown to be equivalent to Abbott's HAVAB-M test (overnight incubation) by the evaluation of serial dilutions of immune sera (cut-off at 1:500). Specificity of the prototype assay is 97%. Coefficients of variation (n = 20) for the non-reactive control were 10.7% (va. 17% for HAVAB-M) and 5.7% (vs. 11.7% for HAVAB-M) for the reactive control.

This prototype assay can be completed in 3.5 hours (compared to overnight for the HAVAB-M method) and exhibits sensitivity, specificity and within-run precision that is comparable to the Abbott assay.

**USE OF CHROMIUM DIOXIDE PARTICLES FOR RAPID DETECTION OF ANTIBODIES SPECIFIC FOR HUMAN IMMUNODEFICIENCY VIRUS.** Robert Diegan and William J. Moran (E. I. du Pont de Nemours & Co., [Inc.], Medical Products Department, Wilmington, DE 19899) (Sponsored by J. A. Bueg). We have developed a chromium dioxide particle-based assay that detects antibodies specific for Human Immunodeficiency Virus (HIV) in serum or plasma. The assay is performed by incubating sample (10 μL), antibody-coated (100 μL) and substrate solution (200 μL) which are covalently coupled with HIV viral proteins, for 30 minutes at 37 °C. The particles are then washed to remove nonspecific antibodies and are incubated with alkaline phosphatase-conjugated IgG (200 μL) for 30 min at 37 °C. After washing to remove unbound conjugate, the particles are incubated with 4-methylumbelliferyl phosphate for 5 minutes at 37 °C. The fluorescence generated is directly proportional to the quantity of HIV-specific IgG bound.

Preliminary evaluations of the performance of the assay indicate that assay precision, sensitivity and specificity compare favorably to existing methods. Typical intra- and inter-assay CV's are less than 10% and, based on test results from 2148 normal donors and 106 western blot confirmed positives, the sensitivity and specificity of the assay are 99.9% and 100%. Respectively. Additionally, no false positives were obtained with samples which were hemolyzed, icteric, contained elevated bilirubin or high levels of rheumatoid factor, or from patients with other viral diseases (EBV, CMV, HBV or rubella).

The use of chromium dioxide particles offers advantages in assay speed and performance, and the potential for many levels of automation.

**CONJUNCTIVE PASSIVE POTASSIUM LEAK AND INTRACELLULAR GLUTATHIONE DEPLETION CORRELATES WITH THE ALTERED DECOMPOSITION AND REDOX STATUS OF MEMBRANE-PROTEIN THIOLS IN RED CELLS.** Juan L. Liu. (CAMS, S. F. State Univ., S. F. CA 94132) and C. Xu, J. Qin, Daniel T. Y. Chu (Bruce Lyon Res. Lab., Children's Hosp., Oakland, CA 94609) (Aided by Tann S. Liu).

1-chloro-2,4-dinitrobenzene (CDNB), an agent capable of forming a thioester conjugate with glutathione (GSH), is commonly employed to deplete cellular GSH of various cell types. However, its possible effect on the functional integrity of membrane has not been studied. Therefore, studies were undertaken to ascertain the possibility that CDNB could directly affect the red cell membrane. First, we observed that incubation of human RBC with various concentrations of CDNB (0.5-5.0 μM) in K-Free PBS solution containing ouabain resulted in a dose-dependent increase in passive K and Li leakages. Concomitantly, cellular stores of GSH were drastically depleted. Taken together, these data implied that membrane damage ensued and the culprit was CDNB. To further pursue the possible site of CDNB in GSH and membrane itself, we prepared membrane ghosts by hypotonic hemolysis with or without CDNB pretreatment. The thiol redox status of the membrane ghosts was measured and the results showed that the CDNB treatment was approximately a 3-fold decrease in thiol concentrations in CDNB-treated membrane ghosts (2 μM n 10 μg/mg protein) as compared to the control (61 μM n 14 μg/mg protein). Further, an osmotic gradient experiments indicated that the deformability index (DI) for CDNB-treated RBC was substantially lower than the DI value of the control (0.28 v.s. 0.48 at 250
We conclude that the modified reagent might distinctly contribute to an improved performance of routine serum fructoseamine assays.


Four hundred and seventy six urinary stones, obtained from 446 patients (48 days to 85 years old) were analysed over a period of 3 years, using infrared spectroscopy with EBR Disc technique. Constituents were identified and semi-quantitatively estimated by comparison with reference spectra prepared in the laboratory.

A knowledge of the chemical composition of renal stones is necessary for prevention of future stones. The method of choice of a poorly-understood mechanism of stone formation. For stone analysis IR spectroscopy is now accepted as the method of choice. Almost 5% stones were pure calcium oxalate dihydrate but none were found with pure calcium oxalate monohydrate.

In this continuing study, calcium oxalate was found to be the most frequent mineral, occurring in 78% of the stones as a major component. This was followed by uric acid in 11%.apatite in 5.5%, struvite in 3.5%, ammonium hydrogen urate in 1%, and cystine in less than 1% stones. Whewellite, uric acid, and its dihydrate were easily identified. Almost 9% stones contained pure calcium oxalate dihydrate but none were found with pure calcium oxalate monohydrate.

Out of 88 visually demarcated nuclei examined, 38 were significantly different in composition from the outer layers. The composition of 33 stones passed by the patients without interference was specifically studied to account for their spontaneous expulsion in contrast to those retained but no distinctive features were discovered.

**DEVELOPMENT OF A SINGLE INCUBATION ENZYME IMMUNOASSAY FOR ANTIBODIES TO RUBELLA VIRUS BASED ON ENHANCED LUMINESCENCE, J. Holian, K. Jones, C. Smith, E. C. Stovb, A. C. The individual (Waresham International plc, Waresham, Bucks, UK) (Spon.: J C Martin)**

The measurement of antibodies to Rubella by enzyme immunoassay is an established technique used in the determination of immune status and as an aid to the diagnosis of recent infection. We report the development of a semi-quantitative, single incubation enzyme immunoassay for Rubella antibodies based on the technology of enhanced luminescence.

The Marelita™ Rubella Ig antibody assay uses microwells coated with inactivated Rubella antigen. 101l of serum, plasma or control is added to the wells together with 100 µl of peroxidase labelled polyclonal antibody to Rubella. The wells are incubated for 1 hour at 37°C with shaking using the Marelita™ Raker/Incubator. The unbound enzyme conjugate and serum are removed by aspiration and washing of the wells. Signal reagent is then added and the light measured in the Marelita™ Analyser. Signals given by samples are expressed relative to the signal given by a borderline reference control to provide a semi-quantitative estimate of antibody levels.

The minimum antibody level required to give a positive result is approximately 1010/l. In a representative study, results for 140 samples were compared with results of a haemagglutination inhibition (HAI) test. 34 samples were negative by HAI, of these, 33 were negative and 1 was borderline in the Marelita™ assay. 106 samples were positive by HAI, of these, 125 were positive and 6 were borderline in the Marelita™ assay.

In conclusion, we have developed an accurate and convenient single incubation enzyme immunoassay for Rubella antibodies for the Marelita™ system based on enhanced luminescence.
The following pages contain descriptions of all full or half day workshops. Please indicate your choice for each day on the registration form and enclose payment.

One day meeting registration is available for those wishing only to attend a full or half day workshop. The cost would be the workshop fee plus the daily fee.

You will be issued a refund only if the workshop is sold out or canceled. There will be on-site registration for workshops with seats still available. Prompt return of the pre-registration form will increase your chance of receiving desired workshops. Seats are limited and space is assigned in order of receipt with full payment.

ACCENT® CATEGORY I CREDIT HAS BEEN GRANTED TO THESE SESSIONS.

SUNDAY, JULY 24

Full Day Workshops

301 CONCEPTS AND PRACTICES IN THE EVALUATION OF LABORATORY METHODS
R. Neill Carey, Ph.D., Peninsula General Hospital, Salisbury, MD
David D. Koch, Ph.D., University of Wisconsin, Madison, WI
Limit: 40 Intermediate (R)

The overall objective of this workshop is to enable the participant to make decisions about the acceptability of method performance. These decisions are based on comparison of the analytic errors of a method to the medically allowable error for the analyte being measured. The following specific topics are considered:
1. Medically allowable analytic error
2. Characteristics of analytical methods that are important in method selection and evaluation
3. Types of analytic errors
4. Factors to consider when designing evaluation experiments
5. Evaluation experiments: preliminary and final
6. Statistical procedures for reducing evaluation data into reliable estimates of analytical errors
7. Criteria for comparing the experimentally observed errors to medically allowable error
8. FDA and NCCLS evaluation protocols

302 PERSONAL COMPUTERS IN THE CLINICAL LABORATORY
Michael L. Bishop MS, Duke University Medical Center, Durham, NC
Kenneth A. Schneider, M.D., Duke University Medical Center, Durham, NC
Lee A. Barbieri, B.S., Duke University Medical Center, Durham, NC
Limit: 35 Introductory - Intermediate (R)

The purpose of this workshop is to introduce laboratory professionals to some of the more productive uses of the personal computer (PC) in the laboratory. This includes scientific sessions, but most of the teaching occurs during hands-on practice sessions.

The lectures touch on a variety of subjects important to PC users: software, hardware, networking, computer literacy. The demonstration sessions teach the fundamentals of PC use, e.g., keyboard, disk operating system, basic "how to use the PC". Then, the sessions demonstrate state of the art software management in three areas: database management, spreadsheets, and math and statistics. Laboratory examples are used to show how these can be used to implement or support quality assurance programs, manage information, and teach employees or students.
WORKSHOPS

303 COST-EFFECTIVE QUALITY CONTROL
James O. Westgard, Ph.D., University of Wisconsin, Madison, WI
Patricia L. Barry, B.S., University of Wisconsin Hospital and Clinics, Madison, WI
Limit: 50 Advanced (R)

This workshop presents an approach for choosing a control procedure based on how it will affect the quality and productivity of the analytical process. It is based on the book Cost-Effective Quality Control: Managing the Quality and Productivity of Analytical Processes.

An understanding of the operation of statistical quality control in clinical laboratories is assumed.

The objectives are to: (a) introduce the principles of industrial quality management to understand the meaning of "quality," "cost," and "cost-effective quality control"; (b) review how analytical goals influence the choice of control procedures; (c) describe the performance characteristics of control procedures in terms of probabilities for rejection and average run lengths; (d) introduce quality-costs models to predict the quality and productivity of an analytical process as a function of the characteristics of its measurement and control procedures; (e) illustrate how a cost-effective control procedure can be chosen based on predictions of defect rate (quality) and test yield (productivity); and (f) discuss the importance of quality management for success in a competitive marketplace.

The program consists of lectures, videotapes, and problem sets. Handout materials include lecture outlines and reprints. The book Cost-Effective Quality Control is available from AACI.

304 GENERAL PRINCIPLES AND CLINICAL APPLICATIONS OF GAS CHROMATOGRAPHY/MASS SPECTROMETRY
Henry G. Nowicki, Ph.D., MBA, Professional Analytical & Consulting Services, Inc., Coraopolis, PA
Dale Deutsch, Ph.D., State University of New York, Stony Brook, NY
E. Howard Taylor, Ph.D., University of Arkansas for Medical Sciences, Little Rock, AR
Limit: 30 Introductory - Intermediate (N)
Developed in cooperation with the Therapeutic Drug Monitoring and Clinical Toxicology Division

This workshop will cover both the basic principles of gas chromatography/mass spectrometry and the clinical applications for drug screening and confirmation analysis. In the first part of the workshop, we will discuss mass spectrometry terminology, and basic components including vacuum systems, sample inlet systems, ion sources, ion collection systems, detectors, and data analysis. We will also discuss basic mass spec techniques such as for tuning and resolution, full vs. selected ion monitoring, use of extracted ion current plots for quantification, use of isotopes, molecular ion tests and their relationship to parent structure, procedure validation, and QA/QC. Justification for mass spec equipment will also be covered.

In the second portion, we will discuss the use of GC/MS for the identification of undervarified drugs (e.g. codeine, meperidine, propoxyphene, methadone, phencyclidine, acetaminophen, barbiturates, tricyclics, cocaine, and ecgonine methyl ester), and derivatized drugs (e.g. Δ-9 carboxy THC, benzoylcoecgonine, amphetamine, and opiates). In addition to the didactic session, there will be a demonstration of samples being extracted, derivatized, and analyzed on benchtop GC/MS instruments available during the workshop.

The workshop will conclude with opinions on how GC/MS should be used in relation to other drug analysis procedures such as thin-layer chromatography and immunochemical techniques for clinical (i.e. hospital or emergency room patients) and industrial (i.e. employee screening) toxicology, and a question and answer period with the faculty.

305 DEVELOPING A PRACTICAL AND COST EFFECTIVE FINANCIAL MANAGEMENT SYSTEM
Annmarie Barros, M.A., CLS, CLD, Health Management Analysts/S.F. State University, Los Gatos, CA
Limit: 50 Advanced (R)

Today's laboratory directors and managers must be able to respond to increasing demands to reduce the cost of services. This requires knowledge and skills in the financial areas of their job responsibilities and an understanding of the overall financial operations at their institutions. This workshop will focus on the development of a cost-effective operation emphasizing financial and productivity monitoring techniques. The workshop will discuss evaluation of the operational systems. The material is designed to provide participants with the opportunity to learn how to:

- Develop a comprehensive understanding of the historical basis for today's competitive approach to the delivery of health care.
- Interpret and apply financial hospital and laboratory financial information.
- Conduct an internal operational audit of their laboratory.
- Compile relevant statistical and operational data for preparation of effective budgetary justifications.
- Develop a cost-accounting system to identify costs appropriate for prospective payment.
- Use productivity data as cost accounting, staffing and competency rating tools.
- Develop strategies and techniques for managing a cost effective and efficient laboratory operation.

The workshop will include lecture, discussion, case studies, exercises and a comprehensive manual.
306 SUBSTANCE ABUSE TESTING — MEETING THE CHALLENGE
Ann Warner, Ph.D., University of Cincinnati Medical Center, Cincinnati, OH
Amadeo J. Pesce, Ph.D., University of Cincinnati Medical Center, Cincinnati, OH
F. Michael Hassan, B.S., University of Cincinnati Medical Center, Cincinnati, OH
Thomas Chamberlain, Ph.D., J.D., VA Medical Center, Memphis, TN
Irving Sunshine, Ph.D., Palo Alto, CA
Tai C. Kwong, Ph.D., University of Rochester Medical Center, Rochester, NY
Limit: 50 Advanced (N)
Developed in cooperation with the Therapeutic Drug Monitoring and Clinical Toxicology Division
In meeting the challenge to the laboratory to provide accurate analysis of abused substances using legally defensible methods and procedures, the workshop will begin with a concise overview of the problem of drug abuse and the history of drug testing, include discussion of recent publicity questioning laboratory accuracy. The remainder of the workshop will be spent describing the current state of the art including: choice of methods for screening and confirmation, specific benefits and limitations of the various choices available, setting of cut-offs, sensitivity limits of the various methods with the effect those limits have on various screening and confirmation combinations, proper utilization of the technology, particularly with regard to the GC/MS, (how it is possible to get bad data from GC/MS and how to avoid the problem), design of a quality control program, laboratory licensing requirements with particular reference to Federal, AACC and CAP guidelines, legal procedures required both before and after the sample is received by the laboratory, and documentation required for court cases.
Along with didactic presentations, participants will evaluate actual data sets, see examples of court presentations, and participate in a discussion of the AACC position paper on drug testing.
Workshop participants will be expected to have the theoretical background for the drug testing methods and will be encouraged to present problems for discussion.
An extensive handout including a bibliography will be provided.

307 ANALYTICAL AND CLINICAL ASPECTS OF BLOOD GAS AND ELECTROLYTE MEASUREMENT
Martin Fleisher, Ph.D., Memorial Sloan-Kettering Cancer Center, New York, NY
Stephen E. Kahn, Ph.D., Loyola University Medical Center, Maywood, IL
Jeffrey S. Groeger, M.D., Memorial Sloan-Kettering Cancer Center, New York, NY
Limit: 55 Intermediate (R)
Developed in cooperation with the Electrolyte/Blood Gas Division
This workshop will consider analytical and physiological concepts of blood gas, electrolyte and ionized calcium measurements with special emphasis on clinical application. The workshop will be presented in three parts.
Part 1 will be a didactic review of acid-based balance and blood gas physiology. The discussion will include gas exchange and transport, oxygen-hemoglobin dissociation, blood buffer system and pulmonary and metabolic compensatory mechanisms. Also discussed will be fundamental aspects of quality assurance and quality control.
Part 2 will consider analytical and physiological aspects of electrolyte measurement. Included in this discussion will be a review of the relationship between pulmonary, metabolic and renal function. The importance of ionized calcium with special reference to the clinical implications of hypocalcemia, and potential areas for laboratory imprecision and/or inaccuracies will be discussed.
Part 3 will integrate the practical and clinical use of blood gas and electrolyte data. Interpretation of pulmonary and renal function will be discussed in the context of life saving clinical intervention.

308 CLINICAL CHEMISTS AS EXPERT WITNESSES
Walter Frajola, Ph.D., WJ. Frajola, Ph.D., Inc., Columbus, OH
Kurt M. Dubowski, Ph.D., University of Oklahoma, Oklahoma City, OK
Patricia H. Field, Ph.D., State Laboratory of Hygiene, Madison, WI
Yale H. Caplan, Ph.D., Office of the Chief Medical Examiner, Baltimore, MD
Limit: 55 Intermediate (N)
Developed in cooperation with the Therapeutic Drug Monitoring and Clinical Toxicology Division
The greatly increased demand for paternity tests, alcohol tests on drinking drivers, blood and urine tests for drugs of abuse and AIDS tests has brought the clinical chemist special public attention. The legal implications of the results of such tests has placed an additional obligation not only on the laboratories providing the tests, but upon the clinical chemist performing the tests. These tests must meet the various forensic requirements. The clinical chemist must become a forensic chemist as well.
This workshop is designed to provide the clinical chemist with the necessary background and information to function as an expert in forensic chemistry. This will be accomplished by discussion of the following topics:
1. the requirements of a good expert witness (knowledge, experience, communicative ability)
2. proper laboratory procedures (chain of custody, analytical variables, reporting safeguards)
3. typical forensic experiences (attorney consultations, depositions, cross-examinations)
4. sources of information (on being an expert witness, on the specific test or subject)
Attendees will be provided with a pamphlet for study prior to the workshop and will be encouraged to be an active participant in the program with questions and comments.
WORKSHOPS

Half Day Workshops

201 HOW TO START AND MAINTAIN A PART-TIME OR FULL-TIME CONSULTING PRACTICE
Christopher S. Frings, Ph.D., Consultant, Birmingham, AL
Limit: 40 Introductory–Intermediate (R)
This workshop is intended for those clinical chemists and laboratorians who are consultants or who would like to become effective consultants. Consulting can be a part of your primary job with the compensation going to your employer, can be a “side line” with extra compensation, or can be full-time. Specific consulting opportunities that will be addressed include contract research and development; evaluation of kits, reagents and instruments; technical advisory panels; seminars; technical writing; videotapes; computers; continuing education; and referee for analytes in controls and calibrators. In this workshop you will learn how to: create demand for your services; identify consulting opportunities; use seminars and lectures to sell consulting; establish fees for your services; avoid giving away your know-how for free; develop marketing strategies that build reputation and cause clients to seek you out; write proposals and reports; network; advertise; and know when to turn down an assignment. This is a practical seminar that has been valuable for those who want to consult.

202 FRONTIERS IN LABORATORY ENDOCRINOLOGY
Joan H. Howanitz, M.D., West Los Angeles VA Medical Center, Los Angeles, CA
Peter J. Howanitz, M.D., University of California, Los Angeles Medical Center, Los Angeles, CA
Limit: 55 Intermediate (R)
The purpose of this workshop is to review recent advances which have occurred in several areas of endocrinology, stressing changes important to laboratory scientists. Emphasis will be placed on the use of the laboratory in diagnosis and patient management for the following areas:

- Thyroid function tests, with emphasis on more “exotic” tests including ultrasensitive TSH assays, TSI, and thyroglobulin.
- Measurements of glucose metabolism including insulin, glycated proteins, C-peptide, and glucagon.
- Hormones of calcium metabolism emphasizing PTH and PTH-like molecules.
- Infertility stressing measurement of the gonadotropins, sex steroids, as well as semen analysis and hamster assay.
- Growth hormone including immunoassays for IGF 1.
- Water metabolism with emphasis on renin, aldosterone, ADH, and atrial natriuretic factor.

A manual will be provided that includes a set of learning objectives, a set of references, and a copy of the material presented on the slides.

203 RECENT ADVANCES IN CYCLOSPORINE MONITORING
Leslie M. Shaw, Ph.D., Hospital of the University of Pennsylvania, Philadelphia, PA
Larry Bowers, Ph.D., University of Minnesota, Minneapolis, MN
Raman Venkataramanan, Ph.D., University of Pittsburgh, Pittsburgh, PA
Timothy Schroeder, M.S., University of Cincinnati, Cincinnati, OH
Limit: 55 Intermediate (R)
Developed in cooperation with the Therapeutic Drug Monitoring and Clinical Toxicology Division
Cyclosporine, which is used in virtually all transplant centers throughout the world, has improved post-surgical care for the solid organ transplant patient by reducing the intensity and duration of rejection episodes and the number of life threatening infections. Cyclosporine is the first immunosuppressant drug for which therapeutic monitoring has become an integral part of the therapeutic drug monitoring that have emerged during the past several years.

The purpose of this workshop is to provide the latest scientific information on critical issues in cyclosporine monitoring that have emerged during the past several years. Critical issues in cyclosporine monitoring will be discussed based on the practical experience of the faculty, each of whom is conducting a cyclosporine monitoring and/or a pharmacokinetics program in their university hospital laboratory. The topics that will be addressed in detail include: choice of specimen for cyclosporine analysis; comparison of the HPLC and RIA methods, including the polyclonal and the monoclonal assays, in relation to clinical outcome; inter- and intralaboratory quality assurance; pharmacokinetics including drug and food interactions with cyclosporine; the relationship between cyclosporine concentration in blood and pharmacological effects; and the significance of cyclosporine metabolites in monitoring immunosuppressant therapy and toxicity.

204 TWO COLOR FLOW CYTOMETRY IN THE DIAGNOSIS OF LEUKEMIAS AND LYMPHOMAS
Gerald M. Penn, M.D., Ph.D., Grant Medical Center, Columbus, OH
Paul Hurtubise, Ph.D., University of Cincinnati Medical Center, Cincinnati, OH
Daniel Pinkel, Ph.D., Lawrence Livermore National Laboratory, Livermore, CA
Limit: 55 Intermediate (R)
Developed in cooperation with the Clinical and Diagnostic Immunology Division
The diagnosis of leukemias and lymphomas is generally made by morphological means. In the case of diagnosing chronic lymphoproliferative processes as well as establishing
the prognosis of acute leukemias and Non-Hodgkin lymphomas, flow cytometry is the procedure of choice.

During this half-day workshop, participants will review the principles of flow cytometry and immunological classification of lymphomas/leukemias and discuss the diagnostic and prognostic implications of flow cytometric data using the case history format. Emphasis will be placed on two-color flow cytometry and the correlation of morphological and cytometric data.

205 WELLNESS TESTING: AN ADVANCING ROLE FOR CLINICAL LABORATORIES IN PREVENTIVE MEDICINE
K. Owen Ash, Ph.D., University of Utah, Salt Lake City, UT
Francis M. Urry, Ph.D., University of Utah, Salt Lake City, UT
Arthur M. Smith, MT(ASCP), Associated Regional and University Pathologists, Inc., Salt Lake City, UT
Limit: 50 Intermediate (N)

Clinical laboratories have traditionally focused on testing for diagnosis and treatment of symptomatic patients; however, escalating costs have mandated changes which place renewed emphasis on preventive medicine. Wellness testing offers expansion opportunities but places new demands on clinical laboratories, e.g. confirmatory tests must support screening procedures which alone are generally insufficient when testing healthy subjects.

Attendees will learn how clinical laboratories can adapt to support or market health promotion efforts at several levels, i.e. national, community, workplace, family and individual. Participants can learn to build on existing expertise to optimize the laboratory contributions to these wellness programs. Emphasis areas will include:

- Drugs of abuse and general health promotion for industry.
- Screening for AIDS, hepatitis, and tumor associated proteins.
- Risk assessment—participants will assess their own risk of cardiovascular disease based on test results, lifestyle, and genetic factors.

We are excited by the potential for clinical laboratories to contribute to wellness efforts and look forward to helping you prepare to realize the opportunities.

Marie Perlstein Rock, Ph.D., Johns Hopkins University, Baltimore, MD
Patricia Garrett, Ph.D., Lahey Clinic, Burlington, ME
Jan S. Krouwer, Ph.D., Ciba Corning Diagnostics Corp., Medfield, MA
Limit: 40 

The workshop will be presented by four speakers. We will review the immunoassay reagents and instrumentation of current interest, evaluation and selection of new methods, quality control and troubleshooting of immunoassay.

The approach is practical. Examples will be given to illustrate the application of the principles to these assays. We will review ways to set acceptance goals for a new method, techniques for evaluating methods before doing lab work and step-by-step recommendations for selection of methods that require minimum lab work. Finally, we will present a quality control scheme for immunoassay and provide troubleshooting guidelines. Specific examples and problems will be discussed and participants are welcomed to contribute.

207 DIAGNOSTIC ENZYMEOLOGY
John A. Lott, Ph.D., Ohio State University, Columbus, OH
Paul L. Wolf, M.D., University of California, San Diego, CA
John C. Griffiths, M.D., Medical University of South Carolina, Charleston, SC
Limit: 60 Intermediate (R)

This is an AACC Workshop on Tour.

The workshop will enable laboratorians to understand:
1. Physiologically important changes in serum and urine enzymes associated with exercise, pregnancy, cachexia, immobilization, aging, drugs, etc.
2. Pathologically important changes in serum and urine enzymes owing to organic disorders, infection, trauma, surgery, malignancy, etc., of the heart, skeletal muscle, pancreas, liver, biliary tract, salivary glands, kidneys, bone, prostate, lung, spleen, etc.
3. Neuer diagnostic and methodological aspects of the clinically important enzymes (ACP, ALD, ALP, amylase, CK, LD, galactosyl transferase, G6PD, GGT, LAP, lipase, 5'N). We will discuss isoenzymes and isoforms and new, clinically relevant applications of these tests in diagnosis, management, and prognosis. Advances in methodology will be discussed for lipase isoenzymes and the isoforms of CK and ALP.

The participants' understanding of the clinically relevant enzyme tests will be developed further by the use of 15 case discussions and examples. Associated with the case studies is an extensive list of reference ranges of most commonly performed tests.

The manual contains 120 multiple-choice, self-study questions with a key. Nearly all of the slides to be shown are
present in the manual, and up-to-date literature citation are included. Each topic is described in depth and goes beyond what can be discussed in the workshop. The 300-page manual is suitable for teaching programs, preparation for board-type examinations, and personal updating.

This workshop rated among top five by attendees last year.

208 WRITE PRECISE AS LAW, CONCISE AS JOURNALISM
Hank Wallace, J.D., Write for Success, Washington, D.C.
Limit: 45 Intermediate (R)

Write with law's accuracy and logic, and journalism's brevity and vividness.

As a clinical chemist, put your talent for both theory and practice into your memos, letters and reports. Topics include:

1. Organization.
   a. Lead with what's most important — usually the future. (Still lead with "In your memo yesterday, you asked. . ." A live TV newscast will show you how to give information in the order your reader wants it.)
   b. Layout for ATTENTION — but also for comprehension!

2. Clarity.
   a. Be consistent. (In a single document, call it a "dish", or a "bowl", but not both!)
   b. Know when clarity demands a proportion rather than an absolute number.
   c. Scrutinize hearsay.

   a. Summarize. (Pure chemistry has neat rules. Clinical Chemistry has rough edges. Can a summary be both accurate and brief?)
   b. Overcome overlap.
      • Superfluous idea
      • Redundancy
      • "Included offense." (Streamline "Separate it by filtration.")
      • Do-nothing word.
      • Self-canceling.
   c. "Verb" your nouns.

4. Tone.
   a. "Be positive" — that's not just a blood type! (Streamline "Do not moisten except with distilled water.")
   b. See how the present tense universalizes your clinical findings.
   c. Write your reader's language: plain English.

All star method: Striking examples—from newspapers, legal documents and clinical chemistry—of how (and how not) to write.

This workshop rated among top five by attendees last year.

209 QUANTITATIVE ASPECTS OF HUMORAL IMMUNOLOGY: HUMAN IMMUNOGLOBULIN ISOTYPES AND SUBCLASSES, PARAPROTEIN, RH FACTORS AND COMPLEMENT NEOANTIGENS
Robert G. Hamilton, Ph.D., University of Texas Medical School, Houston, TX
Oscar E. Beck, Ph.D., Molecular Systems Research Laboratory, Bryan, TX
John D. Tamerius, Ph.D., Cytotech, San Diego, CA
Limit: 55 Intermediate (R)

Developed in cooperation with the Clinical and Diagnostic Immunology Division

Methods for the quantitative measurement of human immunoglobulin and complement protein in serum, urine and other body fluids are becoming increasingly sensitive and accurate due to the improved methods, standards, monoclonal antibody reagents and knowledge of interfering substances. This workshop will contrast classic with new methods and reagents for the quantitation of human immunoglobulin isotypes (IgG, IgA, IgM, IgD and IgE) and subclasses (IgG1, IgG2, IgG3, IgG4, IgA1, IgA2), paraprotein, rheumatoid factors and complement components in human body fluids. Nephelometric turbidimetric and immunological methods for the quantitation and characterization of human immunoglobulins will be initially compared with an emphasis on standards, sample collection, interfering substances and an overview of new reagents and equipment. Second, methods and monoclonal antibody reagents will be examined that allow quantitation of the human immunoglobulin subclasses and human rheumatoid factors in serum. A novel clinical technique will be presented that employs automated isoelectric focusing in combination with passive immunoblot analysis to define monoclonal paraproteins in terms of their isotype, light chain and subclass. Finally, new assays for the measurement of complement neoantigens will be discussed within the context of their potential in the diagnosis and monitoring of patients with selected autoimmune diseases. This is an intermediate level workshop which is intended for laboratory workers who are involved in routine detection and measurement of immunoglobulin and complement protein.

210 HIGH SENSITIVITY OF THYROID STIMULATING HORMONE (TSH)
George G. Klee, M.D., Ph.D., Mayo Clinic, Rochester, MN
Ian D. Hay, M.D., Ph.D., Mayo Clinic, Rochester, MN
David Witte, M.D., Ph.D., Lab Control Limited, Ottenwa, IA
Limit: 55 Intermediate (R)

The recent development of "sensitive" thyrotropin (s-TSH) assays, which can distinguish suppressed values from low normal, greatly expands the clinical utility of measuring serum TSH. When measured with adequately sensitive methods, basal serum s-TSH values can not only confirm
primary hypothyroidism, but also can predict pituitary-thyroid axis response to TRH and can confirm functional hyperthyroidism. Since s-TSH is not beset with the problems of altered binding proteins, it appears to be the most effective single test of thyroid function.

This workshop will outline a clinical approach to thyroid disorders as viewed by an endocrinologist. This will be followed by a discussion of laboratory approaches for thyroid investigation, enumerating the advantages, limitations, and cost effectiveness of total thyroxine, free thyroxine, free thyroxine indexes and s-TSH. After this, analytical and clinical performance standards for evaluating s-TSH assays will be presented, along with a review of various clinical studies of s-TSH assays (see J Clin Endocrinol Metab 64:461, 1987). Then experience of s-TSH as a front-line thyroid function test in a general community hospital will be discussed.

Also, there will be a series of case presentations illustrating various problems in thyroid investigation. Audience participation and general discussion will be encouraged during the case presentations.

211 EFFECTIVE TIME MANAGEMENT
Christopher S. Frings, Ph.D., Consultant, Birmingham, AL
Harold L. Ertman, M.B.A., Christ College, Irvine, CA
Limit: 45 Intermediate (R)

This is an AACC Workshop on Tour.

Time is the least understood, most mismanaged, uncontrolled, and unplanned, but also our most priceless resource. This workshop is designed to teach you how to better use your time and to raise your awareness of how important it is to manage your time. Practical ways to manage your time will be presented. Forms to help the workshop participant get better control of his time and to learn how to manage oneself will be used. Each participant will calculate how much his time is worth. The topics to be addressed include telephones, paperwork, priorities, interruptions, delegation, meetings, planning, goals, motivation, effective use of secretarial help, and procrastination.

This workshop rated among top five by attendees last year.

212 MATERNAL ALPHA-FETOPROTEIN TESTING
A. Michael Spiekerman, Ph.D., Scott and White Hospital and Clinic, Temple, TX
Gary F. Simpson, M.D., Good Samaritan Medical Center, Phoenix, AZ
Linda Bradley, Ph.D., The Genetics Center, Scottsdale, AZ
Limit: 50 Intermediate (N)

Developed in cooperation with the Nutrition Division.

The purpose of this workshop is to provide the critical information needed to perform and interpret maternal serum and amniotic fluid alpha-fetoprotein testing in a clinical laboratory.

The following will be discussed in this workshop:
- Biochemistry, physiology, and metabolism of alpha-fetoprotein (AFP).
- Patient data necessary to perform maternal serum alpha-fetoprotein (MSAFP) testing.
- Maternal age
- Correction factors for maternal weight
- Gestation age, last menstrual period (LMP) vs. ultrasound (US)
- Race
- Insulin dependent diabetes mellitus
- Use of multiple of the median (MOM) in reporting results.
- Analytical performance of current AFP assays and quality control parameters.
- Quality assurance and risk management procedures appropriate for developing MSAFP screening program.
- Decreased levels of MSAFP and maternal age as a predictor of Down's syndrome.
- Use of MSAFP and amniotic fluid alpha-fetoprotein (AFAP) in the diagnosis of neural tube defects, exomphalos, and chromosomal defects in conjunction with use of ultrasonography and amniotic fluid acetylcholinesterase electrophoresis.
- Obstetrician/gynecologist's viewpoint for using MSAFP and AFAP as a means to monitor possible problem pregnancies.
- Medical/legal implications of these assays.

An extensive syllabus covering these topics will be provided.

213 IMPACT OF AIDS ON THE CLINICAL CHEMISTRY LABORATORY
Celia J. Menenzos Botet, Ph.D., Memorial Sloan-Kettering Cancer Center, New York, NY
Frederick Van Lente, Ph.D., Cleveland Clinic Foundation, Cleveland, OH
Herbert N. Rose, Ph.D., Technicon Corporation, Tarrytown, NY
Richard T. O'Kell, M.D., St. Luke's Hospital, Kansas City, MO
Limit: 55 Intermediate (N)

This workshop will focus on the impact Acquired Immune Deficiency Syndrome (AIDS) has had on the Clinical Chemistry Laboratory. Laboratory personnel, always at risk of developing infectious disease from the specimens they process, now face the unique challenge of AIDS, a disease without a known cure or vaccine.

This workshop will discuss the prudent precautionary actions that must be taken in today's laboratories and will answer the following questions: What is the safest approach to the handling of infectious specimens? Are there special techniques for the decontamination of laboratory instruments? Which detergents and germicides are the most...
WORKSHOPS

effective for decontaminating instruments and laboratory surfaces? What are the best commercial products available for testing for the HIV antibody and antigen? What are the safest methods of disposing infectious laboratory waste?

We shall also discuss cases of contamination and regulations concerning confidentiality.

214 NEONATAL CLINICAL CHEMISTRY AND HEMATOLOGY
Jocelyn M. Hicks, Ph.D., Children's Hospital National Medical Center, Washington, D.C.
Naomi L. C. Luban, M.D., Children's Hospital National Medical Center, Washington, D.C.
Barbara M. Goldsmith, Ph.D., St. Christopher's Hospital for Children, Philadelphia, PA
Limit: 50

Developed in cooperation with the Pediatric Clinical Division.
The neonate and premature infant provide unique challenges to the laboratory medicine scientist or physician. It is important to understand the physiological and biochemical development of these patients in order to understand normal and abnormal laboratory test results. It is also vital to develop a symbiotic relationship between the neonatologist and the laboratory medicine specialist.

The newborn and premature infant frequently suffer from cardiorespiratory insufficiency. Therefore an understanding of acid-base balance and the measurement of blood gases is a necessity. Hypocalcemia, hypophosphatemia, and hypoglycemia are common in sick neonates, and many term as well as premature infants experience jaundice in the first week of life. Intravenous alimentation is often necessary in the sick neonate, which can lead to metabolic acidosis, hyperammonemia, hyperglycemia and osmotic diuresis with secondary electrolyte disturbance. The many parameters that need to be measured during this initial period of life will be discussed.

The newborn has a unique oxyhemoglobin disassociation curve secondary to the presence of fetal hemoglobin. Several metabolic phenomena (low pH, temperature) alter this curve in vivo with subsequent changes in oxygen release to the peripheral tissues. Transfusion replaces fetal hemoglobin with hemoglobin A, shifting the curve and increasing oxygen release. Another phenomenon of concern in the newborn is the hyperviscosity/polycythemic syndrome (HVS). Due to increased hematocrit, whole blood viscosity or both, the HVS is associated with a variety of laboratory aberrations that can be corrected with partial exchange transfusion. One percent or more of all nursery admissions are complicated by hemorrhage or thrombosis. The bleeding problems of the newborn demand a careful evaluation. In this workshop our pediatric hematologist will discuss oxygen off-loading, polycythemia, hyperviscosity, reference values and coagulopathies in the newborn. The entire workshop will be supported by case history examples.

The attendee should leave with a better understanding of some of the major concerns of the laboratory physician or scientist serving a neonatal population.

215 BASIC PRINCIPLES AND PRACTICAL APPLICATIONS OF DNA PROBE TECHNIQUES
Dale H. Altimiller, Ph.D., University of Oklahoma Health Sciences Center, Oklahoma City, OK
W. Douglas Scheer, Ph.D., Louisiana State University Medical Center, New Orleans, LA
Limit: 50

The primary objective of this three-part workshop is to provide a basic understanding of gene probe techniques and how this new technology is being applied in the clinical laboratory. Prior knowledge of molecular biology is not required. All terms and concepts will be explained thoroughly.

Part I will lay the foundation. This session will begin with a discussion of the chemical basis of gene probes, followed by a description of molecular "tools" and laboratory equipment used in the performance of gene probe techniques. Part II will cover clinical laboratory applications. The use of gene probes in the detection and identification of infectious agents will be discussed first, followed by a discussion of gene probe techniques used in the diagnosis of specific genetic disorders. Part III will be case studies of genetic disorders.

Participants, working in pairs, will apply what they learned in the first two parts of the workshop to convert "raw" data from gene probe tests into genotype assignments. Results will be discussed in a "question and answer" session following each case.

Each participant will receive a manual containing the text of the lectures, detailed explanations of the case study results, a glossary of terms, a bibliography, and a list of vendors of gene probes and related supplies.

216 FETAL LUNG MATURITY TESTING: PRACTICAL CLINICAL AND LABORATORY CONSIDERATIONS
John F. Chapman, Dr. P.H., University of North Carolina, School of Medicine, Chapel Hill, NC
William N.P. Herbert, M.D., University of North Carolina, School of Medicine, Chapel Hill, NC
Limit: 55

In the "real world", laboratory testing for fetal lung maturity (FLM) typically represents an attempt to balance clinical needs with laboratory capability. With the recognition of the need to achieve such a balance as a central theme, this workshop is designed to provide clinical laboratory professionals with the necessary clinical and analytical information to help them better implement such a service in their own setting.

The workshop will begin with a broad overview of the clinical aspects of FLM testing and end with the discussion of logical strategies for optimizing laboratory utilization while meeting clinical needs. Individual sessions begin with the basics—what is the clinical significance of prematurity and respiratory distress syndrome? Next, a variety of FLM tests will be critically reviewed—the good, the bad, and the promising. Following this overview, various testing strategies...
will be discussed and participants will be given the opportunity to evaluate various approaches using a sample data set. Finally, a number of cases representing special clinical situations will be presented for discussion.

During this workshop, the two faculty members will attempt to share their collective laboratory and clinical experience which derives from several years of joint research in this area. Likewise, sharing of the practical experiences of each participant will be encouraged throughout. A workshop manual, containing lecture notes, selected reprints, problem sets and cases, and references will be provided to each participant.

217 METHODS FOR TRIGLYCERIDE, TOTAL AND HDL CHOLESTEROL DETERMINATION
Joseph D. Artiss, Ph.D., Wayne State University, Detroit, MI
Bennie Zak, Ph.D., Wayne State University, Detroit, MI
G. Russell Warnick, M.S., M.B.A., Northwest Lipid Research Center, Seattle, WA
Donald A. Wiebe, Ph.D., University of Wisconsin Hospital and Clinics, Madison, WI

Limit: 55 Advanced (N)
Developed in cooperation with the Lipids and Lipoprotein Division

There is ample evidence to indicate the direct relationship between lipid levels in blood and the risk of developing atherosclerosis and/or coronary heart disease. Presently there is a strong emphasis not only for everybody to know their cholesterol, but also for the laboratories to provide accurate and reproducible results.

This workshop will provide an overview of the latest guidelines for diagnosing and treating hyperlipidemia and for the associated laboratory measurements as established by the National Cholesterol Education Program. Analytical techniques capable of providing the necessary levels of precision and accuracy will be described. Detailed methods describing state-of-the-art techniques, that are applicable to the routine clinical laboratory for the determination of serum triglycerides, and total and HDL cholesterol will be given. This will include an evaluation of the various chemical-precipitation techniques that are available for the isolation of the HDL fraction. As well the deleterious effects that elevated levels of lipids have on the measurement of other analytes will be discussed in detail.

A prior working knowledge of methods used for chemical separation of the HDL fraction as well as the enzymic techniques for lipid determination would be useful to the attendee.

218 APOLIPOPROTEINS: CLINICAL UTILITY AND MEASUREMENT
Evan A. Stein, M.D., Ph.D., Medical Research Laboratories, Cincinnati, OH

Moti L. Kashyap, M.D., University of California at Irvine, Long Beach, CA
Maryvonne Rosseneu, Ph.D., A.Z. St. Jan, Brugge, Belgium

Limit: 50 Intermediate (N)
Developed in cooperation with the Lipids and Lipoprotein Division

The workshop will 1) focus on the current and potential utility of apolipoproteins in clinical practice; 2) describe, compare and contrast the available analytical procedures for apolipoprotein measurement including a number of commercially available assays; and 3) provide information on current national and international programs to standardize apolipoprotein measurements and assess quality assurance.

In the first part of the workshop, there will be three faculty, each addressing one of the above objectives in a didactic presentation. The second part of the program will present a few select clinical and laboratory problems related to the practical application apolipoprotein measurement. The final part of the program will be an open question-and-answer session and general discussion between participants and faculty.

Workshop materials will include summaries of faculty presentations and case discussions.

TUESDAY, JULY 26

Half Day Workshops

219 APPLICATIONS OF TWO-DIMENSIONAL ELECTROPHORESIS IN CLINICAL CHEMISTRY
N. Leigh Anderson, Ph.D., Large Scale Biology Corp., Rockville, MD
Russell P. Tracy, Ph.D., University of Vermont, Burlington, VT
Denis Hochstrasser, M.D., Geneva University Hospital, Geneva, Switzerland

Limit: 30 Intermediate (N)
Developed in cooperation with the Animal Clinical Chemistry Division

This workshop will review the use of high-resolution two-dimensional electrophoresis in the discovery and implementation of new protein diagnostic tests. The presenta-
tion will be divided into four approximately equal parts:
First, a brief review of 2-D electrophoresis technology and its application in the field of drug effects measurement.
Included will be a detailed look at model studies of changes in tissue protein composition following treatment of experimental animals with drugs.

Second, a description of the approaches required to develop immunodiagnostic tests for protein markers discovered in 2-D electrophoretic studies taking, by way of examples, the development of tests for osteoporosis and related diseases.

Third, a review of novel diagnostic markers observed through 2-D analysis of human plasma proteins. Discussions will focus on quantitative changes in the levels of unknown or poorly-studied plasma proteins associated with physiological and disease status.

Fourth, a demonstration of the major features of 2-D gel electrophoresis equipment and a look at real gels showing representative physiological effects.

Workshop materials will include notes on each presentation, a comprehensive manual on the 2-D electrophoresis technique and a bibliography of important published material in the field.

220 CURRENT CONCEPTS VALUABLE FOR EFFECTIVE CONSULTATION AND INTERPRETATION IN THERAPEUTIC DRUG MONITORING
Benjamin Gerson, M.D., Boston University School of Medicine, Boston, MA
Sum Chan, Ph.D., Nichols Institute, San Juan Capistrano, CA
David Platt, Pharm. D., Hartford Hospital, Hartford, CT
Limit: 60 Intermediate-Advanced (R)
Developed in cooperation with the Therapeutic Drug Monitoring and Clinical Toxicology Division

Clinical laboratorians are called upon to act formally or informally as consultants. The sophistication of laboratory users is improving, leading to more reliance on the laboratory as a technical as well as a clinical resource. This program is intended for clinical laboratorians with at least some drug monitoring experience who wish to focus on certain well defined topics. The topics to be addressed have been chosen by the faculty as some of those about which there are frequent inquiries and about which information is still emerging. The topics to be presented include: (1) Free Drug Monitoring and Related Pharmacokinetic Concepts; (2) Concepts and Applications of Pharmacokinetic Models; (3) Role of Monitoring Trace and Essential Metals. It is the faculty’s intention that the attendees role in providing drug monitoring service will be enhanced.

221 EMERGENCY AND INDUSTRIAL DRUG TESTING
Christopher S. Frings, Ph.D., Consultant, Birmingham, AL
Limit: 50 Intermediate (R)
Developed in cooperation with the Therapeutic Drug Monitoring and Clinical Toxicology Division
This is an AACC Workshop on Tour.

The objective of this workshop is to provide the participants with the necessary information to set up or expand emergency and/or industrial drug testing capabilities in their laboratory. The strengths and weaknesses of TLC, FPIA, EMIT, RIA, GLC and GC/MS for screening and/or confirming the presence or absence of drugs in urine will be discussed. The drugs to be discussed will include, but will not be restricted to, barbiturates, amphetamines, opiates, benzodiazepines, cannabinoids, cocaine, tricyclic antidepressants, salicylates, acetaminophen, phenothiazines, alcohol, and ethchlorvynol. The following important considerations will be discussed: serum vs. gastric contents vs. urine; drug metabolism; screening, confirmation and quantitative tests; chain of custody; how to select a drug testing lab; supervised urine collection; legal aspects; treatment of drug overdoses; specificity of methods; and turn-around time for drug testing. This workshop rated among top five by attendees last year.

222 CLINICAL CHEMICAL APPLICATIONS OF NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY
Gary D. Fullerton, Ph.D., University of Texas Health Science Center, San Antonio, TX
Robert F. Williams, Ph.D., University of Texas Health Science Center, San Antonio, TX
Limit: 55 Intermediate (N)

This workshop is comprised of two distinct, but interwoven, parts. The basic concepts of Nuclear Magnetic Resonance (NMR) and NMR spectroscopy will be reviewed. The application of these concepts to understand Magnetic Resonance Imaging (MRI) and localized spectroscopy will be discussed. Clinical applications will be employed to demonstrate the power and utility of the techniques.

I. Nuclear Magnetic Resonance
A. Basic concepts of NMR
B. High Resolution NMR Spectroscopy

II. Magnetic Resonance Imaging
A. Basic concepts of MRI
B. Tissue Contrast - Relaxation

III. Clinical Applications
A. Magnetic Resonance Imaging
B. Localized Spectroscopy (MRI)
C. High Resolution NMR Spectroscopy

IV. Future Applications
A. Magnetic Resonance Imaging
B. High Resolution NMR Spectroscopy

Workshop material will include a lecture note manual and comprehensive bibliography.
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223 BIOCHEMICAL MESSAGES OF MALIGNANCY: PRACTICAL APPROACHES
John Griffiths, M.D., Medical College of South Carolina, Charleston, SC
Limit: 55 Intermediate (N)
Developed in cooperation with the Clinical and Diagnostic Immunology Division

The objective of this workshop is to share with participants, practical experiences relating to the potential biochemical detection of various major cancers. In addition, after diagnosis to utilize the appropriate biochemical message in control of treatment and for prognosis.

There are four interlocking sequences. The introduction seeks to define criteria for the biochemical message (BMM), with broad references to origin, release and distribution. Several BMM like CEA, AFP and Beta CG have been utilized for at least a decade. Their value in Oncology will be updated, clarifying collection, assay methods and clinical utilization.

In the third section, definitive statements will be offered, relating common cancers to the new generation of BMM, specifically:
- Small cell carcinoma — gamma gamma enolase
- Breast carcinoma — CA 15/3
- Pancreatic carcinoma — CA 19/9
- Ovarian cystadenocarcinoma — CA 125
- Prostatic adenocarcinoma — Prostate Antigen

Finally, the difficulty of diagnosing hepatic metastases remains. There may be alternating patterns of biochemical activity from the metastatic lesion. Utilizing isoenzymes and isoforms, these patterns may be significant. The newer concepts will be described thru methods and practical application.

224 MANAGING PRODUCTIVITY IN THE CLINICAL LABORATORY
Richard B. Passey, Ph.D., University of Oklahoma, Oklahoma City, OK
Limit: 40 Intermediate (N)

This workshop presents tools that will assist you in evaluating and improving productivity in your laboratory. The workshop is divided into three parts.

Part 1 is a didactic presentation on the theory of the College of American Pathologists' Workload Recording Method. We will discuss the recent changes in the method and provide a view of where the Workload Recording Method is headed. Included will be a discussion and presentation of data on non-specified or non-workloaded tasks and how you can improve efficiency by managing time spent in non-workloaded activities.

Part 2 is a series of realistic exercises designed to help you evaluate your laboratories workload, detect inefficiency, determine inaccuracies in calculating your productivity, understand the impact of non-workloaded tasks and paid time off benefits, determine the appropriate number of personnel for a particular situation, and use the workload method to help schedule personnel. Formulas and data will be provided that will assist you in calculating target productivity, number of personnel required for specific situations, and the number of full time equivalents required for your laboratories' work.

The third part of this workshop will be devoted to answering questions from the participants.

225 TOWARD PERFECTION IN ANALYTICAL SYSTEMS: REDUCING NON-SPECIFIC EFFECTS
Melvin R. Glick, Ph.D., Wishard Memorial Hospital, Indianapolis, IN
Kenneth W. Ryder, M.D., Ph.D., Wishard Memorial Hospital, Indianapolis, IN
Oswald Sonntag, Dipl.-Ing., Medizinische Hochschule Hannover, West Germany
Limit: 55 Intermediate (N)

This workshop will focus on one aspect of the less-than-perfect analytical methods currently used in clinical chemistry; namely, the non-specific results obtained with many of the systems available commercially.

Workshop participants will learn how they can estimate the severity, frequency and economic impact of visible and invisible interfering substances contained in serum. Results from a study conducted in a large, general-practice hospital laboratory will be discussed, emphasizing the potential for erroneous results due to many of the non-specific analytical methods now available in automated systems.

An instrument-by-instrument comparison will be presented, showing the quantitative effects of interfering substances, emphasizing the unpredictable interaction between certain reagent and instrument combinations. Analyte-specific interferographs will also be considered, so that participants can modify or select reagents and instrumental conditions to minimize the effect of non-analyte serum constituents. Special attention will be focused on those analytical systems with the greatest promise for improvement in or (analytical) perfection in the near future.

226 CURRENT APPLICATIONS OF NUCLEIC ACID PROBES IN THE CLINICAL LABORATORY
Theodore E. Mifflin, Ph.D., University of Virginia, Charlottesville, VA
Mark A. Lovell, M.D., University of Virginia, Charlottesville, VA
Stephen N. Thibodeau, Ph.D., Mayo Clinic, Rochester, MN
Limit: 55 Intermediate (R)
Developed in cooperation with the Clinical and Diagnostic Immunology Division

The field of molecular biology has provided a wealth of basic data about the gene and its various activities (e.g.)
replication, regulation, transcription). To provide this information, most of these procedures have relied upon molecular probe(s) prepared from labeled DNA or RNA fragments. Only within the last several years however, have DNA/RNA probes methods provided results that are clinically useful. The diagnostic applications of probe technology which have found clinical utility within the clinical laboratory include genetics, infectious disease, and oncology. As these assays now become more popular in diagnostic medicine, clinical laboratory personnel will need to be familiar with various aspects of this new technology (e.g. basic principles, sample preparation, detection reactions, applications, instrumentation, and others).

This workshop is organized to provide participants with: 1) a brief review of nucleic acid chemistry and general techniques, 2) illustrations and explanations of specific applications of this technology in microbiology, oncology, and genetics, and 3) descriptions of future developments. Throughout the presentation, audience participation is encouraged to facilitate better understanding of the discussion topics. Finally, each attendee will receive a workshop manual that summarizes the oral presentation and also contains references, an annotated bibliography, glossary, a list of vendors, and selected procedures.

227 MAKING THE COMPUTER DO WHAT YOU WANT
J. Richard Pearsons, Ph.D., University Hospital, Denver CO
Steven J. Steindel, Ph.D., Prism Associates, Atlanta, GA
Limit: 50 Intermediate (R)
Developed in cooperation with the Laboratory Information Systems Division

Microcomputers have been hailed as great tools for managing and analyzing data. However, sometimes it seems that the computer causes more problems than it solves. This course is designed to help the laboratory professional make more efficient use of the microcomputer.

Emphasis will be placed on learning how to analyze a problem so that the computing requirements can be examined and alternative solutions evaluated. Several example problems will be presented along with a description of different ways to solve each problem and their associated tradeoffs. Alternatives that will be considered include commercially available software packages for database management, spreadsheets and word processing as well as custom programs written specifically for the task at hand.

Participants are assumed to have some familiarity with the use of computers.

228 STATISTICAL METHODS FOR GUARANTEEING ACCURACIES IN THE MANUFACTURING OF IMMUNOASSAY CALIBRATORS
Brian Schlain, M.S., Brian Schlain Associates, Jamaica Plain, MA
Limit: 50 Intermediate (N)

In the immuno-assay industry we are concerned with manufacturing accurate calibrators. A popular approach is to choose a master lot to evaluate new production calibrator lots. Here new calibrators are assayed as unknowns with the master lot serving as calibrating standards. In using the master lot approach, it is not an uncommon rule to reject a standard whose corresponding average assay value is outside 5% of its nominal value. The drawbacks of this rule will be discussed, and an alternative 2-stage procedure for controlling calibrator accuracies will be presented.

Required numbers of assays for accurate value assignments of new production calibrators depend directly on assay precisions. In immuno-assay, precision can change across lots or even across time within lots.

If precision is unknown the popular fixed sample size procedures cannot control calibrator accuracies. The problem of determining required numbers of assays when precision is unknown is solved with the 2-stage procedure: First stage samples are obtained for estimating assay precisions, from which required second stage sample sizes are determined.

The workshop will focus on the case where new and master lot calibrators do not have equivalent analyte values. Through simulated examples, workshop attendees will learn how to assign calibrators floating values and to test for specified target values.

229 BRIEF 1 OR 100: HOW TO TALK TO ANY AUDIENCE
Hank Wallace, J.D., Write for Success, Washington, D.C.
Limit: 55 Intermediate (R)

Brief with the calm of a conversation and the power of a speech. Topics include.
1. You.
   a. Relax
   b. Brief well even if you think you can't relax.
2. Your message.
   a. Give good news the good way.
   b. Earn loyalty even when the news is bad.
   c. Lead with what you know; then show any uncertainty assertively.
   d. Predict, when you can't promise.
   e. Correct your errors the right way — and welcome correction by others.
   f. Contain a crisis.
   g. Strengthen 4 wimp words in this comment reported by Business Week: "We feel he [football star John Riggins]'s helped us convey the image that Ford trucks are tough."
3. Your audience.
   a. Listen.
   b. Ally with an adversary.
   c. Compliment even as you complain.
   d. Capitalize on interruptions.
   e. Overcome demands for a "Yes" or "No" answer.
   f. Get action: "close" your briefing like a sale.

This workshop rated among top five by attendees last year.

230 NEW INSTRUMENT REVIEW FOR CLINICAL CHEMISTRY
Laurence M. Demers, Ph.D., Milton S. Hershey Medical Center, Hershey, PA
Nate Gochman, Ph.D., Beckman Instruments, Inc., Brea, CA
Morton K. Schwartz, Ph.D., Memorial Sloan-Kettering Cancer Center, New York, NY
David H. Vroon, M.D., Emory University, Atlanta, GA

Limit: 55 Intermediate (N)

Advances in instrumentation in clinical chemistry continue to accelerate as manufacturers keep pace with the developing and changing trends of diagnostic medicine. It therefore becomes important to thoroughly evaluate new clinical chemistry instrumentation not only for precision and accuracy determinants but for unique and distinct advantages which match the instrument to a particular laboratory's needs. As decentralization of laboratory services occurs with clinical laboratory extensions found in clinics, doctor's offices and emergency health centers, instrument selection becomes an even more important issue to provide the particular service desired depending on the patient population served.

Immunoassay systems to be reviewed include the Becton Dickinson Affinity System, Abbott IMX, Hybritech Photon Elite, Ciba–Corning Magic Light, Cyberfluor TR-FIA System and Electro-Nucleonics Delfia.

These instruments will be discussed relative to salient features, operating characteristics and performance characteristics in the hospital laboratory setting.

This workshop will review the latest developments with regard to performance characteristics for three new clinical chemistry analyzers and will update the latest information on an array of automated/semi-automated immunoassay analyzers. The clinical chemical analyzers to be discussed include the Technicon, Chem-I, the Beckman Synchron Series 3, 4, 5, and 7 and the Boehringer Mannheim Hitachi 717.

Immunoassay systems to be reviewed include the Becton Dickinson Affinity System, Abbott IMX, Hybritech Photon Elite, Ciba–Corning Magic Light, Cyberfluor TR-FIA System and Electro-Nucleonics Delfia.

These instruments will be discussed relative to salient features, operating characteristics and performance characteristics in the hospital laboratory setting.

231 IONIZED CALCIUM: ITS DETERMINATION AND CLINICAL USEFULNESS
Mary F. Burritt, Ph.D., Mayo Clinic, Rochester, MN
Gary A. Graham, Ph.D., University of Texas Medical Branch, Galveston, TX
John G. Toffaleti, Ph.D., Duke University Medical Center, Durham, NC

Limit: 45 Introductory (R)

Developed in cooperation with the Electrolyte/Blood Gas Division

The measurement of ionized calcium has evolved over the past ten years so that clinical laboratories today are able to perform both routine and emergency determinations. The availability of suitable instrumentation from a variety of manufacturers, coupled with a growing knowledge of calcium homeostasis, have made the determination necessary when patients with suspected calcium metabolic disorders are being evaluated. In addition, ionized calcium values have been shown to be useful in cases of abnormal protein binding, during massive transfusion and in neonates.

This workshop is designed to provide a basic understanding regarding the clinical relevance, specimen handling and instrumentation of ionized calcium determinations. It is oriented towards individuals who are interested in performing these determinations in their laboratories, as well as those wishing more information. Specific topics to be covered include:

- Ionized calcium in renal and parathyroid disease
- Reference intervals and pediatric normal values
- Effect of specimen choice, collection and storage
- Instrumentation
- Ionized calcium in the acute care setting
- Quality control
The Roundtable Program provides an opportunity to have breakfast or lunch with noted investigators in selected areas to discuss and exchange ideas in a relaxed and congenial atmosphere. The same topics are offered at breakfast and lunch.

ONLY 9 PERSONS CAN ATTEND EACH ROUNDTABLE. REGISTER EARLY — THEY FILL UP FAST!

Please indicate up to six (6) choices for series 400 (Breakfast) and/or series 500 (Lunch) on the registration form, using code numbers listed below. Enclose payment for the maximum number of roundtables you plan to attend. THE MAXIMUM YOU CAN ATTEND IN EACH SERIES IS 3. Assignments will be made in order of receipt; there are only nine spaces available in each roundtable. There will be no refunds unless choice(s) are unavailable. Spaces remaining at the close of Pre-registration will be available for sale on-site.

**Roundtables are $20 each for breakfast or lunch.**

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<td>400</td>
<td>7:15–8:15 am</td>
<td>New Orleans Sheraton</td>
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<tr>
<td>500</td>
<td>12:30–1:30 pm</td>
<td>New Orleans Hilton</td>
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ACCENT<sup>®</sup> CATEGORY II CREDIT HAS BEEN GRANTED TO THESE SESSIONS

### MONDAY, JULY 25

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<tr>
<th>Code</th>
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<td>Alpha-Fetoprotein Testing in Prenatal Diagnosis</td>
<td>Norma F. Besch, Ph.D.</td>
<td>Bellaire, Houston, TX</td>
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<td>402</td>
<td>HPLC Electrochemical Detectors: How to Obtain Consistent Results</td>
<td>Steven R. Binder, B.A.</td>
<td>Hercules, CA</td>
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<td>Making Your PC Work for You</td>
<td>Michael L. Bishop, M.S., M.T.</td>
<td>Durham, NC</td>
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<td>404</td>
<td>HPLC/MS: State-of-the-Art</td>
<td>Larry D. Bowers, Ph.D.</td>
<td>Minneapolis, MN</td>
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<td>405</td>
<td>Update on Fetal Lung Maturity Evaluation</td>
<td>Charles Bradley, Ph.D.</td>
<td>Lubbock, TX</td>
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<td>406</td>
<td>Nucleic Acid Probes - Techniques and Applications</td>
<td>Gregory Buffone, Ph.D.</td>
<td>Houston, TX</td>
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<td>407</td>
<td>Do We Need Automation of Non-Isotopic Immunoassays?</td>
<td>Daniel Chan, Ph.D.</td>
<td>Johns Hopkins University Hospital, Baltimore, MD</td>
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563 Dale H. Altmiller, Ph.D.  
Oklahoma Children's Memorial Hospital  
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464 **Clinical Laboratory and Sports Medicine**  
564 Fred S. Apple, Ph.D.  
Hennepin County Medical Center  
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University of Utah A.R.U.P.  
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471 **User-friendly P.C. Software for Automating Clinical Laboratory Information Systems**  
571 Presented in cooperation with the Laboratory Information Systems Division  
Ralph G. Crawford, Ph.D.  
St. Vincent's Hospital  
New York, NY

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James Crowell, Ph.D.  
National Center of Toxicology Research  
Jefferson, AR

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University of Texas Southwestern Medical Center  
Dallas, TX

474 **The Ph.D. as Director of Hospital Laboratories**  
574 Leonard K. Dunikoski, Jr., Ph.D.  
Raritan Bay Medical Center  
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Marvin C. Feil, M.S.  
Instrumentation Laboratory  
Lexington, MA

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576 Frank Fox, Ph.D.  
VA Medical Center  
New Orleans, LA
477 Clinical Chemists as Expert Witnesses
577 Presented in cooperation with the Therapeutic Drug Monitoring and Clinical Toxicology Division
Walter Frajola, Ph.D.
W.J. Frajola Ph.D., Inc.
Columbus, OH

478 Use of Enzyme Labeled Oligodeoxynucleotide Probes for the Detection of Viral and Bacterial Pathogens
Susan M. Freier, Ph.D.
Molecular Biosystems, Inc.
San Diego, CA

479 Specimen Collection and Handling for Ionized Calcium Determinations
Presented in cooperation with the Electrolyte/Blood Gas Division
Gary Graham, Ph.D.
University of Texas Medical Branch
Galveston, TX

480 When Working For Someone Else Doesn’t Work Anymore
Kenneth G. Krul, Ph.D.
The Panda Group, Inc.
Rancho La Costa, CA

481 Kinetic ELISA in Microtiter Plates
Viola T. Kung, Ph.D.
Molecular Devices Corporation
Palo Alto, CA

482 Computer Graphics for Interpretative Reporting in the Clinic Laboratory
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John A. Lott, Ph.D.
Ohio State University Medical Center
Columbus, OH

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Michael P. Murphy, Ph.D.
Providence Hospital
Cincinnati, OH

484 How to Choose a Laboratory Information System
Presented in cooperation with the Laboratory Information Systems Division
Howard A.I. Newman, Ph.D.
The Ohio State University Medical Center
Columbus, OH

485 Grateful Med - Performing Your Own Medline
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Luis E. Remus III, Ph.D.
Center for Clinical Sciences
Nashville, TN

486 The Dexamethasone Suppression Test,
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James C. Ritchie, M.P.H.
Duke University Medical Center
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487 Detection of Anabolic Steroids and Other Doping Agents in Amateur Athletics
R.H. Barry Sample, Ph.D.
Indiana University School of Medicine
Indianapolis, IN

488 Microcomputer-assisted Laboratory Management
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Michael S. Sealfon, Ph.D.
Mt. Sinai Medical Center
Cleveland, OH

489 Training for SPOT Personnel
Valerie Skonie, B.S.
Skonie Marketing Associates, Inc.
San Francisco, CA

490 Viability of Animal Samples Under Various Storage Conditions for Clinical Pathology Tests
Presented in cooperation with the Animal Clinical Chemistry Division
Katherine Stitzel, D.V.M.
Procter & Gamble
Cincinnati, OH

491 Characterization of Monoclonal Gammopathy Paraproteins
J. Robert Swanson, Ph.D.
Oregon Health Sciences University
Portland, OR

492 Medical Examiner Toxicology
Robert M. White, Ph.D.
Diagnostic Services, Inc.
Naples, FL

493 Assessment of Thyrotroph Function Using “Sensitive” TSH Assays
Frank H. Wians, Jr., Ph.D.
Wilford Hall USAF Medical Center
San Antonio, TX
Monday Afternoon—July 25

Session I—Standardization Issues Relating To Ion Selective Electrodes
Developed in cooperation with the Electrolyte/Blood Gas Division

Mary F. Burritt, Ph.D.
Mayo Clinic
Rochester, MN
Chairman

Issues relating to the standardization of ion-selective electrodes have been topics for discussion since the introduction of these sensors in the clinical laboratories. This is particularly true with the development of electrodes capable of measuring analyte activity (usually expressed as concentration) in whole blood. These problems have been compounded by the number of analyzers currently available, the differences in design and in calibrating solutions, and the lack of a reference method and reference materials in some instances. These issues will be discussed in detail together with the ongoing efforts at the National Bureau of Standards to develop a standard reference material and method for the determination of sodium/potassium.

With the recent introduction of combined analyzers which measure blood gases, electrolytes and ionized calcium, new issues arose regarding calibration. Buffers and standard solutions commonly used in individual analyzers were problematic in these new systems due to analyte binding. The final speaker will outline these associated problems and their ultimate resolution.

This program was sponsored by the Electrolyte/Blood Gas Division.

Standardization of Direct ISE Measurements of Sodium and Potassium
Alan D. Cormier, Ph.D.
AMDEV, Inc.
Danvers, MA

There are two different techniques used in the application of the ion selective electrodes: direct measurements where the sample is measured with no added reagents or diluents; and indirect measurements, where in some way the sample is diluted before the measurement is made. Standardization for the two methodologies has quite different requirements. The indirect technique produces answers that resemble those obtained by flame photometry and have the same potential for error as the flame due to abnormal lipid and protein concentrations. Direct ISE measurements are more demanding to make due to calibration requirements, but represent an answer that does not have the same potential for severe error.

Standardization is the key concept that must be resolved for direct ISE measurements to be universally accepted. Requirements for direct ISE calibration will be discussed in detail along with the effect of changing ionic composition and ionic strength.

Each manufacturer has approached the standardization problem in their own manner and, as could be expected, chaos has resulted with respect to analyte concentrations reported on various manufacturer products. A number of manufacturers are working together with the NCCCLS and the NBS to develop a means by which uniformity of results (at least among participating laboratories) can be obtained.

Reference Materials for Sodium/Potassium: National Bureau of Standards' Efforts
William F. Koch, Ph.D.
National Bureau of Standards
Gaithersburg, MD

A research project, sponsored by the National Committee for Clinical Laboratory Standards and funded by eight manufacturers of clinical potentiometric instruments, is being conducted in the Electroanalytical Research Group at the National Bureau of Standards (NBS). The goal of this research is to bring conformity to the direct potentiometric measurements of sodium and potassium in human serum through the development of reference materials. Currently, different manufacturers of clinical analyzers each recommend their own calibration standards. These materials range from simple aqueous solutions to complex mixtures containing bovine serum albumin. Hence, different electrolyte values for the same sample of human serum analyzed by different instruments are not uncommon. A series of inter-laboratory tests has been carried out to quantify the problem and to identify the most suitable reference materials.

Ideally, an accurate calibration standard should simulate the actual sample to minimize the effects of liquid junction, activity, protein volume, and other factors that affect the electrode potential. It also should also be easy to prepare in large quantities, be stable for long periods, and be convenient to use in a hospital laboratory environment. To date, three stages of tests have been completed. The first stage tested the measurement protocol and general sample handling. The second stage consisted of eleven samples: three aqueous standards, two based on bovine serum albumin, and six based on human serum, including three variations of NBS Standard Reference Material 909. This test was designed to narrow the choices for a candidate reference material. The third stage of testing was intended to study the linearity and response characteristics of potentiometric electrolyte analyzers, and their correlation to the reference flame values. This test was based on splits from a single human serum pool that had electrolyte concentrations altered through the use of ultrafiltration and spiking techniques. The results of these tests will be discussed and recommendations will be offered.

The Instrumentation of Ion-Selective Electrodes
Kathleen M. O'Connell, Ph.D.
MediSense, Inc.
Cambridge, MA

In the fifty years since Dr. Arnold O. Beckman introduced the first pH meter, many enhancements have been made to the instrumentation of ion-selective electrodes (ISE's). At the heart of this instrumentation is the electrode itself; which has gone from the more manual, dipping type to the flow through or flow by electrodes compatible with automated systems. The desire to miniaturize and simplify the
The calibration of clinical analyzers based on electrochemical sensing has been the object of development efforts in recent years. Likewise, electrolyte analyzers often required sophisticated instrumentation, since widely different technologies and approaches had to be combined. The years ahead will see the expansion of the test menu available by electrodes in the areas of metabolites, enzymes and immuno assays, as well as continued advances in their ease of operation.

Standardization of Combined pH/Blood Gas and Electrolyte Analyzers
Paul A. D'Onzolzo, Ph.D.
Ciba Corning Diagnostics Corporation
Medfield, MA

The calibration of clinical analyzers based on electrochemical sensing has been the object of development efforts in recent years. Likewise, electrolyte analyzers often required sophisticated instrumentation, since widely different technologies and approaches had to be combined. The years ahead will see the expansion of the test menu available by electrodes in the areas of metabolites, enzymes and immuno assays, as well as continued advances in their ease of operation.

Interfacing: An Instrument Manufacturer's Perspective
George W. Scherer, M.S.
Eastman Kodak Company
Rochester, NY

The role of a laboratory instrument manufacturer is explored from that of the traditional instrument designer/manufacturer to that of the emerging systems designer concerned with developing instrumentation and interfaces that consider the laboratory's work and dataflow requirements. Reasons for this change in focus are discussed, and examples of various systems concepts and modes of instrumentation interface are cited. An outgrowth of system considerations is a realization that standardization of interfaces for data exchange, specimen containers, and container labels is needed. The role of today's laboratory instrument manufacturer is one of working closely with the laboratory personnel to understand and develop laboratory information systems vendors, and with laboratory standards setting committees.

Instrument Interfacing: A Laboratory System Vendor's Approach
Claudia Clifton, B.F.A.
Community Health Computing, Inc.
Houston, TX

Acquiring new instrumentation for the laboratory is a task which must take many items into consideration, including laboratory workflow, specimen workload, and budgetary and operational requirements. Even though instrument based microprocessors have greatly improved the capability and flexibility of interfaces, such software continues to be expensive, problematic, and untimely. Some of the difficulty of developing interfaces relates to technology advances, such as those supporting discrete testing which requires two way interfaces to minimize data entry. Other problems, however, are related to the varied approaches to interfaces, and the poor understanding by both computer vendors and instrument manufacturers as to how interfaces are being used. For example, the K-232 jack on an instrument is often equated to RS-232 interface, ignoring some important issues such as the format of the information flow between the host computer and the instrument. The first two presentations will examine instrument interface issues from the perspective of an instrument manufacturer and a laboratory information system vendor. The third speaker will address some user concerns, including efforts at ASTM in providing guidelines/standards for instrument interfaces so that they will be more "plug compatible." The last speaker will address the more global, but similar issues, of interfacing of various systems and instruments, and the issue of getting information to the end user in a unified fashion independent of data processing complexities in the laboratory.
As a result of Substance Abuse Testing and the attempts to minimize workers' exposure to hazardous chemicals in industrial, environment and other settings, the toxicology laboratory has experienced increasing demands on the volume and the range of testing. In responding to these demands, this selected topic session will focus on four areas of practical interest. From various government agencies and professional organizations, guidelines and lists are being established for safe work practices. By applying the appropriate analytical methodologies, clinical chemists would be helpful in quantifying hazardous chemicals as a measure of the community's exposure. The clinical toxicology laboratory should act responsibly, and should keep thorough record. Further, by joining a certificating program, the laboratory would be able to maintain the desired proficiency, and will be in a better position to offer testimony as an expert witness, if required later on. Various approaches for such preparation will be presented. Another area of concern, sports medicine, has also challenged the chemists due to the constraint on fast-turn-around time for testing, and the ever increasing number of agents. Experience, based on the recent Pan American Game, will be part of the presentation. After attending this session, the attendees will be updated on the above current issues of practical and professional interest to their laboratory experience.

Impact of Industrial Hygiene on Clinical Chemistry

R. Thomas Chamberlain, Ph.D., J.D.
VA Medical Centers
Memphis, TN

Industry has become more and more scrutinized by governmental agencies so as to provide a safe work place. Common Law dictates that there is a basic right for workers to have a safe place to work. The public pressure to enforce such rights has been heightened by such major catastrophes as the Bhopal tragedy. Clinical laboratories have not been directly addressed by the major governmental agencies which promulgate regulations concerning industrial hygiene. However, professional societies have been safety conscious and have developed guidelines. The Occupational Safety and Health Administration has now included laboratories handling small quantities of hazardous substance in its regulations by mandating that all workers have a right to information on substances that may be hazardous to their health. In addition, most states have implemented "right to know" laws. OSHA has proposed rules applicable to the clinical laboratory which will provide guidelines for protecting workers against HBV and HIV infections. Additionally, a "Chemical Hygiene Plan" has been proposed which will require documentation that procedures are in place to avoid overexposure to toxic substances.

Data has been accumulated in a well defined clinical toxicology laboratory to illustrate the fluctuations in organic solvents (e.g., ethyl acetate, chloroform) and how the risk to these materials can be reduced. Methods have been instituted to measure exposure of histopathology personnel to xylene and formaldehyde resulting in the development of precautions to lessen their risk. The clinical chemist has a unique background to impart on industrial hygienic concerns and offer a real service to the laboratory and/or hospital which ultimately could become a community service.

Doping Control in Amateur Athletics—Analytical Challenges

R. H. Barry Sample, Ph.D.
Indiana University School of Medicine
Indianapolis, IN

Athletes have been using drugs to gain a competitive edge for centuries. This practice is both unfair for those athletes choosing not to use drugs and unhealthy for those taking drugs to improve their performance. Stimulants were the first and most widely abused drugs in sports. Starting in the 1950's, anabolic steroids were used in sports requiring strength and endurance and have gradually replaced stimulants as the most widely used doping agent. In order to prevent this misuse of drugs, international sporting federations developed, in the 1960's, a list of banned drugs which included stimulants and anabolic steroids. With the development of radioimmunoassay and GC/MS procedures for detecting anabolic steroids in the early 1970's, anabolic steroids were added to the banned list. However, athletes quickly learned which compounds could be reliably detected and started using other drugs (including veterinary compounds) that were not easily detected. After the the Pan Am games in
When Clinical Toxicology Becomes a Legal Issue
Thorne J. Butler, M.D.
Associate Pathology Laboratories
Las Vegas, NV

Clinical toxicology laboratories are more frequently emblazoned as a party in both civil and criminal legal actions. While almost all toxicological analyses are potential forensic cases, samples collected from the ER (trauma), mental units or the workplace and for TDM control are more commonly at risk. Issues as to patient laboratory privilege, release of information, criteria for a legally defensible test and testimony are important operational factors when a drug test becomes a forensic issue. Ancillary examples will discuss the various issues as to the laboratory's responsibility and risk, should a sample be given special forensic treatment, and what are minimal records. The now being developed certification programs for forensic urine drug testing laboratories should give guidelines to reduce the trauma when the laboratory appears in an evidentiary proceeding.

Expert Witness
Patricia H. Field, Ph.D.
State Laboratory of Hygiene
Madison, WI

Presenting laboratory information in court can be a challenging and even discouraging experience. Litigation involving controversial or potentially damaging results such as alcohol or drug tests is becoming increasingly common. There is little in the laboratory test which could not be material for litigation. Clinical chemists and technologists should be able to present laboratory results confidently and with appropriate documentation and quality assurance information to demonstrate the accuracy and validity of those results.

The presentation will cover preparing for court, what to expect in court, and how to handle potentially embarrassing questions. At the end of this presentation, many participants will find themselves dreading court less, and some will be positively looking forward to it!

Tuesday Afternoon, July 26

Session IV—AIDS and Retroviruses
Donald J. Boone, Ph.D.
Centers for Disease Control
Atlanta, GA
Chairman

Human retroviruses have now been linked with many human diseases, including acquired immunodeficiency syndrome (AIDS). As the list of human immunodeficiency viruses (HIV) that cause disease continues to grow, so does the need for basic and applied research to detect the presence of and prevent the spread of these viruses and to treat people who have already been exposed.

Topics in this session will serve as a review of current information about retroviruses and can be used to anticipate future activity in basic and applied research, in epidemiology, and in modes of diagnosis and treatment. Participants should be better prepared to provide support to the medical community and to assist others in dealing with the increasing social, economic, and medical problems associated with these viral diseases.

Retroviruses: An Overview
Gary Schochetman, Ph.D.
Centers for Disease Control
Atlanta, GA

The retroviruses are comprised of single-stranded RNA that replicate through a DNA intermediate. They are divided into three subfamilies, the oncogenerous, lentiviruses, and spumaviruses. Each having a nucleocapsid containing the enzyme reverse transcriptase.

Oncoviruses form the largest subfamily, are isolated from virtually all vertebrates, and produce leukemias, lymphomas, and breast carcinomas in humans. Lentiviruses induce progressive neurological impairment and chronic pneumonia after a long latent period. Virions are 80 to 100 nm in diameter consisting of a lipid-containing envelope with surface projections surrounding a icosahedral capsid that in turn contains a helical nucleocapsid.

Retroviruses are further classified by the type of budding that they produce from the cell membrane and by the glycoproteins that stud the membrane's surface. The viral genome codes for gag (core protein), pol (reverse transcriptase), and env (envelope protein). In addition, the HIV-1 virus that induces AIDS has a genome that includes four unusual genes known as tat, rev, sor, and Nef that encode proteins that help control the expression of viral genes. Each encoding gene can induce an antibody response following human exposure.

Epidemiology of Human Retroviruses
William L. Atkinson, M.D., M.P.H.
Tulane University School of Public Health
New Orleans, LA

Five pathogenic human retroviruses have been identified since 1978. While distinctly different, these viruses share the common features of an affinity for CD4+ T4 cells and production of a unique viral polymerase, reverse transcriptase. Modes of transmission of three (HTLV-I, HIV-1, HIV-2) are similar: sexual, direct blood contact and mother-to-fetus. Transmission data for two (HTLV-II, HTLV-V) is lacking.

Human T-lymphotropic virus type I (HTLV-I), the first human retrovirus described, has been associated with adult T-cell lymphoma. HTLV-I is highly prevalent in areas of Japan, the Caribbean, Africa and Papua New Guinea. In the U.S. it has been found in blacks in the Southeast, and certain groups of intravenous drug users. HTLV-II has been isolated occasionally from patients with hairy cell leukemia.

Human Immunodeficiency Virus (HIV-1, also called HTLV-III or LAV), isolated in 1983, is the causative agent of AIDS. The virus is highly prevalent in parts of Central Africa and Haiti, and it is prevalent among persons in the U.S. and Europe, such as homosexual men, intravenous drug users, and hemophiliacs. HIV-2 (also called HTLV-IV or SIV) has been primarily found in West Africa, with only a few infections reported in the Western Hemisphere. It may cause an AIDS-like disease.

HTLV-V has been recently described in patients with mycosis fungoides, a type of cutaneous T-cell lymphoma. No epidemiologic information concerning this virus is available.
The Role of Clinical Trials in the Search for Effective Treatment for HIV Infection
Newton E. Hyslop, Jr., M.D.
Tulane University School of Medicine
New Orleans, LA

Potential targets for chemotherapy of HIV-infected patients include CD4 receptors on cells susceptible to infection, enzymes participating in reverse transcription into host DNA, regulatory proteins encoded in the viral genome, enzymes participating in transcription and translation of viral genome in synthesis of viral RNA and proteins, sites of virion assembly, and transport from the productively infected cell into the extracellular environment.

Selection of candidate agents for clinical trials requires evidence of in vitro activity in HIV-infected tissue culture systems, acceptable toxicity in non-primate animals and potential for oral availability of orally administered drugs. Ideally the drug should also show evidence of crossing the blood brain barrier to assure reaching HIV-infected sites in the central nervous system.

Anticancer candidate drugs are reviewed by the NIAID Drug Screening Committee, who choose among members of class agents (e.g. nucleoside analogues) those which should undergo clinical trial.

Clinical trials of agents potentially effective against HIV require initial characterization of pharmacokinetics and safety/toxicity in a small group of volunteers followed by a small study of efficacy. While demonstration of efficacy in HIV infections can be based on virological, immunological or clinical endpoints, the development of acceptable laboratory surrogates (restoration of lymphocyte function, decline of viral antigen, elimination of virus from plasma or cell culture) while demonstrating efficacy and permit more rapid selection of drugs for larger scale clinical trials.

To expedite this process, data from AST clinical trials will be presented.

Laboratory Tests and New Technology for HIV Detection
Thomas A. O'Brien, Ph.D.
E.I. duPont de Nemours and Company, Inc.
Wilmington, DE

The area of HIV testing has evolved rapidly since the licensure of the first antibody ELISA in March 1985. Both scientific advancement and competitive pressures have led to increases in both the sensitivity and specificity of these screening assays. The desire to minimize the reporting of false positive ELISA results has also led to the development and widespread use of supplemental antibody assays for use in testing ELISA reactive samples prior to donor notification.

Recently, HIV antigen assays have become available for use in detection of viral proteins in the serum of infected individuals as well as a replacement for the reverse transcriptase assay currently used to monitor virus in clinical cultures. Finally, nucleic acid probe assays are being developed as an alternative to antigen detection for virus in culture. This presentation will review the current status of HIV antibody, antigen and nucleic acid testing while stressing the likely direction of future assay development.

Utility of Soluble T Cell Markers for Characterization of Disease Activity
Patrick C. Kung, Ph.D.
T Cell Sciences, Inc.
Cambridge, MA

Measurement of T cell responses to diseases can provide valuable information on the nature, severity and progression of a disease. Until recently the involvement of T cells with a pathologic event has typically been assessed by its demonstration that the T cell marker of interest either in peripheral blood or in a biopsy sample: the former yields representative information in a highly disseminated disease and the latter is highly invasive and may also not be representative. It has been demonstrated that certain T cell markers such as interleukin-2 receptor (IL-2R) and the suppressor/cytotoxic phenotypic marker CD8/T8 are released from a functionally active subset of T cells. Measurement of these soluble antigens in serum allows simple and rapid monitoring of the body's immune response to disease process and lymphokine release. Elevated soluble IL-2R has been found helpful in studying malignancies including HTLV-I associated T cell leukemia and lung cancers and in organ transplants. Soluble IL-2R was also shown to have significant prognostic value in monitoring patient response to therapy in rheumatoid arthritis, lupus, and hairy cell leukemia. Soluble T8/CD8 has been shown to correlate with the degree of T8/CD8 cell activation in vivo and in vitro. T8/CD8 is elevated in acute phases of viral infections such as hepatitis, EBV-induced mononucleosis, and HIV infection. Recent evidence that soluble T cell antigen receptor is detectable in serum raises the potential of studying T cell clonal response to a disease. The measurement of soluble T cells in diseases is better understood a comprehensive panel of tools to assess the degree of immune response to disease becomes necessary. Thus soluble T8/CD8 are members of a growing family of released T cell receptors that play an increasing role in better patient management.

Session V—Advances in Cellular Immunology Developed in cooperation with the Clinical and Diagnostic Immunology Division

Gerald M. Penn, M.D., Ph.D.
Grant Medical Center
Columbus, OH
Chairman

This selected topics conference will concentrate on the assessment of cellular immunity as it relates to the clinical chemistry laboratory. Previously this type of assessment was accomplished by measuring T and B cell subsets in flow-cytometry requiring elaborate computer software support. The recent understanding of the role of anti-idiotype, T cells and their effector products in malignancy, autoimmune disease and transplant rejection as well as the use of immunotherapeutic regimes in the treatment of disease, has emphasized the need for the rapid and low cost assessment of these entities. Dr. E. S. Cooper will initiate the conference by reviewing the biochemistry of the HLA system as it relates to transplant rejection. His presentation will be followed by Dr. T. P. Moyer, who will discuss the monitoring of cyclosporine levels during immunosuppression therapy. This concept of therapeutic monitoring will be extended to the therapeutic agents by Dr. G. DeWeese.

He will review the measurement of lymphokines, and point out some of the problems that the clinical chemist may encounter. Dr. P. C. Kung will end the conference by demonstrating how the measurement of T cell markers in fluids may be used in lieu of the more elaborate flow-cytometric methods.

Cyclosporine and Immunosuppression
Thomas P. Moyer, Ph.D.
Mayo Clinic
Rochester, MN

Since the introduction of cyclosporine (CyA), the success rate of organ transplants (measured as length of time or number of surviving grafts) has nearly doubled. CyA has the unique feature of selectively suppressing T-cell proliferation against this antigen, while maintaining the immune system's previously acquired ability to recognize and protect against pathogens. As the benefits of the drug have become apparent, so have observations that its concentration at a specific site is required to achieve effective immunosuppression, and that it causes predictable nephrotoxicity which may not be present for prolonged periods when measured in whole blood. This conference will explore the risk of CyA drug monitoring.

Methods to measure CyA have proliferated. In January of 1988, one can find reports of RIA's and EIA's to analyze serum or whole blood that utilize either polyclonal or monoclonal antibodies. Chromatographic procedures abound. There is a different therapeutic range for each procedure. This presenta-
tion will briefly review the major variables that affect these ranges as described in the Consensus Report on CyA (Clin. Chem. 33:1269, 1987).

Further combating the issue, the synergistic effect between azathioprine, prednisone, and CyA has provided additional clinical benefit over single CyA treatment: 'triple immunosuppression' has become the therapy of choice. The interpretation of results by polyclonal IgG changes because the metabolic fate of CyA changes in the presence of these drugs, and the effective concentration of CyA challenges the limit of sensitivity of chromatographic methods. None of the current commercially available approaches provides a completely satisfactory solution — what fun to be a clinical chemist in the late 1980's.

### HLA Typing and Transplantation

**E. Shanon Cooper, M.D., J.D.**

**Ochsner Clinic**

**New Orleans, LA**

The HLA or Major Histocompatibility Complex antigens on cell surfaces are known to play a major role in transplant rejection as well as focusing certain T lymphocyte actions and antigen presentation. The Class I antigens, known generally as the ABC locus antigens, are extremely polymorphic, relatively easily determined serologically and present on all nucleated cells. The Class II antigens, D, DQ, and DR, are restricted to B cells, macrophages, endothelial cells, activated T cells and a few other cell types. The DQ and DR antigens can be determined readily by standard methods similar to those used for the ABC antigens, but the D antigens at present require complex cellular techniques.

The ABC antigens consist of a heavy and light chain, and therefore resemble a portion of an Fc fragment of antibodies. The HLA antigens in some cases have been subdivided into "splits" and conversely have been lumped into crossreactive groups (CREGS) based on serological variations and similarities respectively.

This highly complex genetic system is being studied intensively in order to optimize graft tolerance and to increase basic knowledge of its role in immune function. The intensive research is progressing in the development of enzyme immunosassay procedures, DNA procedures, and other evolving technologies to complement the serological tests. The structure and function of the HLA system will be reviewed primarily in the context of transplantation biology.

### Laboratory Measurements of Lymphokines as a Guide to Thier Clinical Utility—Problems and Potentials

**Galen D. Marshel, M.D., and Ph.D.**

**Biotherapeutics, Inc.**

**Memphis, TN**

The age of biotherapy of disease is dawning. There is increasing evidence that many diseases will soon be effectively treated with products of the normal immune response. We are currently treating patients having advanced malignancies with lymphokines such as recombinant interleukin-2 (rIL-2) and tumor necrosis factor (rTNF). A major problem associated with this form of therapy is the lack of reliable laboratory assays that reflect accurate serum levels as well as predict clinical activity. The existing quantitative assays depend primarily upon monoclonal antibodies raised against the specific lymphokines and are used in ELISA and radioimmunoassays. The major limiting factors include sensitivity and specificity of the assays, in vitro biological activity of lymphokines have been studied extensively in a variety of systems including proliferative and cytotoxicity assays and, more recently, differentiation of selected cell populations as determined by flow cytometric analysis. The major limitations in these assays are the intrinsic variability of biological assays in general and the overall poor correlation between in vitro and in vivo effects of these molecules. Using a strategy of immunological monitoring of multiple parameters, assay profiles are being designed that should be reliable, reproducible, and relevant reflections of in vivo clinical effects of lymphokines. As this is accomplished, the spectrum of diseases amenable to biotherapy will expand dramatically.

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**Wednesday Afternoon, July 27**

**Session VI—National Cholesterol Education Programs: Current Guidelines for Diagnosis and Treatment of Hypercholesterolemia**

**Developed in cooperation with Lipids and Lipoproteins Division**

**G. Russell Warnick, M.S., M.B.A.**

Northwest Lipid Research Center Seattle, WA

and

**Gerald R. Cooper, M.D., Ph.D.**

Centers for Disease Control Atlanta, GA

Co-Chairmen

Diseases of the cardiovascular system are a major public health problem, accounting for nearly as many deaths as all other causes combined. A Consensus Panel convened in 1984 by the National Institutes of Health concluded from the extensive evidence that high blood cholesterol is indeed a causal factor in coronary artery disease and that a national program of intervention could reduce the associated high rate of death and disability.

The result is the National Cholesterol Education Program (NCEP) which, promoting the theme 'Know Your Cholesterol,' seeks through educating medical professionals and the general public to reduce the prevalence of elevated blood cholesterol. The NCEP has published two reports, one, the Adult Treatment Panel, has recommended cutpoints for total and LDL cholesterol, a rational approach to case finding, and guidelines for dietary and drug therapy (1). The second, the Laboratory Standardization Panel, has developed performance guidelines for cholesterol measurement (2) and an approach to standardization.


### Overview and Adult Treatment Panel Guidelines

**James A. Cleeman, M.D.**

National Institutes of Health

Bethesda, MD

A National Cholesterol Education Program (NCEP), organized by the National Institutes of Health, has the goal of reducing the prevalence of elevated blood cholesterol (CH) and thereby contributing to reducing coronary heart disease morbidity and mortality. The NCEP follows a consensus approach with coordination by a committee including representatives from twenty major medical organizations and will work in partnership with government agencies, professional organizations, and industry to achieve the stated goal.

The Adult Treatment Panel, convened by the NCEP has developed a patient based approach to identify individuals for therapy. Major features include initial identification based on cutpoints for total and LDL cholesterol and treatment decisions based on LDL CH. Cutpoints are as follows:

- CH
  - LDL CH
    - Desirable: <200 mg/dL
    - Borderline: 200-195 mg/dL
    - High: >240 mg/dL

Patients with high CH or those in the borderline range with symptoms of coronary heart disease (CHD) or at least two risk factors require measurement of total and HDL CH and triglycerides with estimation of LDL CH. Risk factors include male gender, family history of premature CHD, cigarette smoking, hypertension, HDL <50 mg/dL, diabetes, history of cerebral or peripheral vascular disease, and severe obesity. Patients with high LDL CH or in the borderline range with symptoms of CHD or two risk factors require therapy with the primary approach being dietary. A Step One diet decreases intake of CH (<300 mg/day) total fat (<30% of calories) and saturated fat (<10% of calories). If necessary, a Step Two diet, preferably given by a dietitian, further reduces CH intake to <200 mg/day and saturated fat to <7% of total calories. Only if intensive dietary therapy for at least six months does not bring CH below the respective cutpoint should drug therapy be considered.

The LDL cutpoints triggering drug therapy are increased by 30 mg/dL. The first line drugs are the bile acid sequestrants and niacinic acid. Since individual cholesterol measurements are subject to analytical and physiological
variability, repeated measurements are recommended both prior to initiating treatment and in monitoring the response to treatment. The NCEP will likely result in more CH measurements by clinical laboratories and will necessitate improvements in the measurements for reliable classification.

Standardization of Cholesterol
Herbert K. Naito, Ph.D.
Cleveland Clinic Foundation
Cleveland, OH

The National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH) recently launched the National Cholesterol Education Program (NCEP). The goal of the program is to contribute to reducing illness and death from coronary heart disease (CHD) in the United States by reducing the number of Americans with high blood cholesterol. Two panels of health care professionals were created by the NCEP to develop guidelines for (a) the detection, evaluation, and treatment of the high blood cholesterol in adults and (b) the standardization of laboratory testing and reporting of cholesterol levels. The Adult Treatment Panel (ATP) of the NCEP issued new cholesterol and LDL-cholesterol cutpoints that define individuals that are at risk for CHD. These nationally derived cutpoints that are being recommended for all laboratories were derived from specialized NHLBI-funded lipid research clinics laboratories which were standardized to the CDC cholesterol reference method and reference materials to ensure an accurate database. The success of the NCEP hinges on all laboratories providing reliable cholesterol measurements. The Laboratory Standardization Panel (LSP) of NCEP recommended that all laboratories standardize their cholesterol measurement for accuracy which are traceable to the NCCLS-National Reference System for Cholesterol.

The presentation will highlight the new guidelines and recommendations of the ATP and LSP. In addition, the current state of reliability of lipid and lipoprotein resources and components necessary for standardization, and the basic how-to implant this process will be discussed. Finally, the presentation will focus on practical issues such as biological and physiological factors, environmental factors, and specimen collection factors which can influence the variability of cholesterol values. The presentation is aimed at both the manufacturers and laboratorians to help ensure more precise and accurate cholesterol testing.

Approach to Achieving Accurate Measurement of Cholesterol
G. Russell Warnick, M.S., M.B.A.
Northwest Lipid Research Center
Seattle, WA

Nationally derived cutpoints defining high risk and borderline levels for total and LDL cholesterol are now available. To achieve reliable classification of patients laboratories must not only achieve acceptable precision but also accuracy. Analytical systems which now are certified to the National Reference System for Cholesterol, which provided the accuracy basis for the national population studies from which the cutpoints were derived. Assessment of method accuracy is best made by comparison with the Abell-Kendall Reference Method (A-K) on actual fresh patient specimens. Quality control and survey materials can provide an approximate assessment of accuracy, but the processing procedures necessary to prepare convenient, economical pools may affect the analytic and matrix properties such that the measurement characteristics are not representative of actual patient specimens. A laboratory might first request documentation of accuracy from the reagent manufacturer. Expect a current direct comparison of the analytical system against the A-K on 50-100 representative patient specimens. With 8 manufacturers supplying approximately 80% of the cholesterol reagent market, it will be most efficient to initiate standardization through them. If the manufacturer does not provide adequate documentation or if a "heterogeneous" analytical system (in-house or with components from several manufacturers) is employed, the laboratory can estimate accuracy from the agreement with the Confirmatory Value (A-K) on the CAP or other proficiency surveys and materials. Caution must be exercised because such pools may not exactly reflect accuracy on patient specimens. The definitive but logically complicated approach is to perform a direct comparison against the A-K on representative patient specimens. Based on the observed relationship, calibration can be adjusted to achieve agreement with the A-K.

Obtaining the necessary accuracy in cholesterol measurement will initially be challenging for many clinical laboratories. In the future, with increased access to the Reference Method and further improvements in control materials, reagents, and instrumentation, performance should improve facilitating reliable classification of patients.

Apolipoprotein Measurements in the Clinical Laboratory
John J. Albers, Ph.D.
University of Washington
Seattle, WA

As a result of a new national thrust to identify and treat hypercholesterolemia, apolipoprotein (apolipoprotein) measurements are of growing clinical interest. Measurement of apoproteins A-I and B are used to predict the risk of developing coronary heart disease and to monitor the progress of dietary and drug intervention. There has been a proliferation of commercial kits for these measurements. However, these apoprotein measurements have not reached their full potential in the clinical laboratory because of problems in methodology, inadequate standardization, the lack of reference methods and population-based reference values.

Considerable differences in values are observed between apoprotein methods. Some of the differences can be attributed to differences in the assigned values of reference materials while some differences between methods are method dependent. Recently, progress has been made in the development of reference methods for apoproteins A-I and B. Also, new population based values for A-I and B are available. Another apoprotein, apoprotein (a) has potential as a biochemical marker for the risk of coronary heart and cerebrovascular disease. Guidelines for the measurement and interpretation of apoprotein measurements in the clinical laboratory will be reviewed.

Session VII—Topics in Clinical Immunology
Developed in cooperation with Clinical and Diagnostic Immunology Division
Vincent A. DeBart, Ph.D.
Chairman

Clinical chemists are emerging as leaders in a field formerly dominated by other laboratory specialists. As our understanding of that field, immunology, develops to a greater extent at the molecular level, it is virtually inevitable that those educated primarily in the profession of chemistry gradually turn their attention toward the study of the immune system. This selected topics session was conceived to provide an overview of four separate areas of immunology in which clinical chemists are actively involved. Included are a state-of-the-art update in a "traditional" area (electrophoresis), two talks on the rapidly-expanding field of "new" aspects of immunochromy such as autoantibodies and circulating immune complexes and a discussion of a practical therapeutic application of immunochromy, the treatment of cancer.

Monoclonal Antibodies and Tumor Imaging
Michael W. Unger, Ph.D.
Hybritch, Inc.
San Diego, CA

The introduction of monoclonal antibody (MoAb) technology has stimulated an enormous interest in using anti-tumor MoAbs to target (direct or guide) diagnostic and/or therapeutic modalities to tumors. While no MoAb based anti-tumor agents have yet been approved for use in the U.S., human clinical trials are underway to test in vivo diagnosis (e.g. imaging with isotopes) in a variety of tumor systems as well as therapy trials using MoAb directed isotopes, drugs, and toxins.

The generation and selection of anti-tumor MoAb's will be discussed. Of major importance is the selection of a MoAb to avoid cross-reactivity with normal tissue components. The production of appropriate quantities and form (e.g. intact antibody versus various fragments) will be discussed. At this time, no general rules for production or form have been validated. Lastly, the conjugation of isotopes, drugs and toxins to the MoAb will be reviewed.

The results of human clinical studies with various MoAb's will be reviewed in order to identify both the problems and potentials for diagnostic and therapeutic use. In general,
while Mob's can target (isotopes to) tumors, the amount of antibody which concentrates in the tumor is low. The amount of Mob delivered to the tumor must be increased in order to expect therapeutic uses to succeed.

Autoantibodies in Systemic Autoimmune Diseases
Vincent A. DeBari, Ph.D.
St. Joseph's Hospital and Medical Center
Paterson, NJ

The systemic rheumatologic diseases with an autoimmune etiology have been variously called collagen diseases, collagen vascular diseases, or, in more modern parlance and more in keeping with current understanding of their causes, the systemic autoimmune diseases (SAD). These diseases have two common features: the presence of autoantibodies (auto-ab) and multi-organ or systemic manifestations and include systemic lupus erythematosus, rheumatoid arthritis, progressive systemic sclerosis (scleroderma), polyposisides/dermatomyositis, Sjögren's syndrome, and mixed connective tissue disease.

Undoubtedly, the most commonly studied of the auto-ab in SAD are those directed against nuclear components of the cell, the antinuclear ab (ANA). These provide the basis for the widely used fluorescent ANA immunocytochemical screening test. A more thorough study of ANA requires characterization of antigen specificity of these auto-ab. For DNA are evaluated primarily by radiometric binding assays and enzyme immunoassays (ELISA). Additionally, the quantitation of ab mass can be accomplished by immuneadsorption/ nephelometry. Ab to the extractable nuclear antigens (ag), particularly Sm, nRNP, SSA/Ro, and SSA/S, have been detected by immunoprecipitin and hemagglutination assays. These suffer from lack of specificity or sensitivity. The use of ELISA methods improves performance. Anti-histone ab may be precisely fingerprinted by the application of western blotting to histone fractions and peptide fragments of histone.

Another group of ab observed in SAD are directed against cytoplasmic components. In addition to mitochondrial and ribosomal ag, the various cytoskeletal elements (microtubules, microfilaments and intermediate filaments) may act as target sites of auto-ab. The detection of these ab requires a knowledge of their distribution as cells under stress, the systemic autoab, and the development of immunoassays for their specific quantitation. ELISAs have been developed for this purpose and may prove useful in the clinical laboratory.

Rheumatoid Factors, Shared Idiotopes, and Antimembrane Antibodies in Immune Complex Disease
Stanley S. Levinson, Ph.D.
Sinai Hospital of Detroit
Detroit, MI

Immune complexes are composed of immunoglobulins attached to antigens. The antigens may be other immunoglobulins. Other serum proteins, such as complement, C-reactive protein, and fibrinectin, may also be attached. In autoimmune and infectious diseases, immune complex deposits, containing immunoglobulins and complement, are often found in tissue in association with damage caused by inflammation. Circulating immune complexes are those found in plasma.

It has been suggested that circulating immune complexes cause tissue damage by a process similar to that seen with serum sickness, becoming trapped in membrane compartments, binding complement, and causing inflammation, vasculitis, glomerulonephritis, neuritis, etc. Conclusions drawn from experience attempting to measure circulating immune complexes have been misinterpreted due to interferences with the assay.

Recent advances in immunology indicate: autoantibodies, such as antiDNA, antinuclear, and lupus anticoagulant have been shown to have affinity for components in which membrane are rich. For example, DNA antibodies bind to phospholipids, TNF-derivatives, proteoglycans, and glycosylipids. 2. Autoantibodies with different antigen specificities and from different patients often react with the same monoclonal antiidiotypic antibody. These autoantibodies are said to exhibit "shared" or common idiotopes. 3. Patients with chronic immunological activation show a greater tendency for antibodies to aggregate than normal. This tendency may be due to increases in IgG, and IgM rheumatoid factor, and to idiotype antibodies. It can be speculated that these factors lead to the formation of immune complexes in situ: Antibodies with appropriate affinities bind to cell membranes. Patients with an increased tendency for aggregation develop immune complexes in local compartments due to facilitated binding to membrane bound antibodies of rheumatoid factors, idiotype antibodies, and possible binding by antibodies to shared idiotopes. The appropriate combination of these ingredients leads to the binding of complement and inflammatory tissue damage.

Electrophoretic Studies in Immunologic Diseases
Sidney N. Kahn, M.B., Ph.D.
St. Luke's Hospital
Bethlehem, PA

Serum protein electrophoresis (SPE) is a qualitative test whose most important and specific diagnostic application is in the detection of immunoglobulin abnormalities. Consequently, the optimal method for SPE must have great sensitivity for detecting such abnormalities; the most suitable routine method currently available is high-resolution agarose gel electrophoresis with visual inspection by a trained observer. Immunofixation electrophoresis (IFE) should be used to characterize any obvious immunoglobulin abnormality or to exclude a subtle immunoglobulin abnormality if there is a high index of clinical suspicion and an apparently normal SPE pattern.

Problems which arise from this approach to clinical SPE include the type of quality control procedure to be used and false-positive and false-negative results for paraproteins. Traditional five-fraction SPE on cellulose acetate with densitometric quantitation has been shown repeatedly to be only semi-quantitative and significantly less sensitive for paraprotein detection than high-resolution electrophoresis. One of the factors preventing wider adoption of qualitative SPE despite its advantages over five-fraction SPE is lack of a suitable method of quality control. Guidelines modified from those proposed by the Protein Commission of the Italian Society of Clinical Biochemistry for quality control of qualitative SPE will be presented in detail.

Paraprotein quantitation by densitometry remains a valid use of quantitative SPE. In the absence of appropriate commercial control material for paraproteins, every laboratory must establish its own internal quality control program. The use of patient samples for this purpose will be presented. SPE can give both false-positive and false-negative results for paraproteins. Patterns which illustrate potential pitfalls in paraprotein detection will be shown.

Immunofixation electrophoresis (IFE) is a powerful adjunct to SPE for the detection and characterization of immunoglobulin abnormalities. The potential drawbacks, pitfalls and limitations of IFE and recommendations for overcoming them will be discussed.

Thursday Afternoon, July 28
Session VIII—Fibrinolysis
Amadeo J. Peace, Ph.D.
Chairman

The inappropriate foundation of clots (thrombi) is part of the process responsible for a variety of human disease from glomerulonephritis to myocardial infarction. Advances in genetic engineering have made it possible to study the components of both regulation and fibrinolytic systems and to elucidate the regulatory mechanisms which balances these complementary processes. Most importantly, these advances have made possible the development of the current intervention therapies which have become extremely important.

The first special topic presentation will give an overview of the components and regulatory nature of the fibrinolytic systems. The second will present current methods of monitoring the system in the use of chronic systemic substrates, an area of importance to the clinical chemist. The third presentation gives a clinicopathologic correlation of functional and immunologic measurements of various components of the fibrino-
lytic system with known disease entities. The final presentation concerns the role of genetic engineering in the preparation of tissue plasminogen activator and its use in human disease, particularly for the treatment of myocardial infarcts.

The Fibrinolytic System: An Overview
Amadeo J. Pease, Ph.D.
University of Cincinnati
Cincinnati, OH

The process by which blood clots are dissolved is termed fibrinolysis and the components of this process are a series of coordinated interactions of proteolytic enzymes and their inhibitors. Fibrinolysis is the product of a cascade of activation reactions much like coagulation.

The activators, which are trypsin-specific serine proteinases, act to convert plasminogen to plasmin which dissolves the blood clot. Two major independent pathways, the intrinsic and the extrinsic, activate plasminogen. The intrinsic system is a relatively undefined activator cascade involving Factor XII, high molecular weight kininogen and prekallikrein. It is believed that the objective of the cascade is to activate single chain urokinase like plasminogen activator which in turn activates plasminogen. The extrinsic system is named for the origin of its activator, tissue plasminogen activator (t-PA), which is released from the vascular endothelium. The fibrinolytic system is regulated both at the level of plasmin and at the level of activator by the specific proteinase inhibitors, plasminogen activator inhibitor (PAI) and alpha-2-PI inhibitor (antiplasmin or alpha-2-PI). In addition the coagulation and fibrinolytic systems are regulated by a "bridging" system involving protein C and protein S.

The strategy of tPA therapy is to attain a high enough level of activator which will overcome the effect of inhibitors, saturating the clot with activator which in turn activates plasminogen at the clot site. Even though considerable progress has been made, such work remains to be done to understand the regulatory mechanisms and interactions of this pathway in both health and disease.

Monitoring the Fibrinolytic System
Ralph A. Magnotti, Jr., Ph.D.
University of Cincinnati
Cincinnati, OH

Approximately 16 components of the fibrinolytic system can be monitored and monitored including tripeptide substrates designed to specifically measure amidolytic proteinases, and ELSA and other immunochemical procedures such as rocket and immunoblotting techniques.

Major technical problems remain with regard to measuring components of the system, such as a lack of reagent specific for the precision and accuracy required in routine clinical assays. A fourth generation of such substrates is currently under evaluation; however, there is a need for standardization of the monitoring techniques. We propose a laboratory improvement program to ensure the quality of the data generated by these assays.

Activase®: A Tissue Plasminogen Activator—An Overview
Elliott B. Grosebardt, M.D.
GENENTECH
South San Francisco, CA

Tissue plasminogen activator (t-PA) is an endogenous glycoprotein serine protease (MW = 65,000) with an affinity for fibrin and with the potential for substantially enhanced activity in the presence of fibrin. Complementary DNA for t-PA messenger RNA was cloned and transfected into a mammalian cell line (Chinese hamster ovary cells). The biosynthetically produced recombinant t-PA (Alteplase, Activase®) was purified and pharmacologically formulated for clinical trials in a variety of vascular thromboembolic disorders including myocardial infarction (M.I.), pulmonary embolism, deep vein thrombosis, peripheral arterial occlusion, strokes, unstable angina and central retinal vein occlusion. On November 13, 1987, the U.S. FDA licensed Activase® for administration to M.I. patients based on its ability to lyse coronary artery thrombi, improve ventricular function and reduce the incidence of congestive heart failure. The fibrin affinity and fibrin-enhanced activity of Activase® produces thrombolytic activity with limited systemic proteolysis, compared to other plasminogen activators. This improved toxic-therapeutic ratio results in fibrinogen sparing which may ultimately be translated into greater safety. An in-vitro artifact due to increased plasma t-PA concentrations during Activase® infusion has been identified. Protection against the artifact, or at least knowledge of its presence for proper interpretation of clotting tests performed at the time of Activase® treatment is necessary.

Fibrinolysis in Medicine: Diagnosis and Therapy
Victor E. Pollak, M.D.
University of Cincinnati Medical Center
Cincinnati, OH

In the genesis of thrombosis, the role of coagulation cascade and platelets is well known, yet treatment is still unsatisfactory. The importance of the fibrinolytic system is only beginning to be understood, fibrinolysis promoing drugs to be used in treatment. Tissue plasminogen activator (t-PA) is released from vascular endothelium, in clot binds fibrin-bound plasminogen converting it to plasmin; the results in clot lysis. In disease, inhibitors of fibrinolysis are important: at least two t-PA inhibitors (PAI) are known. Low t-PA and high PAI levels are common in thrombotic states. Abnormalities have been found in myocardial infarction, stroke, diabetes with complications, glomerulonephritis, nephrotic syndrome and systemic lupus erythematosus. Plasma alpha-2-antiplasmin (α2-AP), the fast reacting specific inhibitor of plasmin, is elevated in severe thrombosis in particular in veins (e.g. renal vein thrombosis; recurrent phlebothrombosis). Full understanding of the diagnostic meaning of plasma levels of t-PA and the inhibitors awaits detailed study. Streptokinase administration results in systemic plasminemia, and may therefore result in bleeding. The effect of therapeutically administered recombinant t-PA on this system is still poorly described. The snake venom enzyme anconid cleaves circulating fibrinogen molecules, giving rise to circulating non-cross-linked anconid-fibrin: this stimulates t-PA release from endothelium. Clot lysis occurs with 3 hours (elevated serum FDP and D-dimer). PAI levels decrease but not to below the normal range. α2-AP levels decrease; systemic plasminemia does not occur, and bleeding is very rarely. This action of anconid occurs when α2-AP levels are normal, not when elevated. Detailed observations with anconid administration show a remarkable correspondence between known understanding of the disordered physiology of clot lysis and the anconid fibrin stimulated release of endogenous t-PA.

Session IX—Alternatives to Laboratory Testing in Animals
Developed in cooperation with the Animal Clinical Chemistry Division

Jon P. Kimball, Ph.D.
Starling Winthrop Institute
Rensselaer, NY
Chairman

An increasing societal concern about the use of animals in research, testing and education is emerging as a national, as well as international, issue in the public, private and industrial sectors. The use of animals is debated and discussed in printed and visual media, and public celebrities have helped dramatize the issues of animal rights and animal activism. Demonstrations protesting animal use, vandalism of animal facilities, and the break-in of facilities and theft of animals has been increasing in frequency and severity. Scientists who use animals in research are aware of the moral and ethical issues, but have a responsibility to scientifically justify and explain
the necessity for animal usage, and to ensure that laboratory animals will be exposed to only the most humane conditions. Proposed and enacted state and federal legislation regulating the use of animals has contributed to minimizing the abuses of earlier periods. Recent technological advances particularly in molecular and intracellular techniques, have provided essential elements for developing alternative in vitro methodologies to complement traditional in vivo techniques. The demonstration of predictive value and validation of these methodologies presents new challenges to the research community. The implications and applications of alternative methods to in vivo animal testing will be described in this symposium.

Why Alternatives or Adjuncts to Animal Testing?
W. Jean Dodde, DVM
New York State Department of Health
Albany, NY

The remarkable accumulation of scientific knowledge over the last fifty years has resulted in greater technical sophistication in experimentation and testing. This has caused more reliance on molecular and intracellular techniques in biomedical investigations and less upon the use of intact, whole animals. The consequences of this shift in experimental emphasis from the traditional in vivo approach to in vitro techniques have been severalfold:

1) to enhance the welfare of animal subjects used in research by implementing the 3 R's of Russell and Burch, namely: reduction in the number of animals used; replacement of animals with nonanimal methods or animals of a lower phylogenetic scale; and refinement of techniques to invoke less harm or ethical cost to the animals.

2) to increase the efficiency and often times the reliability of experimental procedures while at the same time reducing the variability inherent to whole animal use and the overall cost of the research effort.

3) to increase the emphasis on proper animal husbandry, for reliance on fewer animals or the indirect use of animals via their tissues will necessitate more rigorous definition and maintenance of the health of the donor animals.

Ironically, as science becomes more advanced, better testing and research methods will be sought which could eventually depend upon more animals to validate the expanding battery of in vitro "alternatives." Thus, while the public may view the long-term goal of science as total replacement of animals, nonanimal methods will continue to provide important adjuncts but will not fulfill the need for animals, at least in the foreseeable future.

Validity of In vitro Toxicology Testing
J. M. Frazier, Ph.D.
Johns Hopkins Center for Alternatives to Animal Testing
Baltimore, MD

New methodological developments in in vitro toxicity testing hold great promise for revolutionary changes in chemical risk assessment. However, for this potential to be realized the database produced by these new methodologies must be validated. The basic process of validation consists of null phases. The first phase involves preliminary development including identification of potential test systems, selection of test chemicals and definition of the "gold standard," i.e. a database with which to compare the results of new test systems. The second phase, i.e. micromodulation, involves establishing the reliability of a given test within the laboratory which developed the test (the primary laboratory). Next, it must be demonstrated that the technology can be transferred to other (secondary) laboratories, i.e. ruggedness testing. This is referred to as macrovalidation. Finally, an optimization procedure must be undertaken to decrease the battery of tests which provide an adequate spectrum, of data for reliable risk assessment. Several specific tests will be discussed which illustrate the application of these principles as to the validation of in vitro test systems.

Alternative Tests for Carcinogens and Teratogens
David J. Brusick, Ph.D.
Harlestan Laboratories America, Inc.
Kensington, MD

Estimations of product safety have historically been based on the results of experimental animal-based toxicity tests. The questions that have been raised recently concerning animal usage in toxicology have resulted in a resurgence in interest in the development of in vitro toxicity test systems that employed cultured cells. A precedent for the development of in vitro toxicity test systems exists in that cultured rodent and microbial cell systems have been in use for over a decade. Nonetheless, the development of a new generation of in vitro toxicity test systems presents both formidable challenges and opportunities. While cultured cells from various animal species have been successfully employed for many years in investigations of basic biochemical processes, carcinogenesis, and toxicology, the test systems that may have defined molecular bases but that are expressed at the organism system level. The challenge for the in vitro toxicologist is then, to identify the molecular markers relevant to toxicant-induced disease. While investigators working with in vitro cell systems have employed diverse approaches to toxicity test development, tests that used cultured cells may hold particular promise. A specific group of in vitro methods appear to provide adequate prediction for most classes of animal carcinogens and teratogens. An overview of these methods and their advantages and limitations will be presented.

Alternative Methodology in Oculus Irritation Testing
Jeff Everitt, DVM
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Ocular irritation testing ("Drake's eye test") is objectionable to some members of both the public and scientific community. In an effort to curb painful animal research, scientists are promoting the development and use of alternative methods to traditional toxicity testing. This sometimes, but not always, refers to the replacement of whole live animals. Russell and Burch (The Principles of Human Experimental Technique. Methuen Press) enumerated principles of human experimentation which stated that investigators should seek where possible to replace, reduce, and to refine techniques so as to reduce animal pain and suffering. These principles are being applied to ocular irritation testing methodology. Research is presently being conducted to replace albino rabbits in ocular irritation testing with in vitro cell and organ culture systems. In vitro methods are examining cytotoxicity, cellular metabolism, cell and tissue physiology, structure activity relationships, and the use of choriocapillaris membranes in an effort to develop a battery of tests which eventually might replace the in vitro test. The methods currently under development have the potential to be scientifically valid and useful predictive tools for determining risks from human ocular exposure. However, no in vitro tests are yet validated as an alternative to the use of animals in ocular irritation testing. There are methods to reduce animal usage and techniques to minimize animal suffering, by making changes to standard Drake's protocols. Methods include: use of reduced test material volumes; use of anesthetics; prescreening compounds; and staggering study starts. These approaches should always be considered by investigators as well as Institutional Animal Care and Use Committees when reviewing ocular irritation testing protocols.

Session X—Geriatric Clinical Pharmacology
Developed in cooperation with the Therapeutic Drug Monitoring and Clinical Toxicology Division

Joseph N. Miceli, Ph.D.
Chairman

The population in the United States and the Western World is aging. The elderly consumer is more drug than comparable younger populations. This selected topic will present a brief "look" at the realm of geriatric clinical pharmacology, an area in which little information exists. In this session, an overview of age-related changes in pharmacodynamic parameters such as drug absorption, distribution and excretion will be presented first. This will be followed by discussions of drugs that are of particular concern in the elderly. Cardiovascular drugs are probably the most frequently prescribed drugs in geriatric patients. Monitoring of these agents requires an awareness of age-related changes in pharmacodynamics as well as
knowledge of drug-drug interactions because most elderly people consume multiple drugs. Deposition is commonly encountered in the elderly and as a group they receive a disproportionate number of prescriptions for psychotropic drugs. The tricyclic antidepressants are the most frequently used agents to treat depression in the elderly. Other anti-psychotic drugs that are used include haloperidol and chlorpromazine. These two drugs present additional problems in their monitoring because methodology is not commonly available for their determination. The end result is that our knowledge of geriatric pharmacology is very limited and that much more information is required so that drugs can be used in a safe, rational manner in this population.

Changes in Pharmacokinetics and Pharmacodynamics With Age
Joseph N. Lillis, Ph.D.
Morehouse School of Medicine
Atlanta, GA

We are growing older. Life expectancy in the Western World now averages about 70 years for males and 77 years for females. It is predicted that in the U.S. in the year 2000, there will be approximately 12 million people over 75 years old and older. This population will require health care and treatment with therapeutic agents. The elderly have more sickness, hospitalization visits and use more drugs more frequently than does a comparable younger population. The elderly also experience more drug-related side-effects than do the young.

Why are the elderly more susceptible to drug action? Our knowledge of human drug use is based, in part, on human studies which are almost always performed on young, healthy populations; not on elderly patients. Thus, the answers could be related to age-associated changes in receptor function, physiologic changes or both. Most of the scant evidence that exists suggests that changes in physiologic function(s) are predominately responsible.

Clinical pharmacokinetic parameters must be evaluated in the elderly to accurately assess changes in drug handling in this population. These parameters include: absorption, distribution, metabolism and elimination. Additional factors include the status of the immune, renal, endocrine and cardiovascular systems. Examples of changes associated with aging include: decreased gastric secretion, motility, acidity, peristalsis, cardiac output, renal and lung function. Other age-related effects include changes in body composition, effects of cigarette smoking, alcohol consumption, and a decreased ability to maintain homeostasis.

These general concepts and specific examples will be presented. Pitfalls in the interpretation of data will also be discussed. As our population ages, the laboratory will see an ever increasing number of samples from older patients. In order to provide appropriate care in this population, "geriatric" clinical pharmacokinetic studies are needed.

Use of Cardiac Drugs in the Elderly
Thomas Annalisey, Ph.D.
University of Michigan
Ann Arbor, MI

Until the "fountain of youth" is discovered we are all subject to the truth that the human body is going to age and become more susceptible to the diseases processes that accompany the aging process. Fortunately there are multiple drugs available that can be used to moderate or correct many of the physiological defects that accompany such diseases. It should also be recognized that as people age the number of drugs taken increases.

In geriatric patients the most frequently prescribed classes of drugs are cardiovascular drugs, followed closely by analgesic, tranquilizing and gastrointestinal agents. Many geriatric patients have prescriptions for many or all of these simultaneously. Plasma concentrations of antiarrhythmic drugs have been shown to correlate with therapeutic effects, so that it is worthwhile to become familiar with those factors which may influence the disposition of this class of drugs in the older patient. These include: 1) natural changes associated with the aging process, 2) effects of primary and secondary disease states, 3) diet and stress, and 4) concurrent therapy with other drugs. Studies evaluating changes in pharmacokinetics and pharmacodynamics of cardiovascular drugs due to age are few since the action of these drugs generally precludes their administration, especially in the elderly, in the absence of cardiac disease. Despite this limitation, it is evident that current information supports the view that the disposition of many antiarrhythmics is altered in older patients. This presentation will provide a general description of changes in the pharmacokinetics of cardiac drugs that may be observed in geriatric patients.

Psychoactive Agents in the Elderly
David B. Schneider, Ph.D.
Wayne State University
Detroit, MI

Accurate estimates of blood concentrations of psychoactive agents in humans can be accomplished for the antipsychotic agent haloperidol (HAL) and for most tricyclic antidepressants (TC's) in humans. Agents related to chlorpromazine can have more than 25 metabolites and estimates cannot be made of active metabolites. Assay of HAL can be performed by HPLC or RIA. Assay of TC's is accomplished with HPLC or EMIT technology.

Effective clinical therapeutics suggests that blood concentrations of HAL range from 1-15 ng/ml. Pediatric levels are suggested to be 1-3 ng/ml. Adult psychotic levels are maintained from 6-10 ng/ml; schizophrenic behavior can tolerate up to 15 ng/ml. Effective blood concentrations of TC's depend on the specific agent. Therapeutic information must include estimates of both the parent drug (approx. 125 ng/ml) together with an estimate of the single metabolite (100-150 ng/ml).

Complications with side effects and drug interactions are common. Initial dosages produce drowsiness. Antipsychotic or antidepressant actions are seen following 10 days of therapy. Acute toxic psychoactive concentrations produce antiarrhythmia, antiinflammatory, alpha-blockade, allergies, metabolic effects and antihistamine actions.

Overdosage with TC's can be extremely dangerous; depressed patients can be suicidal. Both accidental and deliberate overdose are serious, with patients exhibiting CNS, respiratory, and cardiac problems.

Use of Antidepressants in the Geriatric Patient
Paul J. Orsulek, Ph.D., M.B.A.
University of Texas Southwestern Medical Center at Dallas
Dallas, TX

Depression is the most common psychiatric disorder seen by primary care physicians, and depression in the elderly is one of the most significant treatment problems that these practitioners face. Dealing with depression in the elderly is different than that encountered in younger patient populations because of factors that complicate its diagnosis. Physiological changes associated with aging, differences in depressive symptoms, and difficulty in differentiating the pseudodepsoits of depression from true dementia or Alzheimer's disease all make treating depression in the elderly more difficult.

Individuals who we define as elderly, i.e., those over 65 years of age, currently account for about 11% of the U.S. population, but 25-30% of the health care costs are incurred by this group. The increasing incidence of multiple medical conditions add to the complexity of providing medical care and managing pharmacological treatment in the elderly. Many of these people tend to self medicate and do not follow precisely the treatment recommendations of their physician. Although the elderly make up only 11% of the population, they receive 22% of all prescriptions and are given nearly 25% of the prescriptions for psychotropic drugs.

Because of the frequency with which physicians prescribe psychotropic medications in general, and antidepressants in particular to the elderly and because of the growing number of medications available, it is important to develop appropriate guidelines for their use in this group. This is particularly important because of the concurrent medications that are likely to be present and the complicated medical and neurological factors associated with aging. This presentation will outline current indications for use of antidepressants in the elderly, general pharmacological considerations, and guidelines that should be kept in mind when monitoring patients being treated with these drugs.
Industry Sponsored Workshops

All Industry Sponsored Workshops will be held at the New Orleans Hilton on Tuesday, July 26, and Wednesday, July 27.

Tuesday, July 26, 5:30 p.m.

ESA, INC.
Plasma Catecholamine Determination by the Clinical Laboratory
  Michael Lynch
  Reginald Griffin, Ph.D.
  William F. Oakes
  ESA, Inc.

HELENA LABORATORIES, INC.
Acute Myocardial Infarction, Thrombolytic Therapy, CK isoforms
  Alan H.B. Wu, Ph.D.
  University of Texas
  School of Medicine
  Tipton L. Golias
  Helena Laboratories

MILLIPORE CORPORATION
Membranes in Clinical Diagnostics: Technology and Applications
  Alan Weiss, Ph.D.
  Phil Clark
  Aldo Pitt
  Millipore Corporation

SYVA COMPANY
Emit® Convenience Packs for Random Access TDM/Thyroid Testing on the COBAS MIRA® Chemistry System
  Jay Gorsky, Ph.D.
  Johnny Valdez
  Syva Company

Wednesday, July 27, 5:30 p.m.

BANGS LABORATORIES, INC.
Particle-Based Tests and Immunosays—Why and How
  Leigh B. Bangs, Ph.D.
  Bangs Laboratories, Inc.

BECKMAN INSTRUMENTS
New Protein Markers for Nutritional Assessment: Impact on Hospitalization Length of Stay and Use with Low Birth Weight Infants
  Terry K. Johnson
  Beckman Instruments
  Larry B. Bernstein, M.D.
  Bridgeport Hospital
  A. Michael Spiekerman, Ph.D.
  Scott and White Hospital and Clinic

JCL CLINICAL RESEARCH CORPORATION
Serum Erythropoietin Determination Using Enzyme Immunoassay Method
  Tommye Ann Jordon
  John D. Jordon
  Maxie L. Jordon
  JCL Clinical Research

NICHOLS INSTITUTE DIAGNOSTICS
Cost Effective Strategies for Thyroid Function Testing from a Laboratory and Clinical Standpoint
  Carole Ann Spencer, Ph.D.
  University of Southern California

SYVA COMPANY
A Positive Urine Drug Test Result Through the Eyes of an Attorney
  Robert E. Greenberg, Esq.
  Deso, Greenberg & Thomas, P.C.
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New Products Review

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This information appears here because we believe that gaining information on how to do new things or to do old ones more efficiently is an important part of the educational function of this meeting and the journal. For readers who cannot attend the meeting, more information is provided via our Reader Service Card.

Infectious Mononucleosis Heterophile Antibody

The Ventrescreen® Mono Test from Ventrex Laboratories can be performed with fingertip blood, serum or plasma. This five minute enzyme immunoassay will detect heterophile antibody during early infection with Epstein-Barr Virus. To perform the test, sample and conjugate are added to the bovine antigen coated tube. After 3 minutes, a wash is performed, followed by the addition of the color development solution. If the solution turns blue within two minutes, heterophile antibody is present. All reagents are provided in dropdown vials. The assay is available in 5, 20 and 100 test kits.

VENTREX LABORATORIES, INC. Circle No. 501 on Reader Service Card.

Closure Cap for Evacuated and Culture Tubes

TopCap® Closure is a disposable laboratory device for use as a cap with evacuated blood drawing tubes and culture tubes.

The design allows for the same TopCap® Closure to fit most sizes. The closure system does not leak or pop off under room temperature, refrigeration, or freezer storage. It also can be agitated with test tube rockers without leakage.

TopCap® Closures are color coded with evacuated tubes and are distributed by Curtin Matheson Scientific.

POREX MEDICAL DIV. OF POREX TECHNOLOGIES CORP. OF GEORGIA. Circle No. 504 on Reader Service Card.

Tonometered Co-Oximetry Control

Utilizing the LINEAR KGT-3/4 "cloud" Tonometer, the laboratory can now produce its own CO-Oximetry control from stabilized hemoglobin solutions. You can select your own concentration of HbCO and Methemoglobin. Preparation of 30 days supply requires just a few minutes of technologist time. The cost of the Co-Ox control will be approximately 50–70 cents per milliliter, as compared to over $2.00 per ampule for the conventional sealed sealed products.

LINEAR TONOMETERS, INC. Circle No. 502 on Reader Service Card.

Membrane Products for Clinical Diagnostics

Millipore offers a variety of membrane products for Clinical Diagnostic Kit manufacturers. In addition to membranes for sample clarification and pre-filtration applications, we offer membranes used as solid-phase substrates for ligand immobilization. The Immobilon™-AV Affinity Membrane permits covalent interaction with antibodies, antigens, and other proteins, and can be used in a wide variety of in vitro immunodiagnostic test protocols. We also offer Immobilon nitrocellulose and PVDF transfer membranes for use in hybridization and protein blotting. Millipore will incorporate these membranes into novel working designs. Our device fabrication capabilities allow maximum functionality without compromising membrane integrity.

MILLIPORE CORPORATION. Circle No. 505 on Reader Service Card.

Benchtop Centrifuge Microprocessor Controlled

Jouan announces the introduction of their new 422 series microprocessor controlled benchtop centrifuges.

Two models are available: the CR422 refrigerated model which allows temperature control from –6 °C to 40 °C and the CT422 thermostated model which is capable of controlling chamber temperature from –8 °C to 50 °C.

These units combine high RCF (up to 4200xg with the M4 horizontal rotor) with maximum veratility and capacity.

The microprocessor guides the user through the programming process. The operator just enters the desired values in sequence as the microprocessor requests them. The programs are stored in one of the 10 permanent memories.

JOUAN, INC. Circle No. 503 on Reader Service Card.

Magic® Lite Cardiac CK-MB System

The MAGIC Lite Cardiac CK-MB System is a fast, accurate, highly sensitive non-isotopic immunoassay for detecting CK-MB in suspected myocardial infarction.

The MAGIC Lite CK-MB Assay exhibits no false positives due to cross-reactivity with CK-MM, CK-BB, macro CK, or mitochondrial CK. With results that can be used to monitor TPA.

False negatives are reduced because the chemiluminescent tracer gives the system the best sensitivity available with a minimal detectable dose of 0.65 ng/mL.

The MAGIC Lite Cardiac CK-MB System is cost effective due to the speed, ease and accuracy which does not require confirmation by a second method.

CIBA CORNING DIAGNOSTICS CORPORATION. Circle No. 506 on Reader Service Card.
Model 644 Na/K/Cl Analyzer

Ciba Corning Diagnostics has introduced a Sodium/Potassium/Chloride Analyzer to its line of electrolyte instruments. The Model 644 Na/K/Cl analyzer uses a 65 µl sample of whole blood serum, plasma or urine. Its simple yes or no, menu driven operation makes it easy to use and gives you a result in 35 seconds. Its automatic calibration maintains the unit in a constant state of readiness for handling patient samples.

With the addition of a 604, 40 place autosampler, the 644 becomes a batch analyzer leaving you free for other work.

CIBA CORNING DIAGNOSTICS CORPORATION. Circle No. 507 on Reader Service Card.

280 Blood Gas System

The 280 Blood Gas System from Ciba Corning Diagnostics is the third in a series of analyzers which features maintenance-free electrodes, modular design, powerful menu driven software, onboard diagnostics, and data management communication capabilities.

The 280 measures pH, pCO2, pO2, Hb, BP, and calculates ten other parameters. Membrane changing has been eliminated on the electrodes and the hemoglobin module not only gives clinically reportable results, but requires no routine maintenance.

The instrument was designed for simple operation. It is maintained in a "READY" mode for immediate sample analysis. The 280 also addresses the hazardous waste issue in the laboratory.

Stop by our booth for a demonstration.

CIBA CORNING DIAGNOSTICS CORPORATION. Circle No. 508 on Reader Service Card.

Blood Gas, Electrolytes, Glucose, and Hematocrit Analyzer

The Stat Profile 5 measures glucose, sodium, potassium, chloride, ionized calcium, pH, PO2, PO2 and hematocrit simultaneously on a 250 µl sample in 70 seconds. All measured parameters use electrode technology. Glucose, the most recent addition to the Stat Profile test menu, incorporates a patented new glucose sensor. The glucose analytical range is 0–700 mg/dl with typical C.V.’s of ±2%.

By offering all these critical tests on such a small whole blood sample in such a short amount of time, the Stat Profile 5 truly redefines the concept of stat testing.

NOVA BIOMICROBIAL. Circle No. 509 on Reader Service Card.

Universal™ II Agarose Film

Ciba Corning introduces a new Universal™ II Agarose Film. Featuring 3 chemistries on the same type film, the new Universal II agarose films can be used for performing LD isoenzymes, serum proteins and immunofixation on the same type film. Use of a template eliminates sample application artifacts.

Universal II films are available in 8 and 12 track sizes for maximum versatility and productivity, and no sample preparation or blotting of excess sample is necessary.

For immunofixation it is possible to run two complete profiles on one 12 track plate—a major time and cost savings.

CIBA CORNING DIAGNOSTICS CORPORATION. Circle No. 510 on Reader Service Card.

Quidel Allergy Screens

New regional allergens with serum and whole blood procedures are now available on the QUDEIL Allergy Screen. The 90 minute, visual dipstick enzyme immunoassay can now be performed in patient serum or whole blood with less than 3 minutes hands-on time. The dipstick produces a permanent color change endpoint result and features an integral negative control. Equipment is not necessary to perform this cost-effective test. The Allergy Screens are available for Northern, Southern and Western regions in 4 and 12 test kit sizes. Each 10 allergen dipstick contains the most prevalent regional grasses, weeds, trees and environmental allergens. QUDEIL. Circle No. 511 on Reader Service Card.

Quidel Total IgE Test

The QUDEIL Total IgE Test is a 30 minute dipstick enzyme immunoassay test which is used as a screen to identify the allergic patient. The dipstick produces a colored endpoint result which, using a color chart, can be quantified to high, low and normal Total IgE levels. This cost-effective test does not require instrumentation. The test procedure is simple to perform, requires less than 3 minutes hands-on time and can be used with patient serum or whole blood.

The QUDEIL Total IgE is supplied in 10 and 30 test kit sizes; each dipstick features an integral negative control.

QUDEIL. Circle No. 512 on Reader Service Card.

Carbamazepine Assay for the TDx® Analyzer

Carbamazepine is an automated immunoassay for use on the TDx® Fluorescence Polarization Analyzer. FPR™ Carbamazepine is packaged in a ready-to-use barcoded reagent pack. Each pack contains three vials, antibody, tracer, and pretreatment. Sufficient reagent is provided to perform one hundred determinations of Carbamazepine in serum or plasma. Compared to the TDx® Analyzer manufacturers reagents Colony FPR™ Carbamazepine has a correlation coefficient of .986, at 50–70% less cost. As with all Colony Reagents, FPR™ Carbamazepine is compatible with all TDx® software revisions, including revisions 8.9, 10, and 11.

COLONY LABORATORIES, INC. Circle No. 513 on Reader Service Card.

Urease® EIA for the Detection of Group A Strept Antigen

Visuwell® Strep-A is an EIA based Microwell immunosassay for the detection of Group A streptococcus Antigen in throat swabs. The assay employs UREASE as a substrate, thereby offering the following advantages:

—clear cut colour change from yellow to purple allows for easy visual interpretation. Results can also be read spectrophotometrically.
—Urease is non-toxic enabling safe use and easy disposal.
—Allows for stat or batch testing (up to 20 or so specimens).
—Offers up to 50% cost saving compared to membrane EIA tests.

ALLELIX DIAGNOSTICS, INC. Circle No. 514 on Reader Service Card.

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988 1351
**Urease™ EIA for the Detection of hCG in Urine**

Visuwell™ hCG-Urine is an EIA based Microwell immunoassay for the detection of hCG in urine. The assay employs UREASE as a substrate, thereby offering the following advantages:
- Clear cut colour change from yellow to purple allows for easy visual interpretation. Results can also be read spectrophotometrically.
- Urease is non-toxic enabling safe use and easy disposal.
- Allows for stat batch testing (up to 20 or so specimens).
- Offers up to 50% cost saving compared to membrane EIA tests.

**Fluid Dispensing Cap**

Helena Laboratories introduces a new Tip Top™ dispensing cap to fit standard separating columns for 13 to 16 mm blood collection tubes. Simply centrifuge the sample, insert the serum separating column and slip a Tip Top onto the column. A small opening in the tip then allows serum to be dispensed in a drop-like fashion, without removing the Tip Top. The tube is stored with the Tip Top in place; one Tip Top can be used to dispense the same sample again and again. Tip Tops protect laboratory personnel from infectious agents by minimizing sample handling and risk of accidental spills.

**HELENA LABORATORIES.** Circle No. 519 on Reader Service Card.

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**Cardiac Isoenzymes**

REP CK/LD Combo Kits are now available for the Helena REP (Rapid Electrofhoresis) System. Twelve CK and 12 LD samples can be separated simultaneously on a single REP gel, cutting time and expense by 50%! Complete results (finished, dried and scanned gels) are ready for both CK and LD isoenzyme tests in 20 minutes. The REP Combo CK/LD Gel is the first truly simultaneous isoenzyme method—and it gives the confidence of electrophoretic separation, in 20 minutes, at $1.25 per test. Automation also improves reproducibility; CVs of 2 to 3% for CK-MB are typical with REP.

**HELENA LABORATORIES.** Circle No. 516 on Reader Service Card.

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**CK Isoforms**

An automated method for rapid electrophoresis of CK-3 isoforms is available from Helena Laboratories. CK-3 isoforms can be separated on the REP (Rapid Electrofhoresis) system in only six minutes. Complete reportable results are ready in 16 minutes with less than 2 minutes of hands-on time. With the increased use of thrombolytic therapy, CK-3 isoform analysis could be a valuable addition to the cardiac enzyme profile. CK-3 isoforms can support earlier diagnosis of AMI and assess reperfusion after thrombolysis. The REP automates or eliminates the labor-intensive, technique-dependent steps of conventional electrophoresis, making STAT CK-3 isoform testing possible.

**HELENA LABORATORIES.** Circle No. 517 on Reader Service Card.

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**Laboratory Water System Continuous Deionization**

The new Millipore Ionpure™-12 Lab Water System produces water of 1 megohm quality or better at up to 12 liters per hour for qualitative analyses, production of buffers and reagents, and glassware washing and rinsing. The Ionpure-12 system uses an exclusive continuous deionization (CDI) technology which combines ion exchange resins, selectively permeable membranes, and electric current. The Ionpure-12 system does not require the labor-intensive cleaning required with a still or the regular tank exchanges required with conventional D.I. systems. Depending upon feed water quality, the Ionpure-12 system can operate from 1 to 2 years or more without a module change.

**MILLIPORE CORPORATION.** Circle No. 518 on Reader Service Card.

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**Thyroid Symposium Program**

A Comprehensive Thyroid Symposium Program In Videotape (VHS) Format Sponsored by Ciba Corning Diagnostics Corp.

A Guide to Understanding Thyroid Function Testing for the Primary Care Physician is a Four Part Presentation Featuring:
- Circulating Thyroid Hormones
  Sidney H. Inghbar, M.D.
- Sensitive TSH Assays
  Carole Ann Spencer, Ph.D.
- Thyroid Antibodies and Thyroglobulin
  Leslie J. DeGroot, M.D.
- Thyroid Stimulation Immunogobulins
  J. Maxwell McKenzie, M.D.
- Moderator
  Lewis E. Braverman, M.D.

Proceeds Donated to the Education Funds of the Endocrine Society and the American Thyroid Association.

**CIBA CORNING DIAGNOSTICS CORP.** Circle No. 522 on Reader Service Card.
Uniflex Speci-Gard™ Specimen Transfer Envelope

Uniflex introduces Speci-Gard™, a plastic specimen transfer envelope to physicians, hospitals and laboratories. Speci-Gard's roomy double section/double seal envelope provides safer handling of potentially infectious specimen. Separately sealed sections in one envelope prevents paperwork and personnel from contact should the specimen container leak. The clear section allows visibility of specimen; the partially opaque section provides confidentiality if desired. Snap-off perforation allows for easy opening after permanent sealing. Available with temporary or permanent adhesives, Speci-Gard may be custom imprinted and accepts ball point pen writing.

UNIFLEX, INC. Circle No. 523 on Reader Service Card.

Reactive Screen Immobilizes Antibodies, Antigens and Enzymes

Unique polyvinylbenzylchloride (PVBC) screen permits immobilization of antibodies, antigens, or enzymes in very convenient form. Amine groups on these ligands bind readily and directly to the reactive PVBC screen to produce affinity chromatography supports or immunoassay substrates. The ligand-bound screen may be cut, rolled, and formed into imaginative products.

Polymeric monofilament screen fabrics of 40 and 100 mesh were grafted with monomers to create polystyrene (PS) or polyvinylbenzylchloride (PVBC) surfaces permitting covalent binding of a variety of chemicals. Sulfonation of the PS screen and amination of the PVBC screen have been used to produce ion exchange resins in screen form.

BANGS LABORATORIES, INC. Circle No. 524 on Reader Service Card.

Simple Immunoturbidimetric Tests for Apolipoproteins

Most methods for determining apolipoproteins A-I and B are too cumbersome for busy laboratories. However, an immunoturbidimetric technique has been introduced that is extremely simple to perform. Tests require combining serum with antisera specific for apolipoprotein A-I or B and measurement of turbidity at 340 nm. Assays may be carried out using popular discrete analyzers or manually with most spectrophotometers. A series of six calibrators is provided for standardization and two controls are included for quality assurance purposes.

SIGMA DIAGNOSTICS. Circle No. 525 on Reader Service Card.

Simplified EIA Assays and Reader for Panel of Rapid Tests

EIA minilab™ simplifies quantitative EIA testing in all laboratory settings. Strips of antigen-coated microwells are used with dropwise technology. Stable color is measured on any spectrophotometer or on miniaturized EIA minilab™ Reader. Tests for rubella, anti-DNA, anti-DNP and amebiasis antibodies also toxoplasma IgG and IgM, cytomegalovirus IgG and IgM, herpes type 1 and 2, rheumatoid factor and IgG immune complexes. Stable liquid reagents used at room temperature. Standard and control (±1-) provided. EIA minilab™ Reader rapidly prints out automated microwell measurements.

SIGMA DIAGNOSTICS. Circle No. 526 on Reader Service Card.

Serum Protein Reagents for BM/Hitachi Systems

Tina-quant® B System Pack reagents are available from Boehringer Mannheim Diagnostics for the automated determination of IgA, IgG, IgM, C3, C4, CRP and transferrin. These reagents, used on the Boehringer Mannheim/Hitachi 704, 705 and 717 Analyzers, provide the laboratory with a rapid and economical random access method for measuring serum proteins.

Tina-quant® B reagents offer one-point calibration and ready-to-use reagents. The six week working reagent stability makes the reagents economical to use with even the most varied numbers of samples and testing schedule. The assays are based on an immunoturbidimetric principle and demonstrate high precision and accuracy.

BOEHRINGER MANNHEIM DIAGNOSTICS. Circle No. 527 on Reader Service Card.

Boehringer Mannheim/Hitachi 717 Clinical Chemistry System

The Boehringer Mannheim/Hitachi 717 analyzer is a discrete, fully selective, walk-away analyzer that can perform as many as 35 parameters from one sample, including Na⁺, K⁺, and Cl⁻, by a flow-through ISE module in a 24-hour, high throughput basis. Six hundred to 750 tests per hour throughput (with electrolytes) are possible with an average volume of 350 uL per test and 3-20 uL of sample volume. The automatic rerun feature utilizes reassembly with reduced sample volume without operator intervention.

BOEHRINGER MANNHEIM DIAGNOSTICS. Circle No. 528 on Reader Service Card.

Porex Format Diagnostic System

Porex Technologies proudly introduces the POREX® Format for OEM diagnostic applications. These devices, using a single piece of MEMPOR® Porous Plastic, combine the functions of microporous membranes, supports, and reservoirs for flow through particle based diagnostics. Porex has Formats available in several capture configurations and can also assist with custom designs.

POREX Formats are available in three standard capture configurations, 0.3, 0.8, and 1.2 micrometers. The total fluid capacity is approximately 4 milliliters. Custom colors are available for the housings.

POREX TECHNOLOGIES. Circle No. 529 on Reader Service Card.

Roche Cobas Fara II

COBAS FARA II, the only instrument to offer multiassay capability with six flexible measuring modes... Absorbance, Fluorescence Polarization, Fluorescence Intensity, Nephelometry, Turbidimetry, and Ion-Selective Electrodes.

COBAS FARA II, the "Specialist" Chemistry System is a fully programmable, compact, robotic system enabling full automation of more assays than any other system.

COBAS FARA II offers automated procedures for therapeutic drug monitoring and toxicology assays, lipids, proteins, coagulation, research and routine chemistries and electrolytes.

As a TDM Specialist, COBAS FARA II can accommodate up to 30 immunoassays with simultaneous testing capability and a ready-to-run menu of Roche TDM FP assays.

ROCHE DIAGNOSTIC SYSTEMS, INC. Circle No. 530 on Reader Service Card.
Rapid Assays for Drug Abuse

Roche Diagnostic Systems announces the introduction of Abuscreen® OnTrak® Rapid Assay for Drug Abuse. Simple, convenient and cost effective, Abuscreen OnTrak can be performed by anyone, anywhere, at any time—with absolutely no instrumentation. The OnTrak assays are designed to provide reliable, accurate screening results in as little as 3 minutes. Results are clear, unequivocal and require no interpretation—negatives form particles (agglutination), and positives have a smooth, milky appearance. Abuscreen OnTrak assays are available for Cocaine, THC, Morphine, Barbiturates and Amphetamines, with assays for Benzodiazipines, Methadone and Phencyclidine to follow. ROCHE DIAGNOSTICS SYSTEMS, INC. Circle No. 531 on Reader Service Card.

Coagulation Reagents & Controls

A complete family of 23 reagents and controls for the ACL Automated Coagulation Laboratory is now available from Fisher Medical Division. They feature exclusive, easy-to-open safety flip-cap seals to eliminate cut fingers and the associated risk of infection. The unique PT-FIB Reagent provides a fibrinogen result with each PT at no added cost, when run on the ACL system. The new aPTT Reagent is a complete kit with both particulate activator and calcium chloride—optimally sensitive to intrinsic factors, heparin, lupus. The new Assess™ controls and reference calibrator allow comprehensive QC. FISHER MEDICAL DIVISION. Circle No. 532 on Reader Service Card.

Laboratory Information System

CITATION data management and laboratory information systems and application software will be distributed by the Fisher Medical Division of Instrumentation Laboratory. CITATION systems link IBM Personal System/2™ workstations with Novell file servers into local area networks (LAN) to create a high-performance, low-cost alternative to mini- and mainframe computer-based networks. Systems can be expanded in affordable increments by adding IBM PS/2 workstations. A CITATION network can link up with virtually all hospital information systems, as well as with most laboratory diagnostic systems. CITATION also offers software programs for microbiology, blood bank and anatomic pathology applications. FISHER MEDICAL DIVISION. Circle No. 533 on Reader Service Card.

Platelet Aggregation Test

Streck Laboratories, Inc. introduces a new diagnostic platelet aggregation test, P.A.T. P.A.T. utilizes (K³) EDTA blood samples and a whole blood platelet counting instrument. The ability of platelets to aggregate in response to certain agonists is commonly used to determine normal and abnormal platelet function. P.A.T. reagents include ADP, collagen, epinephrine, ristocetin, arachidonic acid and a control.

Patient samples are collected in EDTA. The EDTA inactivates the platelet by sequestration of calcium. However, P.A.T. reagents contain calcium to reverse this effect.

Percent aggregation is calculated from the following:

% aggregation = \[
\frac{\text{beginning platelet count} - \text{final platelet count after 5 minutes}}{\text{beginning platelet count}} \times 100
\]

STRECK LABORATORIES, INC. Circle No. 534 on Reader Service Card.

Glucose Analyzer Utilizing Immobilizing Enzyme Technology

AMDEV, Inc. is exhibiting its new APEC GLUCOSE Analyzer which uses innovative immobilized enzyme technology to produce accurate and precise (C.V. 1%) glucose results in under 30 seconds on 10 ul serum or plasma. Sampling is automatic, either from a cup or tube, or from the optional turntable.

The only reagent, buffer diluent, needs neither preparation nor refrigeration. Maintenance is minimal with no set-up or shut-down procedures. Easy replacement of the unique, accessible molded reagent/resealable waste pack eliminates risk of contact with waste bottle contents. AMDEV's new APEC GLUCOSE Analyzer is always ready, requiring calibration once every 4 hours. AMDEV, INC. Circle No. 535 on Reader Service Card.