PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of the kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.

3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.

4. In order to reduce the occurrence of potentially hazardous substances, gloves should be worn when handling kit reagents and human specimens.

5. All kit reagents and specimens should be kept to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.

6. A calibrator curve must be established for every run.

7. The controls should be included in every run and fall within established confidence limits.

8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may result in the above-mentioned. Values for the controls do not reflect established ranges.

9. When reading the microplate, the presence of bubbles in the wells or the failure to see any color in a well may indicate that bubbles were not completely removed before reading the test.

10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue-violet color in which case the assay should be discarded.

11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.

12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.

13. Do not mix various lot numbers of kit components within a test method however, can offer complete assurance that HIV, reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No testing for antibodies against any other virions is provided. The presence of antibodies to hepatitis B virus or any other infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

14. Kit reagents must be regarded as hazardous waste and should be handled with caution.

INTENDED USE

For the direct quantitative determination of Insulin-Like Growth Factor Binding Protein-1 by an enzyme immunoassay in human serum.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test format involves a typical two-step ‘sandwich’ type assay. The assay makes use of two highly specific monoclonal antibodies: a monoclonal antibody specific for IGFBP-1 is immobilized onto the microplates, endogenous monoclonal antibody specific for a different region of IGFBP-1 is conjugated to horse radish peroxidase (HRP). The added sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured by the microplate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of IGFBP-1 in the sample.

A set of standards is used to plot a standard curve from which the amount of IGFBP-1 in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

Insulin-like growth factor binding protein-1 (IGFBP-1) is one of six proteins that specifically bind insulin-like growth factors I and II (IGF-I and II) in body fluids and tissues. IGFBP-1 contains 234 amino acids, with a predicted molecular mass of 25 kDa. The major sites of IGFBP-1 synthesis are the fetal and decidualized endometrium. Serum levels of IGFBP-1, which reflect its synthesis by the liver, exhibit considerable diurnal variation. Circulating IGFBP-1 levels are highest during pregnancy and lowest in the evening. The levels are high in the fetus and newborn, but decline steadily until puberty. The level in healthy adults is 4.4 µg/L (range 0.6–14.4 µg/L). After about 65 years of age, serum IGFBP-1 levels begin to increase. There is a linear increase in serum IGFBP-1 levels at various age groups. The mean level of IGFBP-1 in healthy adults is 4.4 µg/L (range 0.6–14.4 µg/L). After about 65 years of age, serum IGFBP-1 levels begin to increase. There is

1. The most important regulator of circulating IGFBP-1 is insulin. During the secretory phase of the menstrual cycle, serum IGFBP-1 levels are highest early in the morning and lowest in the evening. Serum levels of IGFBP-1, which reflect its synthesis by the liver, exhibit considerable diurnal variation. Circulating IGFBP-1 levels are highest during pregnancy and lowest in the evening. The levels are high in the fetus and newborn, but decline steadily until puberty. The level in healthy adults is 4.4 µg/L (range 0.6–14.4 µg/L). After about 65 years of age, serum IGFBP-1 levels begin to increase. There is a linear increase in serum IGFBP-1 levels at various age groups. The mean level of IGFBP-1 in healthy adults is 4.4 µg/L (range 0.6–14.4 µg/L). After about 65 years of age, serum IGFBP-1 levels begin to increase. There is

2. Fasting insulin and IGFBP-1 concentrations are inversely correlated. During a 3-h glucose tolerance test, there is a decrease of about 50% in serum IGFBP-1 levels. Eating a meal also has a decreasing effect.

3. In insulin-dependent diabetes (IDDM), serum IGFBP-1 levels are elevated. In non-insulin-dependent diabetes mellitus in which insulin levels are high, serum IGFBP-1 is decreased. Low levels of IGFBP-1 have also been observed in the following cases: acromegaly, Cushings’ syndrome, and polycystic ovarian syndrome (PCO).

4. In insulin-dependent diabetes (IDDM), serum IGFBP-1 levels are elevated. In non-insulin-dependent diabetes mellitus in which insulin levels are high, serum IGFBP-1 is decreased. Low levels of IGFBP-1 have also been observed in the following cases: acromegaly, Cushings’ syndrome, and polycystic ovarian syndrome (PCO).

5. In children, serum IGFBP-1 levels are lowest during the perinatal period (within the 1st week of life) and increase steadily until puberty. The mean level of IGFBP-1 in healthy adults is 4.4 µg/L (range 0.6–14.4 µg/L). After about 65 years of age, serum IGFBP-1 levels begin to increase. There is a linear increase in serum IGFBP-1 levels at various age groups.

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1. Calculate the mean optical density of each calibrator

2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.

3. Pipette 25 μL of each calibrator, control, and specimen sample into correspondingly labelled wells in duplicate.

4. Pipette 100 μL of assay buffer into each well. (We recommend using a multichannel pipette.)

5. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.

6. Wash the wells 3 times with 300 μL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)

7. Pipette 100 μL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)

8. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.

9. Wash the wells again in the same manner as step 6.

10. Pipette 100 μL of TMB substrate into each well at timed intervals.

11. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD).

12. Pipette 50 μL of stopping solution into each well at the same timed intervals as in step 10.

13. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

ASSAY PROCEDURE

Specimen Pretreatment: None.

All reagents must reach room temperature before use. Calibrators, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solution of the anti-IGFBP-1 conjugate and wash buffer.

2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.

3. If a sample reads more than 220 μg/L, then dilute it with calibrator A at a dilution of no more than 1:10. The result obtained should be multiplied by the dilution factor.

4. Spiked samples were prepared by adding defined amounts of IGFBP-1 to three patient serum samples (1:1). The results (in μg/L) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Uncalibrated</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ 6.5</td>
<td>5.8</td>
<td>5.75</td>
<td>100.9</td>
</tr>
<tr>
<td>+ 35</td>
<td>20.2</td>
<td>20.0</td>
<td>100.0</td>
</tr>
<tr>
<td>+ 174</td>
<td>90.9</td>
<td>89.5</td>
<td>100.6</td>
</tr>
<tr>
<td>2 Uncalibrated</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ 6.5</td>
<td>14.1</td>
<td>13.3</td>
<td>105.3</td>
</tr>
<tr>
<td>+ 35</td>
<td>29.3</td>
<td>24.5</td>
<td>81.8</td>
</tr>
<tr>
<td>+ 174</td>
<td>100.0</td>
<td>97.1</td>
<td>103.1</td>
</tr>
<tr>
<td>3 Uncalibrated</td>
<td>110</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ 6.5</td>
<td>62.0</td>
<td>58.3</td>
<td>106.3</td>
</tr>
<tr>
<td>+ 35</td>
<td>80.0</td>
<td>72.5</td>
<td>110.3</td>
</tr>
<tr>
<td>+ 174</td>
<td>155.0</td>
<td>133.0</td>
<td>116.5</td>
</tr>
</tbody>
</table>


REFERENCES


5. DBC-Diagnostics Biochem Canada Inc. 384 Neptune Crescent London, Ontario, Canada N6A 1A1 Tel: (519) 681-8731 Fax: (519) 681-8734 e-mail: dbc@dbc-labs.com www.dbc-labs.com An ISO 13485 Registered Company

SYMBOLS

e: European Conformity

C: Contents sufficient for in vitro tests

R: Storage temperature

L: Legal Manufacturer

A: Authorized representative

X: Use by Lot number

C: Dilute 1:9 before use

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