turnover. This ratio also appears to be related to the risk of developing osteoporosis (2) and has been approved by the Food and Drug Administration for use in monitoring antiresorptive therapies. A more complete assessment of a patient’s osteoporosis status requires, among other things, both bone mineral densitometry (a measure of current bone status) and measurement of a biochemical marker of bone turnover (a measure of the rate of change in bone status). Because NTx and other bone markers are measures of the rate of turnover (as opposed to extent), they can reflect changes in bone turnover that occur within a relatively short period of time, often as little as 4 weeks. Bone densitometry measurements, although quite precise, are a static measure. Often 1–2 years pass before marked bone loss can be detected by densitometry. This makes NTx useful in assessing response to therapeutic interventions (3, 4) such as estrogen replacement and bisphosphonates.

The reflectance of specific test zones on each strip is measured by a 4-channel reflectometer, and the clinical results are displayed as a ratio of NTx nmol BCE (bone collagen equivalent) per mmol creatinine (as calculated by an on-board microprocessor). The microprocessor also performs corrections for lot-specific reagent characteristics and several forms of optical variation, in addition to tests for proper electrical functioning and adequate sample volume. In measurements of standard gray-scale materials (Munsell Color, GretagMacbeth), the miniaturized reflectometer had very good stability (<=0.1% CV over 270 s, n = 73 for each channel), high reflectance reproducibility (<=0.5% CV, n = 10 for each channel), and excellent linearity (signal vs gray scale r² >=0.999, slope = 1.00 ± 0.01, intercept <0.01 in units of reflectance). The range of NTx that can currently be measured extends from 30 to >1000 nmol/L BCE and from ~1 to 25 mmol/L for creatinine. Clinical precision (CV of the analyte concentration test result) for NTx and creatinine is in the range of 5–9% (NTx assay precision measured at 300 nmol/L; creatinine assay precision measured at 4 mmol/L; n = 20). The precision (CV) of ratioed results (NTx/creatinine) from 30 prototype DRx tests performed over a 3-month period with a buffered aqueous calibrator solution was 10.9% (38.3 ± 4.19 nmol BCE/mmol creatinine). Comparison of clinical sample test results (n = 20, performed in duplicate) to an NTx microtiter plate ELISA (Osteomark™, Ostex International, Inc.) and to a Boehringer-Mannheim creatinine assay yielded correlation coefficient (r) values of 0.946 and 0.967, respectively. Correlation plot slopes and intercepts were (for NTx) 1.15 ± 0.06 and 40.4 ± 49.3 nmol/L BCE (S_y|x = 169.4), and (for creatinine) 1.04 ± 0.04 and 1.28 ± 0.53 mmol/L (S_y|x = 1.74), respectively. The correlation plot slope and intercept values for the ratio of NTx/creatinine were 0.998 ± 0.036 and 2.86 ± 3.36 (S_y|x = 12.8), respectively, with an r value of 0.976.

We conclude that DRx NTx has performance that approaches that of clinical laboratory systems and can provide high-quality analytical information in a timely fashion at the point of care.

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References

Simultaneous Detection of Multiple Analytes Using Copalis Technology: A Reduction to Practice, Michael J. Benencky, Kevin L. McKinney, Kimberly M. Peterson, and John Q. Kamerud* (DiaSorin USA, 1990 Industrial Blvd., Stillwater, MN 55082; * author for correspondence; fax 612-773-1519, e-mail John.Kamerud@Incstar.com)

Coupled Particle Light Scattering (Copalis) is a homogeneous immunoassay technology that enables the simultaneous determination of multiple analytes in serum, plasma, or whole blood (1). The Copalis system measures changes in light-scattering properties of particles when they form antibody-mediated complexes. The system can measure two types of events: polystyrene microparticle aggregation and polystyrene-gold colloid microparticle coupling. In the first format, polystyrene microparticles are differentiated from their complexed aggregates as a function of antigen concentration or serological response. The second format measures the broadening of the microparticle light scatter histogram as gold particles are attached. In both cases, light scattering analysis is performed as particles flow singly through a finely focused laser beam. The ability to differentiate various sizes of polystyrene microparticles allows the system to measure multiple analytes or serological responses simultaneously. This capability has been demonstrated by the ToRC assay (toxoplasma, rubella, cytomegalovirus), in which antibodies to three different infectious agents are detected simultaneously (2). In the present work, we demonstrate the application of Copalis technology to a variety of areas of diagnostic medicine in which the simultaneous measurement of multiple analytes or variables may be advantageous, including infectious disease, autoimmunity, and reproductive endocrinology.

LATEX-LATEX FORMAT

This format is useful for detecting the presence of antibodies to infectious agents or autoantigens, which are coated onto latex microparticles. By using only forward-
scatter measurement, Copalis instrumentation can distinguish between different-sized particles, as well as between monomers and aggregates. The presence of specific antibodies is detected as a reduction in the number of monomeric particles coated with the corresponding antigen (see Fig. 1A). In addition to the aforementioned ToRC assay, we have applied this format to the diagnosis of syphilis, Epstein-Barr viral infection, and SSA/SSB autoimmunity.

The typical algorithm for syphilis testing involves first screening with a nonspecific test such as the VDRL (Venereal Disease Research Laboratory) agglutination assay, followed by confirmatory testing of positive specimens by immunofluorescence assay (IFA) or ELISA using a specific treponemal antigen (3). By coating the treponemal antigen and a VDRL-like antigen on microparticles of different sizes, we have designed a Copalis assay that combines the screening and confirmatory tests. The Copalis syphilis assay has been evaluated by comparison with commercially available kits for treponemal (Zeus IFA and Centocor Captia ELISA) and nontreponemal (MacroVue
RPR by Becton Dickinson) antigens. The relative sensitivity and specificity were as follows: treponemal: 92.2% sensitivity (n = 51), 93.6% specificity (n = 94); and nontreponemal: 94.2% sensitivity (n = 52), 97.6% specificity (n = 96).

Another area of infectious disease testing to which Copalis has been applied is Epstein-Barr serology. In this case, three different antigens from the Epstein-Barr virus (EBV) were coated onto three different sizes of polystyrene latex microparticles. The antigens represented are the Epstein-Barr nuclear antigen, the viral capsid antigen, and the early antigen. Reduction in the size of any of the three peaks indicates the presence of antibodies against the corresponding antigen. Such testing may be useful in the differentiation of new infections, immune status, and reactivation of latent infections.

The systemic autoimmune diseases are characterized by the presence of antibodies against a number of different autoantigens (4). The simultaneous determination of these autoantibody specificities might be helpful in the differential diagnosis of diseases such as systemic lupus erythematosus, Sjogren syndrome, mixed connective tissue disease, and systemic scleroderma. The application of Copalis technology to the detection of autoantibodies is demonstrated by an assay for the simultaneous detection of antibodies to the autoantigens SSA (Ro) and SSB (La). For evaluation of the SSA Copalis assay, 23 ELISA-positive samples and 44 healthy blood-bank donors were run. Relative to ELISA, the sensitivity and specificity of the Copalis assay were 96% and 93%, respectively.

**LATEX-GOLD FORMAT**

Utilizing both forward-scatter and side-scatter parameters, this format is useful for the quantitative detection of analytes with greater sensitivity than that achieved using the latex-latex format. Antigen-mediated binding of colloidal gold to antibody-coated latex microparticles causes a broadening of the latex side-scatter histogram (2). The extent of this broadening can be measured and correlated to analyte concentration (see Fig. 1B).

The Latex-Gold format has been applied to the assessment of human fertility. Antibodies against different epitopes on luteinizing hormone (LH) were coated separately onto latex particles and onto colloidal gold. The same was done for follicle-stimulating hormone (FSH), using a different size of latex. When these reagents are combined and incubated with patient sample, the presence of either analyte leads to formation of a “sandwich” between the antibody on the gold and that on the latex. The coupling of gold and latex in turn causes a broadening of the LH and FSH side-scatter peaks, the magnitude of which is dependent on the concentration of the corresponding analyte.

Using this method for simultaneous measurement of LH and FSH, we obtained the following correlations with the Abbott IMx methods. For LH, Copalis = 0.16 + 0.83 (IMx); r = 0.95; and n = 64. For FSH, Copalis = 0.196 + 0.98 (IMx); r = 0.98; and n = 36.

**ADDITIONAL FEATURES**

Utilizing the forward- and side-scatter detection parameters, Copalis instrumentation is capable of differentiating and enumerating various cell populations and thus could function as a hematology analyzer, as well as an immunooassay analyzer. In addition, using electronic gating to mask the scattering signal from blood cells, Copalis II should have the capability of performing immunooassay analysis on whole-blood specimens.

**CONCLUSIONS**

Copalis is a versatile technology that may be applied to many areas of immunooassay, including infectious disease, autoimmunity, and endocrinology. A key feature of this technology is the ability to measure multiple analytes in the same reaction. This has now been demonstrated with multiplex assays for ToRC (toxoplasmosis, rubella, and cytomegalovirus), EBV (Epstein-Barr nuclear antigen, viral capsid antigen, and early antigen), syphilis (treponemal and nontreponemal antigen), autoimmunity (SSA and SSB), and fertility (LH and FSH). Several other technologies for multianalyte detection have been proposed (5). For example, Fulton et al. (6) report a system that also utilizes latex particles as solid phase, with fluorometric detection on a flow cytometer. Advantages of Copalis over these other techniques include the homogeneous format (no washing steps) and detection by light scattering. These features allow Copalis instrumentation to be relatively simple and inexpensive.

**References**


**A Rapid, Sensitive, Multiplexed Assay for Detection of Viral Nucleic Acids Using the FlowMetrix System, Perry L. Smith,1 Cindy R. WalkerPeach,2 R. Jerrold Fulton,1 and Dwight B. DuBois2 (1 Luminex Corporation, 12212 Technology Blvd., Austin, TX 78727 and 2Ceneteron Diagnostics, 2170 Woodward St., Austin, TX 78744; * address correspondence to this author at: Luminex Corporation, 1638 Osprey Dr., DeSoto, TX 75115; fax 972-224-9689, e-mail perrys@luminexcorp.com)

Sensitive assays for viral nucleic acids are important tools for the accurate diagnosis and treatment of viral diseases.