Enzyme Immunoassay

Insulin

Cat # 1606Z

NAME AND INTENDED USE

The Diagnostic Automation Inc. INSULIN QUANTITATIVE is a solid phase enzyme-linked immunosorbant assay (ELISA). This test is designed for in vitro quantitative measurement of insulin in human serum and plasma. (For Professional Use Only)

SUMMARY AND EXPLANATION OF TEST

Insulin is the principal hormone responsible for glucose metabolism. It is synthesized in the cells of the islets of Langerhans as the precuror, proinsulin, which is processed to form C-peptide and insulin and both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chairs, the A chain (21 amino acids) and the B chain (30 amino acids), which are linked by two inter-chain disulphide bridges. There is, in addition, a single intra-chain disulphide bridge in the A chain. The sequence of insulin is highly conserved in mammalian species, and is homologous with the insulin-like growth factors IGF-I and IGF-II.

Secretion of insulin is mainly controlled by plasma glucose concentration and the hormones have a number of important metabolic actions. Its principal function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagons, epinephrine(adrenaline), growth hormone and cortisol. Insulin concentrations are severely

2°C-8°C  Σ=96 tests  #1606Z
reduced in insulin-dependent diabetes (DDM) and some other conditions such as hypopituitarism. Insulin concentrations may be raised in non-insulin-dependant diabetes (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing’s Syndrome and Acromegaly. The main clinical utility measurement is in the investigation of hypoglycaemia. Insulin assay have been used in the following applications:

1. To assess the residual cell function, especially in newly diagnosed cases of IDDM.
2. As an aid to the discrimination between IDDM and NIDDM.
3. The diagnosis of insulinoma.
4. In the investigation of the pathophysiology of diabetes mellitus.

Insulin assays are the essentials in various dynamic tests, such as oral of intravenous glucose tolerance tests (OGTT and IVGTT), to determine the insulin response of the pancreas and the degree of insulin resistance.

In many applications, insulin measurements may be complicated by cross-reactivity with partially degraded insulin, proinsulin and split forms of proinsulin. Immune complexes of these molecules are essentially problematic in patients who have developed anti-insulin antibodies through animal insulin administration. The DIAGNOSTIC AUTOMATION INC. INSULIN assay measures biologically active insulin with a high degree of specificity, using a pair of specific antibodies.

**PRINCIPAL OF THE ASSAY**

The Diagnostic Automation Inc. INSULIN is a solid phase enzyme-linked immunosorbant assay (ELISA). The wells are coated with monoclonal antibody with higher activity for insulin. When the samples, and controls are incubated in the wells with enzyme conjugate, which is another antibodies linked to horseradish peroxidase to form a sandwich complex bound to the well. Unbound conjugate are then washed off with wash buffer. The amount of bounded peroxidase is proportional to the concentration of the insulin present in the sample. Upon addition of the substrate and chromogen, the intensity of color will develop in proportional to the concentration of insulin in the samples.

**WARNING AND PRECAUTION**

1. The Diagnostic Automation Inc. INSULIN quantitative is designed for in vitro use only.
2. The components in this kit are intended for use as an integral unit. The components from different lots should not be mixed and used.
3. References contains human serum should be treated as potentially infectious. All human bases products should be used appropriate precautions.

**MATERIALS PROVIDED**

1. Microwell Strips (96 wells): Monoclonal Anti-Insulin Antibody coated wells.8x12 strips
2. Enzyme Conjugate (11ml): Anti-Insulin Antibodies conjugated to horseradish peroxidase.
3. Reference Standard Set (0.70 ml/each): Human Insulin References: 0, 5, 25, 50, 100, 200 uIU/mL calibrated against 1st WHO IRP of insulin (66/304). Conversation of insulin unit to S.I. units (pmol/L, 1uIU=6.0 pmol).
4. TMB Solution (11ml): Buffer solution containing hydrogen peroxide and TMB.
5. Concentrated wash buffer (100x) 10 mL (add 10 mL Wash Buffer to 990 mL distilled water)
6. Stop solution (11mL): 2N HCl.
7. Well holder for securing individual well.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microwell reader with wavelength at 450 nm.
2. Pipetor with tips for measuring 25 ul, 100 ul.

STORAGE AND STABILITY

1. Store the kits at 2-8°C and keep microwells in a dry bag with desiccants.
2. Unopened reagents are stable until expiration of the kit. TMB Solution should be colorless. If the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture. Allow to clot and separate the serum by centrifugation at room temperature. If sera cannot be assayed immediately, they can be stored at 2-8°C for up to 3 days or frozen at 20°C for up to a month. Avoid repeated freezing and thawing of serum specimen. Do not store in self-defrosting freezer. Do not use hyperlipemic, hemolyzed, contaminated, heat inactivated or EDTA plasma sample as they may cause erroneous results.

PREPARATION FOR ASSAY

1. Bring all reagents and samples to room temperature (20-25°C) and mix gently before beginning the test.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun, it must be performed without any interruption to get the most reliable and consistent results.
3. Use new disposable tips for each specimen.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Mark date sheet with sample identification.
2. Dispense 25 ul of serum sample, controls and reference into the assigned wells.
3. Dispense 100 ul of Enzyme conjugate into each well and mix for 5 seconds.
4. Incubate for 30 minutes at 25°C
5. Remove incubation mixture and rinse the wells five times with washing buffer.
6. Dispense 100 ul of TMB Solution into each well.
7. Incubate for 15 minutes at R.T.
8. Stop reaction by adding 50 ul Stop Solution to each well and read O.D. at 450 nm with a microwell reader in 5 minutes.

**PROCEDURAL NOTE**

1. Wash the microwells and remove water thoroughly to get the Best results.
2. Pipet all reagents and samples into bottom of the well. Vortex-mixing of shaking is not required.
3. Absorbance is the function of time and temperature of incubations. It is recommended to have all reagents and sample caps removed, all needed wells secured in holder and assigned. It will ensure the equal elapsed time for each pipetting without interruption.
4. For the same reason the size of the assay runs should be limited. It is suggested to run no more than 20 patients samples with a set of Reference Standards in duplicate.

**QUALITY CONTROL**

Each laboratory should utilize internal controls at several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values of the control. Controls can be obtained from commercially available sources but should not contain sodium azide as preservatives.

**CALCULATION OF RESULTS**

Any microwell reader capable of determining absorbance at 450 nm may be used. The insulin value of patient is obtained as follows:

1. Plot the concentration (X) of each Reference Standards against its absorbance (Y) on graph paper.
2. Obtain the insulin values of samples by reference to the Standard curve.

<table>
<thead>
<tr>
<th>Well No.</th>
<th>Description (uIU/mL)</th>
<th>Absorbance (450 nm)</th>
<th>Insulin (uIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>5</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>25</td>
<td>0.303</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>50</td>
<td>0.600</td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>100</td>
<td>1.183</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>200</td>
<td>2.152</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>Patient A</td>
<td>0.189</td>
<td>15.8</td>
</tr>
<tr>
<td>H1</td>
<td>Patient B</td>
<td>1.316</td>
<td>113.2</td>
</tr>
</tbody>
</table>
REFERENCE RANGE

Fasting: 5-35 uIU/mL
30 min. after oral glucose 30-230 uIU/mL
120 min. after oral glucose 14-160 uIU/mL

PERFORMANCE CHARACTERISTICS

**Precision**
Intra-assay: three pool sera were assayed of 8 in a single run.
Inter-assay: three pool sera were assayed in duplicate in three days.

<table>
<thead>
<tr>
<th>Serum Sample</th>
<th>Mean (uIU/mL)</th>
<th>Intra-assay</th>
<th>Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.D.</td>
<td>CV%</td>
<td>S.D.</td>
</tr>
<tr>
<td>1</td>
<td>15.2</td>
<td>1.59</td>
<td>1.54</td>
</tr>
<tr>
<td>2</td>
<td>51.4</td>
<td>3.23</td>
<td>6.29</td>
</tr>
<tr>
<td>3</td>
<td>124</td>
<td>10.53</td>
<td>6.87</td>
</tr>
</tbody>
</table>

**Accuracy**
A serum containing 200 uIU/mL of INSULIN was diluted with series of INSULIN free serum.
The dilutions were tested and the insulin recoveries were compared with the expected concentrations.
<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Insulin level expected (uIU/mL)</th>
<th>Insulin level Measured (uIU/mL)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.6</td>
<td>125</td>
<td>125.3</td>
<td>100.2</td>
</tr>
<tr>
<td>1:1</td>
<td>100</td>
<td>104.4</td>
<td>104.4</td>
</tr>
<tr>
<td>1:2</td>
<td>66.7</td>
<td>60.8</td>
<td>91.2</td>
</tr>
<tr>
<td>1:4</td>
<td>40</td>
<td>43.0</td>
<td>107.5</td>
</tr>
<tr>
<td>1:6</td>
<td>28.6</td>
<td>28.5</td>
<td>99.7</td>
</tr>
<tr>
<td>1:7</td>
<td>25</td>
<td>27.3</td>
<td>109.2</td>
</tr>
<tr>
<td>1:9</td>
<td>20</td>
<td>20.4</td>
<td>102</td>
</tr>
<tr>
<td>1:12</td>
<td>15.4</td>
<td>15.4</td>
<td>100</td>
</tr>
</tbody>
</table>

Known insulin samples were spiked with different concentrations of insulin. Samples were then tested and the insulin recoveries compared with the expected concentrations as illustrated: (Unit uIU/mL)

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Insulin Spiked</th>
<th>Expected Value</th>
<th>Measured Value</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>100</td>
<td>52.5</td>
<td>47.7</td>
<td>90.9</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>102.5</td>
<td>101.2</td>
<td>98.7</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>30.0</td>
<td>31.4</td>
<td>104.7</td>
</tr>
<tr>
<td>50</td>
<td>25</td>
<td>37.5</td>
<td>35.2</td>
<td>93.9</td>
</tr>
<tr>
<td>50</td>
<td>200</td>
<td>125.0</td>
<td>124.9</td>
<td>99.9</td>
</tr>
<tr>
<td>50</td>
<td>40</td>
<td>45.0</td>
<td>45.4</td>
<td>100.1</td>
</tr>
</tbody>
</table>

Sensitivity

The sensitivity obtained from this study was less than 1.5 uIU/mL. The minimal detectable concentration of insulin is estimated to be 0.5 uIU/mL.

Specificity

There is no cross reactivity with C-peptide at the concentration of 5000 pmo/mL, with intact human proinsulin (biosynthetic) 0.3%. High concentrations of lipid or bilirubin do not interfere in the DIAGNOSTIC AUTOMATION INC. INSULIN assay. Purified hemoglobin up to 50 ug/mL has been shown not to interfere the test. No interference for rheumatoid factor or human anti-mouse antibodies (HAMA) was observed.

REFERENCES

2. Clark PMS and Hales CN (1994)
How to Measure Plasma Insulin.
Diabetes/Metabolism Reviews, 10:79-90

Enzyme Immunoassay for Intact Human Insulin in Serum or Plasma. Clin Chem 38:578-582


<table>
<thead>
<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008-11-02</td>
<td>DA-Insulin-2008</td>
</tr>
</tbody>
</table>

DIAGNOSTIC AUTOMATION, INC.
23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383
ISO 13485-2003

Revision Date: 1/15/09