Diabetes is one of the leading causes of disability and death in the U.S. It affects an estimated 16 million Americans - about one third of them do not even know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors play a significant role. The disease is marked by deficiencies in the body’s ability to produce and properly use insulin, the hormone that controls and regulates the amount of sugar in the bloodstream.

2.0 SUMMARY AND EXPLANATION OF THE TEST

In-vitro determination of insulin and C-Peptide levels help in the differential diagnosis of liver disease, acromegaly, Cushings’ syndrome, familial glucose intolerance, insulinoma, renal failure, gestational diabetes, secondary diabetes mellitus, gestational diabetes mellitus, insulinoma, renal failure, gestational diabetes, secondary diabetes mellitus, multiple endocrine neoplasia, and type 2 diabetes. In the most common forms of diabetes mellitus, the body is resistant to insulin even though it properly uses insulin. The most common forms of diabetes are type 1 and type 2. The incidence of type 1 diabetes is about 1/200 in type 2, in which the body is resistant to insulin even though it properly uses insulin. The most common forms of diabetes are type 1 and type 2. The incidence of type 1 diabetes is about 1/200 in type 2. In type 2 diabetes, the body is resistant to insulin even though it properly uses insulin.

2.1 PROCEDURE:

The specimen shall be, blood or serum, plasma, or urine; and the unknowns in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning specimen should be obtained. The blood should be collected in a plain red-venipuncture tube without additives or anticoagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for 1 hour before centrifuging the specimen to separate the serum or plasma from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be utilized within this timeframe, the sample(s) may be stored at -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When used, duplicate (1.00mL (100µg) of the specimen is required.

3.0 REAGENTS

A. Equipment:

1. Wash: Decontaminate wash solution tokees substrate solution to all wells (see “Reagent Preparation”).

2. Pipette 0.05mL (50µL) of the appropriate calibrator, controls and antibody to the wells.

3. Add 100µL (100ng) of the insulin or C-Peptide Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the microwell.

4. Add 0.100mL (100µg) of the C-Peptide micropipette to gently for 20–40 s.

5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

6. Add 0.350mL (350µl) of wash buffer (see “Reagent Preparation”), (dip, tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instructions for proper usage. If a squeeze bottle is used, fill well enough to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat two (2) additional times.

7. Add 0.100mL (100µl) of substrate solution to all wells.

8. Incubate at room temperature for fifteen (15) minutes.

9. Incubate at 450nm (using a reference wavelength of 620-630nm to minimize well-to-well variations between wells.

10. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well-to-well variations between wells.

11. The absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well-to-well variations between wells.

12. Note: Do not use the working substrate if it looks blue.

13. Note: Do not use reagents that are contaminated or have bacteriological growth.

4.0 REAGENT PREPARATION:

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1 or Example 2.

2. Plot the concentration of each duplicate serum reference versus the corresponding insulin or C-Peptide concentration in pM/L on linear graph paper (do not average the duplicate results of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.

4. Determine the concentration of insulin or C-Peptide for an unknown, locate the average absorbance value of the duplicate samples of the unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pM/L) from the horizontal axis of the graph. (The duplicate values of the unknown may be averaged as indicated).

5. In the following example, the average absorbance value of 0.405 (intersects the dose response curve at 66.8 µIU/ml (0.82ng/ml). In the following example, the average absorbance value of 0.405 (intersects the dose response curve at 66.8 µIU/ml (0.82ng/ml). In the following example, the average absorbance value of 0.405 (intersects the dose response curve at 66.8 µIU/ml (0.82ng/ml). In the following example, the average absorbance value of 0.405 (intersects the dose response curve at 66.8 µIU/ml (0.82ng/ml).

6.1 Required But Not Provided:

1. One (1) vial containing enzyme labeled affinity purified monoclonal C-Peptide IgG in buffer, dye and preservative. Store at 2-8°C.

2. Reagent to each well.

3. Note: Do not use the working substrate if it looks blue.

4. Note: Do not use reagents that are contaminated or have bacteriological growth.

9.0 TEST PROCEDURE:

Before proceeding with the assay, bring all reagents, reference calibrators and controls to room temperature (20-27°C).

**Test procedure should be performed by a skilled individual or trained professional**

1. Format the microplates’ wells for calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 4°C.

2. Pipette 0.50mL (50µl) of the appropriate calibrators, controls and patient serum.

3. Add 100µL (100ng) of the insulin or C-Peptide Enzyme Reagent to each well. Use an automatic or manual plate washer can be used. Follow the manufacturer’s instructions for proper usage. If a squeeze bottle is used, fill well enough to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat two (2) additional times.

4. Incubate at room temperature for fifteen (15) minutes.

5. Add 0.100mL (100µl) of substrate solution to all wells.

6. Incubate at room temperature for fifteen (15) minutes.

7. Read the absorbance at 450nm (using a reference wavelength of 620-630nm to minimize well-to-well variations between wells.

8. Draw the best-fit curve through the plotted points.

9. Determine the concentration of insulin or C-Peptide for an unknown, locate the average absorbance value of the duplicate samples of the unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pM/L) from the horizontal axis of the graph. (The duplicate values of the unknown may be averaged as indicated).

10. In the following example, the average absorbance value of 0.405 (intersects the dose response curve at 66.8 µIU/ml (0.82ng/ml). In the following example, the average absorbance value of 0.405 (intersects the dose response curve at 66.8 µIU/ml (0.82ng/ml).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized the validation of the software should be ascertained.

*The data presented in Example 1-2 and Figure 1-2 are for illustration only and should not be used in lieu of a dose response curve prepared with each assay.
10.0 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrators 0 µIU/ml should be < 0.1.
2. The absorbance (OD) of calibrators 300 µIU/ml or 10ng/ml should be ≥ 1.3.

3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of sample should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should be discarded. If the same sequence to eliminate any time-deviation during reaction.
4. Plate readers measure vertically. Do not touch the bottom of the wells.
5. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor recoveries.
6. Use components from the same lot; no intermingling of reagents from different batches.
7. Patient samples with insulin concentrations above 300µIU/ml (Insulin) or 10ng/ml (C-Peptide) may be diluted with the zero calibrator and reassayed. Multiply the value obtained by the dilution factor to obtain the correct value.

12.2 Interpretation

1. It is important to keep in mind that establishment of a range of values, which can be measured by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method of the analysis. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an application and subsequent results can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Ins/C-Pep VAST® AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 1 and Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Well Number</th>
<th>Abs (A)</th>
<th>Mean Abs (B)</th>
<th>Value (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool 1</td>
<td>24 (20)</td>
<td>10.7 (1.43)</td>
<td>0.89 (0.11)</td>
<td>8.3% (7.7%)</td>
</tr>
<tr>
<td>Pool 2</td>
<td>24 (20)</td>
<td>48.2 (5.07)</td>
<td>2.07 (0.46)</td>
<td>4.3% (9.0%)</td>
</tr>
<tr>
<td>Pool 3</td>
<td>20 (20)</td>
<td>10.3 (1.81)</td>
<td>1.46 (0.57)</td>
<td>7.2% (6.1%)</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate over ten days.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/ml calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.182 µIU/ml for insulin and 0.020 ng/ml for C-Peptide.

14.3 Accuracy

The Ins/C-Pep VAST® AccuBind® ELISA Test System was compared with a reference coated tube radioimmunoassay assay for insulin. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from 0.01µIU/ml – 129µIU/ml). The total number of such specimens was 104. The data obtained is displayed in Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool 1</td>
<td>11.8 (1.27)</td>
<td>1.33 (0.12)</td>
</tr>
<tr>
<td>Pool 2</td>
<td>48.5 (4.94)</td>
<td>2.46 (0.54)</td>
</tr>
<tr>
<td>Pool 3</td>
<td>46.2 (7.69)</td>
<td>2.77 (0.64)</td>
</tr>
</tbody>
</table>

*Correlation coefficient* (r) was 0.942.

15.0 REFERENCES

2. Gerbitz VRD, “Pancreatiae B-zeilen: Peptide, and Immunological Properties of Proinsulin, Insulin and C-Peptide in Plasma and Urine Protein Fractionation.” Clin Chem 1988: 3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
3. Kahn CR, Rosenthal AS, "Immunologic cross-reactivity of Insulin and C-Peptide with autoimmune insulin antibodies: a problem for all immunoassays' Clin Chem 1988; 3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid results, certain criteria and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are interpreted incorrectly. Monobind shall have no liability.

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