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Immunoassay

REF **CMG0101/CMG0102/CMG0103/CMG0104/CMG0105**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

Insulin CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative detection of Insulin in human serum.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Introduction

The pancreas is located behind the lower part of the stomach. It makes insulin and enzymes that help the body digest and use food. Throughout the pancreas are clusters of cells called the islets of Langerhans. Islets are made up of several types of cells, including beta cells that make insulin. Insulin is a hormone that helps the body use glucose for energy.

Human insulin is a peptide hormone composed of 51 amino acids and has a molecular weight of 5808 daltons.[1]

Insulin levels are most frequently ordered following a low glucose and/or when someone has acute or chronic symptoms of low blood sugar (hypoglycemia), Insulin and C-peptide are produced by the body at the same rate as part of the conversion of proinsulin to insulin in the pancreas. Both may be ordered to evaluate how much insulin in the blood is made by the body (endogenous) and how much is from exogenous sources. The test for insulin measures insulin from both sources while the C-peptide test reflects insulin produced by the pancreas (endogenous insulin).

Insulin concentrations tend to be higher in obese individuals, particularly those with an increased proportion of visceral (abdominal) fat.[2,3]

Measurement of circulating insulin concentrations may be useful in the diagnostic evaluation of several conditions.[4] High circulating insulin concentrations may be involved in the pathogenesis of hypertension and cardiovascular disease. Conversely, low insulin concentrations in the presence of hyperglycemia suggest insulin-deficiency, e.g. insulin-dependent or Type 1 diabetes mellitus. Measurement of immediate or first-phase insulin secretion after an acute glucose load may be predictive of Type 1 diabetes mellitus.[5]

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, Insulin antibodies coated microparticles and enzyme-labeled Insulin antibodies are combined. During the incubation, Insulin present in the sample is allowed to react simultaneously with the two antibodies, resulting in the Insulin being sandwiched between the coated microparticles and enzyme-linked antibodies. After washing, a complex is generated among the coated microparticles-antibodies, the Insulin within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate are then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the concentration of Insulin in the patient sample.

Materials Provided

1. Calibrators

6 vials of lyophilized calibrator A through F. The matrix is Tris-HCl buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Allow the reconstituted material to stand for at least 5 minutes. Then invert the calibrator to mix it completely.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3 mL*2	2.3 mL*5	1.2 mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

- Microparticles Solution

Recombinant Insulin antibody coated microparticles in PBS (phosphate buffered saline) buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish peroxidase labeled mouse anti-human IgG monoclonal antibodies in a Tris-HCl buffer containing bovine serum. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needles
6. Wash Buffer used in the washing procedure
7. Distilled water or deionized water

Metrological Traceability of Calibrators

The product calibrators are manufactured using lyophilized Insulin powder and signal matched to our working calibrators, which are also signal matched to a higher order calibrator purchased from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products), China, at each concentration level.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. Ensure the microparticles are resuspended before loading it on the analyzer.
12. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
13. Do not substitute any reagent in this kit from other manufacturers or other lots.

14. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store all components of the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10 ℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the reconstituted calibrators in aliquots and freeze at -20 ℃ immediately after the experiment. Avoid multiple freeze-thaw cycles.
5. When storing the calibrators, be certain the vials are securely sealed.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples and samples with obvious microbial contamination. Do not use sodium azide preservative in samples.
3. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit in creased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
4. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
5. Insufficient processing of the sample or disruption of the sample during transportation may cause depressed results.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
8. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
9. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
10. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
11. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit
- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample cups or tubes on the sample rack, 50 μL of sample for each test. But considering the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of Insulin in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and substrate solution with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the instrument are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with Insulin value exceeding 300 μIU/mL may be diluted manually.
Calibrator A is used to dilute the samples.
After dilution, multiply the result by the dilution factor

- The concentration of the sample after dilution should not be less than 5 μIU/mL.

Measurement Results

The sample test results are determined automatically by the system software. The amount of Insulin in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored results.
Conversion formula: 1μIU/mL × 7.175 = 1pmol/L

Biological Reference Interval

A normal range of 1.5 – 25 μIU/mL (95% confidence interval) was obtained by testing serum samples from 182 individuals defined as normal by a clinician. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. 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. This kind of samples is not suitable to be tested by this assay.
4. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
5. This test measures concentrations within the range of 1.0 - 300 μIU/mL. If insulin concentrations above the measuring range to be expected, it is recommended to dilute samples with Calibrator A, the maximum dilution is 1:4 of this test, allowing samples to be quantitated up to approximately 1500 μIU/mL.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in replicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean	Within-run	Total
			%CV	%CV
1	80	19.58	3.04	4.96
2	80	70.86	3.65	5.25
3	80	103.68	5.04	5.18

*Representative data; results in individual laboratories may vary from these data

2. Analytical Sensitivity

Limit of Blank=0.1μIU /mL
Limit of Detection=0.3μIU/mL
Limit of Quantitation= 0.5μIU/mL with a coefficient of variation of ≤ 20%.

3. Analytical Specificity

This assay is designed to have cross-reactivity test with the substances listed below, at the concentration levels listed, in Tris-HCl buffer containing BSA.

Substances	Concentration tested	Cross-reactivity %
C-Peptide	10 μg/mL	n.d.*
Glucagon	10 μg/mL	n.d.
Somatomedin (In-like growth factor 1- IGF-1)	50 ng/mL	0.035
Procine insulin	0.5ng/mL	95.9

*n.d. = not detectable
The concentrations of proinsulin and split products of fasting healthy subjects are 100 times lower than the Insulin concentrations and therefore the cross-reactivity is of no clinical significance.

4. Interference

The following substances and concentrations were tested and found not to interfere with the test.

Interferent	Concentration
Bilirubin	60 mg/dL
Hemoglobin	250 mg/dL
Triglyceride	3000 mg/dL

5. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and Insulin Test which was already available on the market. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	158	-1.6681	1.0454	0.9942

Literature References

1. Chopra IJ, Ho RS, Lam R. An improved radioimmunoassay of triiodothyronine in serum: its application to clinical and physiological studies. *J. Lab. Clin. Med*. 1972;80(5):729-739.

2. Young DS. Effects of drugs on clinical laboratory tests. *Ann. Clin. Biochem*. 1997;34(Pt 6):579-581.

3. Santini F, Pinchera A, Ceccarini G, et al. Evidence for a role of the type III-iodothyronine deiodinase in the regulation of 3,5,3'-triiodothyronine content in the human central nervous system. *Eur. J. Endocrinol.* 2001;144(6):577-583.

4. Nikkilä EA, Kekki M. Plasma triglyceride metabolism in thyroid disease. *J. Clin. Invest*. 1972;51(8):2103-2114.