

Immulite 2000

The Immulite 2000 Automated Immunoassay Analyzer is a continuous random-access instrument that performs chemiluminescent immunoassays. The system uses serum, plasma, and/or urine samples for *in vitro* diagnostic testing. The Immulite 2000 Analyzer automates the entire testing procedure and accommodates high volume testing (generating up to 200 test results per hour). Primary, secondary, and microsample test tubes may be loaded directly on the instrument. This system interfaces with the Hospital's LIS, but the results are not available in Carecast. Core Lab staff provide LIS-style result sheets to the PI by email or fax.

The Immulite 2000 uses specific antibody-coated polystyrene beads as the solid phase. A bead is dispensed into a specially designed Reaction Tube, which serves as the vessel for the incubation, wash, and signal development processes.

After the sample is incubated with an alkaline phosphatase-labeled reagent, the reaction mixture is separated from the bead by spinning the reaction tube at high speed along its vertical axis. The fluid is transferred to a Coaxial Sump Chamber which is integral to the Bead/Tube Wash Station. Four discrete washes occur within seconds, allowing the reaction tubes to be processed sequentially with uniform timing. The bead remains in the reaction tube with no residual unbound label.

The bound label is then quantified using the dioxetane substrate to produce light. Light is emitted when the chemiluminescent substrate reacts with alkaline phosphatase label bound to the bead. The amount of light emitted is proportional to the amount of analyte originally in the samples. The light emission is detected by the Photomultiplier Tube (PMT), and the results are calculated for each sample.

The Immulite 2000 is supported by Siemens Healthcare Diagnostics Inc. in Deerfield, IL.

ELISA

ELISA is an EIA used for analyte measurements. It is a heterogeneous, solid phase assay that requires the separation of reagents. ELISA has two available techniques for analyte measurement - the sandwich technique and the competitive technique. The sandwich or double antibody technique begins with the analyte (or antigen) in the blood or urine sample binding to a specific antibody attached to a polystyrene well. An enzyme conjugate-antibody complex is then added to the well and binds to a different epitope of the antigen. Finally, a substrate is added to the enzyme conjugate-immune complex. A color change in the well will occur in direct proportion to the concentration of analyte in the sample.

For the antigen competitive inhibition assay, a sample (blood or urine) is added to an antibody-coated polystyrene well. The unlabeled analyte (or antigen) in the sample compete with an antigen-enzyme conjugate for available binding sites to the antibody

attached to the well. After formation of an immune complex, unbound antigen is removed by washing and a substrate is added. A color change in the well will occur in inverse proportion to the concentration of analyte in the sample

The Core Lab uses the ELx 50 Auto Strip Washer and EI800 Automated Microplate Reader manufactured by BioTek. The washer is a self-contained, automated microplate strip washer that comes with an 8-, 12-, or 16-channel manifold designed to wash individual strips and 96- or 384-well microplates.

The plate reader is a single-channel, automated, benchtop, general purpose enzyme immunoassay analyzer which performs *in-vitro* diagnostic analyses of samples. The data analysis software is Gen5 used for all applications in absorbance, fluorescence, and luminescence.

Instrumentation for the ELISA is from BIO-TEK INSTRUMENT, INC, Highland Park, Box 998, Winooski, Vermont.

Bio-Plex Multiplex System

In recent years, the development of protein microarrays have allowed for the measurement of several biomarkers within a single sample. These Multiplex systems utilize combinations of antibody-coupled color-coated microspheres and fluorescent-tagged secondary antibodies within a single microtiter plate well. The system uses a liquid suspension array of up to 100 sets of 5um beads, each internally dyed with different ratios of two spectrally distinct fluorophores to assign it a unique spectral address. Each set of beads can be conjugated with different capture antibodies; the beads are mixed and incubated with sample in a microplate well to allow binding to specific analytes. To detect and quantitate each analyte, a fluorescently labeled reporter molecule that specifically binds the analyte is added. Following incubation, the contents of each microplate well are automatically drawn into a flow-cytometer, and the beads are aligned in single file where two lasers excite the beads individually. The red laser excites the dyes in each bead, identifying the spectral address for each analyte. The green laser excites the reporter molecule associated with the bead which allows quantification of each analyte. System software records the fluorescent signals simultaneously for each bead translating the signals into quantitative data based on standard curves included within each microplate.

Multiplex kits are available from various manufacturers for endocrine, cardiovascular, apolipoproteins, adipocytes/adipokines, sepsis/apoptosis, skin, tumor markers and signal transduction proteins. Since most panels only require 10-25ul of serum/plasma, the system allows for measurements using experimental models where limitations in sample volume were previously not feasible (i.e., time course or repeated measure studies in children or adolescents). Currently available panels can be customized for individual investigators.

In-house validation studies have been performed on urine samples for the R&D Cytokine Multiplex Kit. Results showed that urine samples are acceptable for IL-6 and IL-10, but not IL-b, IL-2 or TNF alpha.

Related GCRC Services

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