CHEM.CENTAUR.ASSAY.14.0 INSULIN BY ADVIA CENTAUR

PRINCIPLE

The ADVIA Centaur Insulin assay is a two-site sandwich immunoassay using direct chemiluminescent technology which uses constant amounts of two antibodies. The first antibody, in the Lite Reagent, is a monoclonal mouse anti-insulin antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-insulin antibody, which is coupled to paramagnetic particles.

The system automatically performs the following steps:

1. Dispenses 25 ul of sample into a cuvette.
2. Dispenses 50 ul of Lite Reagent and incubates for 5.0 minutes at 37°C.
3. Dispenses 250 ul of Solid Phase and incubates for 2.5 minutes at 37°C.
4. Separates, aspirates, and washes cuvettes with reagent water.
5. Dispenses 300 ul each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.
6. Reports results according to the selected option, as described in the system operating instructions or the online help system.

A direct relationship exists between the amount of insulin present in the patient sample and the amount of relative light units (RLUs) detected by the system.

DOCUMENT OWNER

Manager, Chemistry & Special Chemistry

RELATED DOCUMENTS

CHEM.CENTAUR.ASSAY.2.0 Advia Centaur Operating Procedure

SPECIMEN COLLECTION

A. Specimen Requirements
   1. Serum
B. Stability
   1. Room Temperature – 8 hours
   2. Refrigerated (2-8°C) - 2 days.
   3. Frozen (≤ - 20°C) – 6 months
C. Minimum volume – 100 µL
D. Special Instructions
   1. Before placing samples on the Centaur, ensure that:
      a. Samples are free of fibrin or other particulate matter

b. Samples are free of bubbles.
c. Separate serum from red blood cells before storage at 2-8°C or -20°C

REAGENTS

A. ADVIA Centaur Reagents
1. Lite Reagent (5.0 ml). Contains monoclonal mouse anti-insulin antibody (~0.24 ug/mL) labeled acridinium ester in buffered saline with bovine serum albumin, sodium azide (<0.1%) and preservatives.
a. Store at 2-8°C.
b. Stable until the expiration date on the pack label.
c. Onboard stability 21 days.
d. Calibration interval 14 days.
2. Solid Phase (25 ml). Contains monoclonal mouse anti-insulin antibody (~6 ug/mL) covalently coupled to paramagnetic particles in buffered saline with bovine serum albumin, sodium azide (<0.1%) and preservatives.
a. Store at 2-8°C.
b. Stable until the expiration date on the pack label.
c. Onboard stability 21 days.
d. Calibration interval 14 days.
3. Insulin Diluent (10.0 mL). Contains buffered saline with casein, potassium thiocyanate (3.89%), sodium azide (<0.1%) and preservatives.
a. Store at 2-8°C.
b. Stable until the expiration date on the pack label, or 21 consecutive days after accessing the ancillary pack.

EQUIPMENT

ADVIA Centaur XP

CALIBRATION

A. Standard: Insulin uses Insulin Calibrator
B. Standard Preparation: Refer to calibrator insert
C. Storage: Refer to Calibrator Insert
D. Concentration: Refer to Calibrator Insert for calibration deffination
E. Standard Curve: Insulin requires a two-point calibration
F. Acceptable Tolerance: Accept if ADVIA Centaur accepts curve, and controls are within acceptable range.
G. Frequency:
1. Calibration Interval is every 14 days
2. A reagent lot number has changed

3. There is an observed shift in controls.
4. Major preventative maintenance has been performed
5. A critical part has been replaced

H. Refer to the ADVIA Centaur Operators Manual for further detail on calibrating the ADVIA Centaur.

QUALITY CONTROL

A. Refer to MACL Regional Chemistry QC Testing Intervals for appropriate control material and testing intervals.

B. Refer to MACL Regional Chemistry QC Policy for criteria on accepting/rejecting patients.

PROCEDURE

Refer to procedure CHEM.CENTAUR.2.0, Advia Centaur Operating Procedure, for specific details.

DILUTIONS

A. Specimens with insulin levels > 300 mU/L shall be diluted.

B. Patient samples can be automatically diluted by the system, or prepared manually.

1. For automatic dilutions, ensure the ADVIA Centaur Insulin Diluent (IRI) is loaded and set the system parameters as follows:
   a. Dilution point: ≤ 300 mU/L
   b. Dilution factor: 2, 5
2. Manually dilute the patient samples when the patient results exceed the linearity of the assay using automatic dilution, or when laboratory protocol requires manual dilution.
3. Use Insulin Diluent to manually dilute patient samples, and then load the diluted sample in the sample rack, replacing the undiluted sample.
4. Ensure that results are mathematically corrected for dilution. If a dilution factor is entered when scheduling the test, the system automatically calculates the results.

REPORTING RESULTS

A. Reference ranges: 0 - 17 mIU/mL

B. Critical Value: None

C. Linearity: 0.5 – 300.0 mIU/mL

D. Extended Linearity: 0.5 – 1500.0mIU/mL

RESULT ENTRY

A. Sunquest Computer Entry

1. Function: OEM or MEM
2. Worksheet: CI
3. Test Code: INSUL

B. QLS Computer Entry

1. Common Pathway: 3, 3, 1
2. Worklist: CIM2
3. Test Code: 561

NOTE: If HIS or LIS system is down, see appropriate Laboratory Computer Downtime Policy.

PROCEDURE NOTES

A. Clinical Application

Insulin is a protein hormone that is synthesized, stored, and secreted by the beta cells located in the islets of Langerhans in the pancreas. Insulin is responsible for regulating glucose concentrations in the blood. Insulin is released in response to the presence of glucose in the blood typically after the ingestion of a meal. A normal healthy individual produces 40-50 units of insulin each day. The half-life of insulin in serum or plasma is 5-10 minutes. Approximately 50% of the insulin released into the portal circulation is cleared by the liver. Insulin binds to receptor cells located on cell membranes of target tissues. The target tissues are primarily liver, fat, and muscle tissue. Insulin lowers glucose concentrations in the blood by stimulating glycogenolysis in the liver, triglyceride synthesis in adipose tissue, and protein synthesis in muscle. Recent studies have indicated that insulin and insulin receptors may play a role in learning and memory. The interruption of insulin and insulin receptor activity may lead to deficits in learning and memory formation. Increased insulin production is common in the development of cancers. If insulin production is not stimulated, blood glucose levels will not be lowered and hyperglycemia results. Fasting hyperglycemia supports the diagnosis of diabetes mellitus. Insulin therapy is used for insulin-dependent diabetes mellitus (IDDM) patients and many non-insulin-dependent diabetes mellitus (NIDDM) patients. In Type I diabetes (IDDM), there is a deficiency of insulin. This can be a result of autoimmune destruction of beta cells or the presence of autoantibodies to insulin. Many factors can play a role in the development of Type II diabetes (NIDDM). Type II diabetes (NIDDM) can result if there is a decreased biological response to circulating insulin (insulin resistance) or if there is decreased or diminished insulin secretion due to beta cell failure. Insulin levels are not typically used to diagnose or treat diabetes patients. Insulin levels can be useful in evaluating patients with fasting hypoglycemia, in determining insulin resistance in the general population, and in assessing abnormalities in beta cell secretory function. Insulin levels are used in studying the pathophysiology of diabetes.

LIMITATIONS

A. Interfering substances:

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

Serum specimens that are….	Demonstrate <6% change in results up to…
Hemolyzed	125 mg/dL of hemoglobin
Lipemic	1000 mg/dL of triglycerides
Icteric	20 mg/dL of bilirubin
Proteinemic	12 g/dL of protein

REFERENCES

See ADVIA Centaur Assay Procedure, procedure 128323 Rev. B, 2002-10