**Qualitative Results:**

1. Do not add the stop solution to the wells. Blue color is much easier to interpret than the yellow color that shows after the addition of stop solution.
2. Compare the blue color in the sample well, to the color in the well assigned to Calibrator ‘B’ (25 mIU/mL).
3. If the color in the sample well is less than the color in the ‘B’ well the sample should be considered Negative because it has HCG concentration < 25 mIU/mL at the time the sample was taken.
4. If the color in the sample well is more than the color in the ‘B’ well, the sample should be considered Positive because it has HCG concentration > 25 mIU/mL at the time the sample was taken.

**INTERPRETATION OF RESULTS**

**Semi-Semi-quantitative Determination of hCG in Human Serum or Urine by a Microplate Immunoenzymometric assay for early detection of pregnancy.**

**SUMMARY AND EXPLANATION OF THE TEST**

Human chorionic gonadotropin (hCG) concentration increases dramatically in blood and urine during normal pregnancy. hCG is secreted by placental tissue, beginning with the primitive ophallus almost from the time of implantation, and serves to support the corpus luteum during the early weeks of pregnancy. hCG or hCG similar glycoproteins can also be produced by wide variety of non-placental tumors. The measurement of hCG, by assay systems with suitable sensitivity and specificity has proven great value in the detection of pregnancy and the diagnosis of early pregnancy disorders.

**REAGENTS**

A. hCG Calibrators – 1ml/vial - Ions A-E (Lyophilized) (A-E) Five (5) vials, of references for hCG Antigen at levels of (A). 25(B), 50(D), 100(D) and 250(E) mIU/mL. Store at 2-8°C. Reconstitute each vial with 1.0 ml of distilled or deionized water.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd IS (75/537).

**PRINCIPLE**

**Immunoenzymometric assay:**

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (signal and capture), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the calibrator, control or patient sample is added to the wells coated with anti-hCG antibody. HCG from the sample binds to the Anti-hCG (MoAb) on the wells. Subsequently an enzyme labeled Anti-HCG is added to the wells. HCG from the sample forms a sandwich complex between the two antibodies. Excess enzyme and sample is removed via a wash step. The interaction is illustrated by the following equation:

\[
\text{Enz}_{A}\text{Ab}_{p} + \text{AghCG} + \text{Ab}_{m} \rightarrow \text{Enz}_{A}\text{Ab}_{p} + \text{AghCG} + \text{Ab}_{m}\]

\[
\text{Ab}_{m} = \text{Anti-HCG (MoAb)(On the Microwells in Excess Quantity)}
\]

\[
\text{AghCG} = \text{Native Antigen (Variable Quantity)}
\]

\[
\text{Enz}_{A}\text{Ab}_{p}\text{Enzyme labeled Goat } \alpha \text{ hCG (P) (Excess Quantity)}
\]

\[
\text{k}_a = \text{Rate Constant of Association}
\]

\[
\text{k}_d = \text{Rate Constant of Dissociation}
\]

The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

A suitable substrate is added to the wells to generate color in varying intensity depending upon the concentration of hCG in the wells. The intensity of the color in the sample can be visually compared to the known calibrators to obtain qualitative results or the color development can be read with the help of a microplate spectrophotometer to obtain semi-quantitative results.

**REAGENT PREPARATION AND STORAGE:**

**Wash Buffer:**

Dilute contents of Wash Concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C).

**Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C).**

**Calibrator ‘B’ well the sample should be considered because it has hCG concentration < 25 mIU/ml at the time the sample was taken.**

**4. If the color in the sample well is more than the color in the ‘B’ well, the sample should be considered Positive because it has HCG concentration > 25 mIU/mL at the time the sample was taken.**

**TEST PROCEDURE**

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

1. Format the microplate wells for each serum reference, control or patient specimen to be assayed.

2. Dispense pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 100µl of hCG-Enzyme Conjugate solution to all wells.

4. Swirl the microplate for 5-10 seconds to mix and incubate at room temperature for 10 minutes.

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

6. Add 350µl of wash buffer (see Reagent Preparation Section) decant the wash and store at 2-8°C.

7. Add 100µl of hCG-Conjugate solution to all wells.

8. Incubate at room temperature for 5 minutes.

9. Add 350µl of wash buffer (see Reagent Preparation Section) decant the (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 instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat two (2) additional times.

10. Add 100µl of substrate solution to all wells.

**DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**

11. Incubate 10 minutes at 20-27°C.

**For Semi-quantitative results go to step 11 below.**

12. Deposit contents of Wash Concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C) for up to 60 days.

**SPECIMEN COLLECTION AND PREPARATION**

- **Urine Sample:** Collect urine sample in a clean container.

- **Serum Sample:** Collect blood sample in a clean container. For most accurate results it is advisable to collect first morning urine sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05 ml of the specimen is required.

**Example 1**

<table>
<thead>
<tr>
<th>Serum Sample</th>
<th>Cal A</th>
<th>Cal B</th>
<th>Cal C</th>
<th>Cal D</th>
<th>Cal E</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.029</td>
<td>0.373</td>
<td>0.646</td>
<td>1.097</td>
<td>1.654</td>
</tr>
<tr>
<td>B1</td>
<td>0.025</td>
<td>0.373</td>
<td>0.646</td>
<td>1.097</td>
<td>1.654</td>
</tr>
<tr>
<td>C1</td>
<td>0.025</td>
<td>0.373</td>
<td>0.646</td>
<td>1.097</td>
<td>1.654</td>
</tr>
<tr>
<td>D1</td>
<td>0.025</td>
<td>0.373</td>
<td>0.646</td>
<td>1.097</td>
<td>1.654</td>
</tr>
<tr>
<td>E1</td>
<td>0.025</td>
<td>0.373</td>
<td>0.646</td>
<td>1.097</td>
<td>1.654</td>
</tr>
</tbody>
</table>

**Results**

- **Cal A:** Reference 1.000 mIU/mL
- **Cal B:** Reference 0.373 mIU/mL
- **Cal C:** Reference 0.646 mIU/mL
- **Cal D:** Reference 1.097 mIU/mL
- **Cal E:** Reference 1.654 mIU/mL

**Interpretation Only**

- **Note 1:** Do not use reagents beyond the kit expiration date.
- **Note 2:** Above reagents are for a single 96-well microplate assay.
- **Note 3:** For Semi-semi-quantitative Determination of hCG in Human Serum or Urine by a Microplate Immunoenzymometric assay for early detection of pregnancy.

**QUALITY CONTROL**

Each laboratory should assay controls at levels in the low, medium and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Qualilty control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed, if acceptable limits are not met, a sample deviation from the established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.
**TABLE 3**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>5.5</td>
<td>0.35</td>
<td>6.4%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>20.5</td>
<td>0.95</td>
<td>4.6%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>91.6</td>
<td>7.07</td>
<td>7.7%</td>
</tr>
</tbody>
</table>

**TABLE 4**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>10</td>
<td>6.1</td>
<td>0.52</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>22.3</td>
<td>1.63</td>
<td>0.36</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>85.1</td>
<td>6.17</td>
<td>0.36</td>
</tr>
</tbody>
</table>

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**REFERENCES**


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**Q.C. PARAMETERS**

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

1. The absorbance (OD) of calibrator E should be > 1.0.
2. Four out of six quality control pools should be within the established ranges.

**LIMITATIONS OF PROCEDURE**

**A. Assay Performance**

1. The Monobind Accubind™ Rapid HCG Microwell Elisa test is intended to be used for early determination of pregnancy and should not be used for monitoring of advanced pregnancy. Monobind Accubind™ HCG Elisa (Cat # 825-300) should be used for that purpose.
2. It is important that the time of reaction in each well is held constant for reproducible results.
3. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and the Stop solution should be added in 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 replication and spurious results.
6. Sample(s), that are contaminated microbiologically, should not be used in the assay. Highly lipemic or hemolysed specimen(s) should similarly not be used.
7. For semi-quantitative determination the patient specimens with HCG concentrations above 250 mIU/ml may be diluted with normal male serum (hCG < 1 mIU/ml) or normal male urine and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.

**B. Interpretation**

1. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
2. False positive results may occur in the presence of a wide variety of trophoblastic and nontrophoblastic tumors that secrete hCG. Therefore, the possibility of an hCG secreting neoplasia should be eliminated prior to diagnosing pregnancy.
3. Also, false positive results may be seen when assaying specimens from individuals taking the drugs Pergonal® and Clomid®. Additionally Pergonal will often be followed with an injection of HCG.
4. Spontaneous micro abortions and ectopic pregnancies will tend to have values which are lower than expected during a normal pregnancy while somewhat higher values are often seen in multiple pregnancies (4, 5, 6).
5. Following therapeutic abortion, detectable hCG may persist for as long as three to four weeks. The disappearance rate of hCG, after spontaneous abortion, will vary depending upon the quantity of viable residual trophoblast (4, 5, 6, 7).

**REFERENCES**


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**QB. Accuracy**

The Accubind™ Rapid HCG ELISA Microplate Test System was compared with a predicated Elisa immunoassay, Biological specimens from normal and pregnant women were assayed. The total number of such specimens was 244. The least square regression equation and the correlation coefficient were computed for the hCG ELISA in comparison with the reference method. The data obtained is displayed in Table 4.

**TABLE 4**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (x)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>This Method</td>
<td>25.57</td>
<td>y = 1.035x + 0.9474x</td>
<td>0.956</td>
</tr>
<tr>
<td>Predicate</td>
<td>24.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Only slight amounts of bias between the Accubind™ HCG ELISA Microplate Test System and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

**C. Sensitivity**

The Accubind™ Rapid HCG ELISA Microplate Test System has a sensitivity of 2.5 mIU/ml.

**D. Specificity**

The cross-reactivity of the Accubind™ Rapid HCG ELISA Microplate Test System to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Chorionic Gonadotropin needed to produce the same absorbance.

**Substance**

<table>
<thead>
<tr>
<th>Cross Reactivity Concentration</th>
<th>Chorionic Gonadotropin (hCG) 1.0000</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG subunit</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Folliculotropin (FSH)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lutropin Hormone (LH)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Thyrotropin (TSH)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**E. High Dose Hook Effect**

The Monobind Accubind™ Rapid HCG ELISA Microplate Test System will detect samples with hCG concentrations up to 25,000 mIU/ml as positive qualitatively.