Evaluation of an Engineering Model of the "EKTACHEM" Analyzer for Glucose and Urea Assay

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We report results of a 21-week evaluation of an engineering model of the Kodak "EKTACHEM Glu/BUN Analyzer." This novel clinical instrument measures glucose and "urea nitrogen" (actually, urea) in a minimum of 30 μL of body fluids by use of the principles of thin, multilayer film analysis and reflectance spectrophotometry. We compared the performance of the instrument to that of the Dupont acca and the Beckman Glucose Analyzer for glucose, and to the Dupont acca and the Technicon SMA 12/60 for urea. We also measured glucose in cerebrospinal fluid, with use of aqueous standards. Instrument stability during 39 days without recalibration was evaluated. Results from the EKTACHEM compared favorably with those from the other instruments with respect to precision, accuracy, analytical recovery, instrument and reagent stability, and (non-drug) interferences. General instrument performance was excellent, with little downtime. The instrument was enthusiastically accepted by our laboratory personnel.

Additional Keyphrases: inter-instrument comparison · multilayer film analysis · enzymic methods · variation, source of · cerebrospinal fluid

The "EKTACHEM Glu/BUN Analyzer" (Eastman Kodak Co., Rochester, NY 14650) is a novel clinical instrument, designed to measure urea nitrogen and glucose by use of Kodak's thin, multilayer film technology (1, 2). Glucose is measured by a modification of Trinder's procedure, with glucose oxidase (EC 1.1.3.4) and peroxidase coupling of a naphthol compound to amionoptazirine. For urea, the reaction is urease (EC 3.5.1.5) catalyzed hydrolysis of urea, liberating ammonia, which reacts with a protonated merocyanine compound to form an indicator dye. Buffers, enzymes, and indicators are incorporated into multiple, thin, dry layers, which are applied to a 16-mm square transparent support base (the "slide").

The desk-top instrument is microprocessor-controlled, and automatically dispenses and processes slides from 50-test slide cartridges. Micro-scale samples of body fluids are delivered to the slides with use of a discrete-sampling, air-displacement pipetting device and a unique sample cup; after incubation, optical measurements are made by reflectance colorimetry. The microprocessor is used to control various instrument functions, trouble-shoot malfunctions, and calculate results.

This report gives our evaluation of an engineering model of the EKTACHEM Analyzer and the thin-film technology during 21 weeks of use in a general-hospital laboratory.

Materials and Methods

Equipment

The instrument we used was an engineering model of the "EKTACHEM Glu/BUN Analyzer," supplied by Eastman Kodak, which was virtually identical to the production model except for minor differences in the software package.

We compared the following instruments with the Kodak Analyzer: Dupont acca (Automated Clinical Analyzer; E. I. Du Pont de Nemours & Co., Wilmington, DE 19898); SMA 12/60 (SMA 12/60 AutoAnalyzer System; Technicon Instrument Corp., Inc., Tarrytown, NY 10591); and the Glucose Analyzer (Beckman Instruments, Inc., Fullerton, CA 92634). Unless otherwise specified for a particular experiment, each instrument was operated as prescribed in the operator's manual supplied by the manufacturer.

Methods

The SMA 12/60 urea procedure was the unmodified diacetyl monoxime method. Glucose and urea procedures used with the acca were the standard enzymic methods provided by DuPont. Glucose was measured by the oxygen rate method with the Beckman Glucose Analyzer.

Reagents

Glucose and urea reagents for the Kodak instrument were supplied by Kodak in cartridges containing 50 slides. Unless otherwise specified, the cartridges were stored at 4 °C until the day of use. Standard acca reagent packs for glucose and urea obtained from the DuPont Co. were stored at 4 °C until use. Reagents for the SMA 12/60 urea method were obtained from the Technicon Corp. Reagents for the Beckman Glucose Analyzer were obtained from Beckman Instruments. Glucose and urea obtained from the National Bureau of Standards (NBS) as Standard Reference Materials, were used for studies of linearity and analytical recovery.

Calibration

The Kodak instrument was calibrated as recommended by the supplier; three concentrations of calibrators were run daily, in duplicate. The calibrators were Kodak lyophilized human-serum-based calibrators, Level 1 (lot no. 2942-500), Level 3 (lot no. 2944-500), and Level 4 (lot no. 2949-500). The calibration values were those assigned by Kodak for each instrument. For urea assay with the SBA, it was calibrated with SBA Reference Serum 3, lots B7E392, B7F358, B7F376, and B7L767. The set point value was used as provided by Technicon for the particular lot numbers. Glucose and urea were calibrated in the DuPont acca with Versatol Full Range Calibration Reference materials, lot no. 4D115. Beckman Aqueous Glucose standard (1500 mg/L) was used to calibrate the Glucose Analyzer.

Controls

We used the following lyophilized controls: Kodak Human...
Serum Controls, Glu/BUN Level 1 (lot no. 2942-400), Level 2 (lot no. 2943-400), and Level 3 (lot no. 2944-400); LederRate N, B, P, Kinetic Test Control Serum—Human, lot no. 2912-641R1, 2913-642R1, and 2914-643R1 (Lederle Diagnostics, American Cyanamid Co., Pearl River, NY 10965); Versatol Full Range Quality Control Set, Kit no. 4D115 (General Diagnostics Division of Warner-Lambert Co., Morris Plains, NJ 07950); and Ortho Spinal Fluid Control, lot no. 10R158 (Ortho Diagnostics, Inc., Raritan, NJ 08869).

Patients’ Samples

Serum. Routine patients’ samples for SMA 12/60 analysis from our hospital and outpatient population were used in the serum study. The specimens were collected in evacuated blood-collection tubes (Vacutainer Tubes; Becton-Dickinson Co., Rutherford, NJ 07070), centrifuged within 15 min of clotting, and separated from the clot either by decantation or with filter separators (Technicon “Seraclear”). Serum was aliquoted and assayed on the comparison instruments, the interval of testing between instruments usually being less than 2 h.

Plasma. Specimens for plasma study were collected in Vacutainer Tubes containing sodium heparin, sodium fluoride, or ethylenediaminetetraacetate.

Spinal fluid. Spinal-fluid samples were obtained from specimens submitted for routine spinal fluid analysis. Samples were stored at 4 °C and assayed within 24 h, otherwise frozen at −20 °C. All specimens were assayed within 60 min of specimen collection in the case of the comparison instruments.

Results

Between-Run Precision

Table 1 summarizes the between-run precision studies. The data are presented for a baseline study and for daily quality-control data collected during two months after the baseline study. The baseline study or performance-check period accorded with the NCCLS protocol (3).

The object of the baseline study was to establish a reference data base for future comparative studies. Kodak and Leder-Rate controls (three concentrations each) were assayed in triplicate twice daily for 10 working days. As specified in the NCCLS protocol (3), controls were interspersed with randomly chosen patients’ samples. Over a wide range of concentrations, the between-run precision for glucose and urea on the Kodak Analyzer was excellent.

Within-Run Precision

Eleven experiments (n = 24 to 59) were performed during six weeks with use of pooled reconstituted lyophilized controls in the low, medium, and high range for glucose and urea. The CV’s for glucose were consistently less than 1.5%; the CV’s for urea ranged from 1.0 to 3.4%.

Linearity

For the low-range glucose study we used a saline solution of human albumin, 41 g/L, and human globulin, 25 mg/L (Sigma Chemical Co., St. Louis, MO 63178), with glucose added to the solution. The solution (glucose, 1400 mg/L) was analyzed five times for glucose in the Kodak Analyzer. Appropriate dilutions were made with the initial protein-based solution to provide five glucose concentrations ranging from 230 to 1400 mg/L; each was assayed five times with the Kodak Analyzer. For the high-range study, we used a pooled specimen of serum, assayed for glucose five times with the Kodak Analyzer (glucose = 1080 mg/L). We added glucose to a portion of this pool to give a final glucose concentration of 5920 mg/L. Appropriate mixtures with the serum pool gave a range of six concentrations. These samples were each assayed five times with the Kodak Analyzer. Glucose concentration was linearly related to instrument reading from 230 to 5920 mg/L.

Urea linearity was studied in two separate experiments. In the first, the range covered 270 to 1210 mg/L. A fresh human serum pool was prepared (urea by acc, 270 mg/L). A second solution was prepared by adding urea to a portion of the initial pool (urea by acc, 1210 mg/L). Appropriate mixtures of the two gave six concentrations over the range, and each was assayed five times for urea with the EKTACHEM Analyzer. The second linearity study included the range 40 to 380 mg/L. Kodak Control II (urea by acc, 380 mg/L) and fresh human serum (urea by acc, 40 mg/L) were mixed to provide seven concentrations over the range; each was assayed five times in the Kodak Analyzer. Readings for urea with the EKTACHEM were linearly related to concentration from 40 to 1210 mg/L.

Analytical Recovery

A fresh pooled specimen of human serum was analyzed five times for urea and glucose with the Kodak Analyzer (urea = 256 mg/L, glucose = 1082 mg/L). Glucose (108, 200, and 480 mg) and urea (25, 50, and 94 mg) were weighed into 100-mL aliquots of the pool and each solution was analyzed five times. Analytical recovery for both glucose and urea was 96 to 102%.

Interference

We tested the effect of hemolysis, lipemia, and icterus.

We added known amounts of hemoglobin (0–5600 mg/L), assayed in a fresh hemolysate, to a serum pool and then measured five times for glucose (1000–5000 mg/L) and urea (200–600 mg/L).

For the lipemia study, 14 turbid serum samples were assayed in duplicate for urea (70–400 mg/L) and glucose (750–5000 mg/L) with the Kodak Analyzer, the acc, and the Beckman Analyzer. Cholesterol, when assayed, ranged from
2.63 to 10.24 g/L and triglycerides, when assayed, from 3.28 to 13.4 g/L.

For the icterus study, samples from 29 patients with values for total bilirubin ranging from 14 to 174 mg/L were assayed in duplicate for glucose (700–6000 mg/L) and urea (80–1000 mg/L) with the four instruments.

We observed no effect on results for glucose or urea over a wide range of hemoglobin, lipid, and bilirubin concentrations.

Reagent Stability

We analyzed Kodak controls no. 1, 2, and 3 daily, in duplicate, during four weeks, using reagent slides (same lot number) left at room temperature. These data were compared to similar data from refrigerated reagents (same lot numbers). Room temperature ranged from 20 to 26 °C and the relative humidity from 2 to 77%. We observed no effect on urea measurements. We found a good correlation for glucose during the first two weeks of the study. The glucose values were inconsistently increased during the last two weeks. Reagent slides kept for extended periods should be stored refrigerated. For short-term use of up to two weeks, they may be stored at room temperature.

Calibration Stability

The purpose was to determine how frequently the Kodak instrument should be re-calibrated. The instrument was calibrated for glucose and urea on day 1 according to the standard protocol of Kodak, and not again for 39 days. Fresh Kodak and LederRate controls were reconstituted each working day and analyzed in the morning and late afternoon. The same lots of cartridge packs were used during the experiment, the results of which are presented in Table 2. CV’s for this test period were consistently lower and the mean for each control was essentially the same as with daily recalibration (refer to Table 1). Evidently there is no loss of precision or accuracy if the instrument is not recalibrated daily.

Sample Size

We determined that the minimum volume necessary to perform the two tests was 30 µL, or 15 µL for only one test.

Anticoagulant Study

We assayed 75 samples (sodium fluoride anticoagulant) for glucose, in duplicate, with the Kodak and Beckman Analyzers. Glucose ranged from 280 to 5560 mg/L. There was good correlation between instruments for glucose values up to 3000 mg/L. The Kodak instrument gave lower results (3 to 7%) for glucose >3000 mg/L (n = 6). The same group of fluoride-treated plasma samples were analyzed for urea in the Kodak Analyzer and the aca. There was good correlation for normal range. Results for the EKTACHEM were up to 20% lower when urea exceeded 400 mg/L.

In a separate study, we compared serum and fluoride-treated plasma on the Kodak Analyzer for 35 patients’ samples, with glucose ranging from 260 to 5570 mg/L. Glucose results for two of the plasma samples with glucose >3000 mg/L were 5 and 7% lower than for serum. We conclude that samples anticoagulated with sodium fluoride should not be used for urea. Our data for glucose indicate fluoride-treated plasma will be satisfactory; however, we had few samples with glucose >3000 mg/L.

In a more limited study involving 12 patients, we compared urea and glucose in serum and plasma in the Kodak Analyzer, with use of EDTA and heparin as anticoagulants. For both glucose (570–1950 mg/L) and urea (70–540 mg/L), such plasma samples gave results that compared favorably with those for the serum samples.

Protein Effects

In a study of 43 patients’ serum samples with low total proteins (39–54 g/L; glucose, range, 840–5340 mg/L; urea range, 40–1060 mg/L) there was good correlation between the Kodak instrument and the other three instruments. Two patients’ samples with high total protein (82 and 99 g/L) showed good correlation on all four instruments. We prepared a more concentrated pooled specimen of human serum by molecular filtration (Immersible Molecular Separator; Milipore Corp., Bedford, MA 01739) and diluted it with isotonic saline to obtain a range of total proteins from 0 g/L (saline) to 150 g/L (concentrated pool). The glucose and urea concentrations were adjusted by adding urea and glucose so that the glucose and urea concentrations were respectively the same in each solution. For those solutions with total protein between 30 and 120 g/L, glucose was found to be within 10% of the weighed-in value; for those with <30 g/L or >120 g/L, the results obtained for glucose were significantly below the expected. When the data were normalized for variation in sample-spot size, glucose values were within 5% of the expected value for protein concentrations of 0–120 g/L.

Results were similar for urea: values were within 10% of the expected value when protein concentration was 30–120 g/L. In a separate experiment (protein range 0–150 g/L) the urea showed a consistent 15% negative bias over the entire range of protein concentration.

Protein effects are attributed to variation in spot diameters at extreme protein values (viscosity effects) and to protein interaction with the thin film matrix (personal communication, Eastman Kodak Co.). We believe that some of the var-
Glucose in cerebrospinal fluid: comparison between EKTACHEM (calibrated with aqueous standards) and the Beckman Glucose Analyzer

Albumin Solutions as Diluents

In six studies we used commercially available albumin, with water or saline as a diluent. We found a wide range of negative bias on comparing the Kodak Analyzer to the other instruments; we concluded that no commercial albumin solution is satisfactory for use as a diluent with the Kodak Analyzer.

Glucose in Cerebrospinal Fluid

Analysis of samples for glucose were initially performed with the EKTACHEM according to the recommended calibration procedure for serum. Values ranged consistently less (approximately 20%) than those obtained with the aca and the Glucose Analyzer. In an attempt to correct this discrepancy, we calibrated the Kodak Analyzer with aqueous standards (NBS glucose concentration 625, 2500, and 5000 mg/L) and repeated the experiments. The interval of testing was within 1 h on the various instruments. The data are presented in Figures 1 and 2.

Ortho Spinal Fluid Control (glucose, 590 mg/L) was analyzed with the EKTACHEM (calibrated with aqueous standards) five times a day for nine days. The mean value obtained was 582 mg/L (CV 1.7%). We conclude that the EKTACHEM can be used to measure glucose in spinal fluid when calibrated with aqueous standards, and that Ortho Cerebrospinal Fluid Control is a satisfactory quality-control material.

Patient Correlation Study

We compared results during 21 weeks for glucose and urea determined in 160 patients' sera with the Kodak Analyzer, the aca, and the Beckman Analyzer for glucose, and the aca and the SMA 12/60 for urea. All samples were run in duplicate on the instruments except an occasional SMA-12 specimen which was run single. The glucose range was 210–5730 mg/L, the urea range 50–1200 mg/L. Figures 3–6 show the results. There was...
those obtained with the other instruments, for both glucose and urea in patients' sera. Accuracy, precision, and recovery were well within generally accepted limits. The EKTACHEM Analyzer is modular in design, simple to operate, and easy to troubleshoot. Laboratory technologists were trained to operate the instrument in about 45 min. Operator communication with the instrument is through a special-purpose keyboard by use of function codes. These are used routinely for specimen processing, calibration, analysis of unknown samples, daily maintenance, and troubleshooting. The program allows determination of both glucose and urea, or a single test on each specimen. The instrument requires a minimum of 30 μL for both tests or 15 μL for either test alone. The maximum throughput of our engineering model was 120 samples per hour, 60 for glucose and 60 for urea. The instrument was operated in both batch and emergency-use modes.

We thank Eastman Kodak Co. for providing the EKTACHEM Analyzer and all necessary reagents, calibration materials, and controls used for correlation studies on the comparison instruments.

References