Enzymatic Immunoassay for the qualitative determination of Human Chorionic Gonadotropin in human serum

INTENDED USE
For the rapid determination of human chorionic gonadotropin (hCG) in urine or serum specimens. This test kit is used to obtain a visual, qualitative result.

SUMMARY AND EXPLANATION OF PROCEDURE
Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected in serum as early as 7 days following conception (1-4), doubling every 1.3 to 2 days. At the time of the first missed menstrual period, serum hCG concentration is about 100 mLU/ml (2-5), and peak levels of 100,000 - 200,000 mLU/ml are seen at the end of the first trimester. The appearance of hCG soon after conception and its subsequent rise in concentration during early gestational growth make it an excellent marker for the early detection of pregnancy. Elevated serum hCG levels comparable to those observed in early pregnancy may also be associated with trophoblastic or non-trophoblastic neoplasms (6-7) such as hydatidiform mole, choriocarcinoma; therefore, the possibility of such diseases should be ruled out before a positive hCG result is considered diagnostic for pregnancy. The DIMA Pre-View HCG immunoassay kit is a rapid test to detect the presence of hCG in urine or serum specimens in a qualitative format sensitive to 25 mLU/ml. The test utilizes a monoclonal antibody reagent specific to the beta subunit of hCG. The immunological specificity of the test kit virtually eliminates cross-reactivity interference from the structurally related glycoprotein hormones hFSH, hLH and hTSH at physiological levels.

PRINCIPLE
The DIMA Pre-View HCG assay is a qualitative, sandwich enzyme immunoassay (8-9) for the determination of human chorionic gonadotropin in urine or serum. The method employs an unique combination of monoclonal antibodies to selectively identify hCG in urine or serum with a high degree of sensitivity. In less than 10 minutes, elevated levels of hCG as little as 25 mLU/ml can be detected.

The patient urine or serum to be tested is allowed to react with the antibody enzyme conjugate and the antibodies on the solid phase simultaneously. In the presence of hCG, a specific antibody-antigen-antibody-enzyme complex will be formed on the surface of the microtiter well. After removing unbound enzyme conjugate by rinsing under a stream of tap water, the well is incubated with the substrate solutions. The development of blue color in the well indicates the presence of hCG. Comparing the color intensity developed by patient samples with that of the provided known reference, the amount of hCG can be visually estimated to be greater or less than 25 mLU/ml.

REAGENTS AND MATERIALS SUPPLIED
1. Microtiter Reaction Wells : Microtiter reaction wells containing goat polyclonal antibodies (IgG) directed against hCG. Store at 2-8 °C.
2. Enzyme Conjugate : One bottle, containing mouse monoclonal antibody-peroxidase conjugate in protein stabilizer and 0.02 % thimerosal. Store at 2-8 °C.
3. Substrate Reagent A : One bottle, containing hydrogen peroxide and stabilizer. Store at 2-8 °C.
4. Substrate Reagent B : One bottle, containing substrate chromogen, Store at 2-8 °C.
5. Negative reference : One bottle, of buffered protein solution containing, 1% sodium azide, store at 2-8 °C.
6. Positive reference : One bottle, of buffered protein solution containing approximately 150 mLU/ml hCG and 1% sodium azide, store at 2-8 °C.

Note: All the provided reagent bottles are specially made, so there is no need to use droppers additionally.

PRECAUTION
1. FOR IN VITRO DIAGNOSTIC USE ONLY
2. Do not mix reagents from different lots and do not use kit components beyond expiration date.
3. Do not mix reagent bottle caps.
4. Do not expose the substrate reagent B to strong light during storage or use.
5. All human specimen should be treated and handled as if being capable of transmitting infectious diseases.

SPECIMEN COLLECTION
The urine sample must be collected in a clean, dry container, either plastic or glass, without preservative. Specimens collected at anytime may be used, however, the first morning urine generally contains the highest concentration of hormone. Urine specimens may be refrigerated (2-8 °C) and stored up to 72 hours prior to assay. If samples are refrigerated, they must be equilibrated to room temperature before testing. Urine samples exhibiting visible precipitates should be filtered, centrifuged, or allowed to settle and clear aliquots obtained for testing.

Serum Sample (0.5 ml): No special preparation of the patient specimen is required. Additives such as preservatives should be avoided. Limited sample studies indicated that plasma sample prepared from EDTA can be used in lieu of serum. Sera not asayed immediately must be stored in the refrigerator (2-8 °C, up to 72 hours) or frozen (-20 °C for at least 3 months). Bring these specimens to room temperature before assay. Do not freeze and thaw repeatedly.

ASSAY PROCEDURE

SPECIMEN COLLECTION
The urine sample must be collected in a clean, dry container, either plastic or glass, without preservative. Specimens collected at anytime may be used, however, the first morning urine generally contains the highest concentration of hormone. Urine specimens may be refrigerated (2-8 °C) and stored up to 72 hours prior to assay. If samples are refrigerated, they must be equilibrated to room temperature before testing. Urine samples exhibiting visible precipitates should be filtered, centrifuged, or allowed to settle and clear aliquots obtained for testing.

Serum Sample (0.5 ml): No special preparation of the patient specimen is required. Additives such as preservatives should be avoided. Limited sample studies indicated that plasma sample prepared from EDTA can be used in lieu of serum. Sera not assayed immediately must be stored in the refrigerator (2-8 °C, up to 72 hours) or frozen (-20 °C for at least 3 months). Bring these specimens to room temperature before assay. Do not freeze and thaw repeatedly.
Material Provided:
1. Microtiter reaction wells
2. Enzyme conjugate
3. Substrate reagent A
4. Substrate reagent B
5. Negative reference
6. Positive reference

Materials Required But Not Provided:
1. Specimen collection containers
2. Timer
3. Flowing tap water
4. Absorbent paper towels

Procedure Instructions:
All reagents including patients samples, controls and reference material should be brought to room temperature (20-30 C) and mixed thoroughly before beginning the assay.

1. Place the antibody coated wells for your test on the holder.
2. Dispense 1 drops of patient sample (urine or serum) or 1 drops of positive or negative reference samples into appropriately labeled reaction wells.
3. Add 1 drop of enzyme conjugate to each well.
4. Incubate 3 minutes at room temperature.
5. Remove content by flicking the microtiter plate holder into sink, followed by rinsing the wells 5 times with running tap water (or distilled water).
   Note: Take care to avoid well to well contamination from water overflow during the first rinse. Separate wells on the well holder would help.
6. Add 1 drop of Substrate solution A and 1 drop of Substrate solution B into each well and incubate at room temperature. Note: Substrate solution A must be added before addition of Substrate B.
7. Between 2 to 5 minutes after the addition of Substrate solution B, Compare the color developed in sample wells to that of the positive Reference well against a white background.
   Note: A. Depending on the concentration of hCG in the sample the color may develop instantaneously.
   B. Prolong incubation of the Substrate Solutions beyond 5 minutes may result in a slight shade of blue much less intense than that of the Positive Reference. This should be considered as negative result.

INTERPRETATION OF RESULTS

1. Positive: Wells showing blue color.
2. Negative: Wells showing no color at the end of the second 5 minute incubation indicate a non-detectable amount of hCG in the sample, or a negative result. A slight blush tinge, much lighter than the Positive reference well, may result from insufficient wash and should be considered negative. If patient sample shows less color than the Positive Reference and Pregnancy is suspected, the test should be repeated using a fresh sample obtained 48-72 hours later.
3. Invalid: When negative reference results in clearly blue color, improper wash procedure may occur. The test should consider invalid.
PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity
The analytical sensitivity of the DIMA HCG test has been set at 25mIU/ml (calibrated to the 2nd International Standard).

Specificity of the DIMA Pre-View HCG test was determined from cross reaction studies with known amounts of Luteinizing Hormone (hLH), Follicle Stimulating Hormone (hFSH), and Thyroid Stimulating Hormone (hTSH). 300 mIU/ml hLH, 1000 mIU/ml hFSH and 1000 uIU/ml hTSH all gave negative results.

Accuracy
A. Analysis of Urine Samples: Urine samples from five known non-pregnant subjects were spiked with hCG to the concentrations of 0, 25, 50 mIU/ml. Total of 75 of these samples were blind labeled and tested with Pre-View HCG tests. Results are summarized in Table 1.

<table>
<thead>
<tr>
<th>hCG added (mIU/ml)</th>
<th>0</th>
<th>25</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sample</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

B. Analysis of serum samples: Serum samples from four known non-pregnant subjects were spiked with hCG to the concentrations of 0, 25, 50 mIU/ml. Total of 72 samples with different levels of hCG were blind labeled and tested with DIMA HCG tests. Results are summarized in Table 2.

<table>
<thead>
<tr>
<th>hCG added (mIU/ml)</th>
<th>0</th>
<th>25</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tests</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

C. Correlation with Qualitative Visual Tests - Urine: 80 randomly selected urine samples were analyzed by DIMA HCG test procedure in parallel with a commercial available visual method. Out of these samples, 37 were negative and 43 were positive. A complete agreement of the results was observed.

D. Correlation with Qualitative Visual Tests - serum: 60 randomly selected serum samples were analyzed by DIMA HCG test procedure in parallel with a commercial available visual method. Both results indicated 22 negative, 38 positive and agree completely.

Interference Testing:
The following substances were added in hCG free and 25 mIU/ml hCG spiked urine or serum samples. None of the substances at concentrations tested interfered in the assay.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Acetylsalicylic Acid</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Glucose</td>
<td>2 g/dl</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1 mg/dl</td>
</tr>
</tbody>
</table>

REFERENCES

BioTina GmbH
Elseyer Str. 59, 58119 Hagen, Germany