

Assays:

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Institute: UCSF

Study: Health ABC

Date: 10-7-13

Analyte: Various

Type of Sample: Human serum

Number of samples: 982

Method: ISYS Analyzer (Luminescence)

Manufacturer: Immunodiagnostic Systems

Catalog#:

Units:

Detection Limit:

Range:

Interassay variability:

Intra-assay variability:

Normal Range

The threshold for interference from lipemic, hemolyzed or icteric samples is as follows:

	P1NP	IGF-1	IGFBP-3
Catalog#:	IS-4000US	IS-3900	IS-4400
Units:	ng/mL	ng/mL	ng/mL
Detection Limit:	1.0 ng/mL	4.4 ng/mL	50 ng/mL
Range:	2 -230 ng/mL	10-1200 ng/mL	80-10,000 ng/mL
Interassay variability:	4.6%	5.1%	6.4%
Intra-assay variability:	2.9%	2.2%	1.9%
Normal Range	27.7-127.6 ng/mL	116-353 ng/mL	See Kit Insert
Lipid	2803 mg/dL	3000 mg/dL	3000 mg/dL
Bilirubin	200 mg/dL	20 mg/dL	200 mg/dL
Hemoglobin	500 mg/dL	500 mg/dL	500 mg/dL
Biotin	NA	NA	300 nmol/L
RBC	NA	NA	0.4%

Methods

P1NP, IGF-1 and IGFBP-3 were quantified in human serum samples using the iSYS immunoassay analyzer (Immunodiagnostic Systems, Scottsdale AZ).

Calibrators and controls for each analyte (P1NP, IGF-1 and IGFBP-3) were tested as required by the manufacturer

Following successful instrument calibration and quality control checks, sample vials were thawed, vortexed and a 125uL volume transferred into 500uL sample cuvettes for testing.

Sample cuvettes were loaded onto the iSYS sample carousel. Sample positions were linked to sample identification numbers by scanning the barcoded label on each vial.

Sample volumes for each test (20uL for P1NP, 10uL for IGF-1 and 6uL for IGFBP-3) were automatically pipetted from the sample cuvette

and transferred to individual reaction cuvettes for testing

Luminescence measurements were carried out in the luminometer directly in the reaction cuvettes

For each test, the Relative Luminescence Units (RLU) were converted to analyte concentration in ng/mL

iSYS Assay Description

The P1NP, IGF-1 and IGFBP-3 assays are based on chemiluminescence technology.

Samples and calibrators are diluted in a diluent and a portion of this is incubated with a biotinylated anti-P1NP monoclonal antibody, an acridinium labelled monoclonal antibody, streptavidin labelled magnetic particles and an assay buffer. The magnetic particles are captured using a magnet and a wash step performed. Trigger reagents are added and the resulting light emitted by the acridinium label is directly proportional to the concentration of intact P1NP in the original sample.

For the IGF-1 assay, samples are first incubated with an acidic solution to dissociate IGF-1 from its binding proteins

The acidic solution is subsequently neutralized and the reaction carried out as described above.

iSYS Principles

Luminescence measurements

By-products of luminescent acridinium esters are used as detection markers (DMAE - dimethylethanolamine). The acridinium esters emit light after reacting with hydrogen peroxide and an alkaline solution. IDS-iSYS Trigger A contains hydrogen peroxide in a dilute acid medium, and IDS-iSYS Trigger B contains a solution of dilute sodium hydroxide. The analyzer automatically injects trigger solutions A and B into the reaction cuvette, which results in the oxidation of the ester into an excited form. The return to a stable state is accompanied by the emission of light which is measured and is expressed in relative light units (RLU) by the luminometer integrated in the analyzer.

Assays are carried out using either a one-site or two-site method.

One-site (competitive) method assay

The assay is based on competition between an unknown quantity of analyte in a sample with labelled analyte in the kit.

In a sample where no analyte is present, maximum binding of the labelled analyte is possible. With the increasing analyte concentrations, decreasing binding of labelled analyte is observed. The signal generated by the labelled analyte in the luminometer is therefore inversely proportional to the concentration of analyte in the sample.

Two-site (sandwich) method assay

This technique uses two antibodies that detect and bind different portions of the analyte molecule. Incubation of these antibodies with the sample results in the formation of a 'sandwich' complex, where the analyte is specifically bound by both antibodies. Incubation with coated magnetic particles allows capture of these complexes. The signal generated by detection of the captured complexes is directly proportional to the concentration of analyte in the sample.

Calculation of results

The results are calculated in comparison to a calibration curve.

The specific reference curve (master curve) for a reagent lot is in the file containing all the data for that lot, that is provided on the CD accompanying the cartridge. This information is registered in the analyzer's database when the CD is introduced on the controlling computer.

If a new reagent lot is used, this curve must be registered