## 1.0 INTRODUCTION

The Circchorionic Gonadotropin Concentration in Human Serum by a Microparticle Enzyme Immunoassay, Chemosensivity

### 2.0 SUMMARY AND EXPLANATION OF THE TEST

Human chorionic gonadotropin (hCG) concentration increases dramatically in blood and urine during normal pregnancy. hCG is secreted by the placenta beginning with the first trimester. The test measures hCG concentration in the early weeks of pregnancy. The measurement of hCG by this assay is more sensitive and specific than other techniques used to detect pregnancy and the diagnosis of early pregnancy disorders.

According to the literature, hCG is detectable as early as 10 days after ovulation, reaching 100 mIU/ml by the first month period. At the time for the next ovulation, the hCG level is 200 mIU/ml (approximately 28 days after conception). A peak of 50,000 or 100,000 mIU/ml is attained by the third month, then a plateau is reached.

### 3.0 PRINCIPLE

#### 3.1 Immunoenzymometric assay (Type 3):

The essential reagents required for an immunoenzymometric assay are antibodies, specific antibodies, and a conjugate. The assay results are obtained by adding the antigen to the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
\text{EnzAb} + \text{Ag} \rightarrow \text{EnzAb-Ag} \rightarrow \text{EnzAb-Ag-BtnAb(m)}
\]

where \(\text{EnzAb} = \) Enzymelabeled Monoclonal Antibody, \(\text{Ag} = \) Native Antigen, \(\text{BtnAb} = \) Biotinylated Affinity Purified Antibody, and \(\text{m} = \) Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

\[
\text{EnzAb-Ag-BtnAb(m)} + \text{Streptavidin} \rightarrow \text{Streptavidin-Immobilized complex}
\]

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the 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.

### 4.0 REAGENTS

#### 4.1 Materials Provided:

- A: hCG-XR Calibrators – 1 ml/vial – Icos A-F
- B: hCG-XR Tracer Reagent – 13 ml/vial – Icos
- C: Light Reaction Wells – 96 wells – Icos
- D: Wash Solution Concentrate – 20 ml/vial – Icos
- E: Wash Buffer
- F: Signal Reactant A – 7 ml/vial – Icos
- G: Signal Reactant B – 7 ml/vial – Icos
- H: Wash Buffer
- I: Wash Buffer
- J: Signal Reactant B
- K: Signal Reactant B
- L: Wash Buffer
- M: Wash Buffer
- N: Wash Buffer
- O: Wash Buffer
- P: Wash Buffer
- Q: Wash Buffer
- R: Wash Buffer
- S: Wash Buffer
- T: Wash Buffer
- U: Wash Buffer
- V: Wash Buffer
- W: Wash Buffer
- X: Wash Buffer
- Y: Wash Buffer
- Z: Wash Buffer

#### 4.2 Reagents:

- A. hCG-XR Calibrators – 1 ml/vial – Icos A-F
- B. hCG-XR Tracer Reagent – 13 ml/vial – Icos
- C. Light Reaction Wells – 96 wells – Icos
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- R. Wash Buffer
- S. Wash Buffer
- T. Wash Buffer
- U. Wash Buffer
- V. Wash Buffer
- W. Wash Buffer
- X. Wash Buffer
- Y. Wash Buffer
- Z. Wash Buffer

#### 4.3 Product Instructions

**Note 1:** Do not use reagents beyond the expiration date.

**Note 2:** Avoid extended exposure to heat and light. Opened reagents are stable for forty-five (45) days when stored at 2-8°C. Kit and component stability are identified in the Reagents Section.

**Note 3:** Above reagents are enough for a single 96-well microplate assay.

### 5.0 PRECAUTIONS

#### 5.1 For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all handling procedures for preparing the reagents should be as potential, hazardous and capable of transmitting disease. Good laboratory practices include using rubber gloves, a constant work area, and wearing goggles. These precautions can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components according to local regulatory and statutory requirement.

### 6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be serum, in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood shall be collected in a plain red top venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Sample may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assessed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices.

Avoid contamination of the signal reagent. Each well should be washed after signal addition. Do not re-use reagents.

### 7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium, and high levels for monitoring assay performance. These controls should be treated as unknowns and values determined in the same manner as an unknown. The kit should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain that the quality control performance can indicate unnoticeable change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

### 8.0 REAGENT PREPARATION

#### 8.1 Wash Buffer

Diluted Wash Buffer Concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store diluted Wash Buffer at 2-30°C for up to 60 days.

#### 8.2 Signal Reactant A

Dilute Signal Reactant A – 1 ml/vial. Store at 2-8°C.

#### 8.3 Signal Reactant B

Dilute Signal Reactant B – 1 ml/vial. Store at 2-8°C.

#### 8.4 Wash Buffer

Store at 2-8°C.

### 9.0 PROCEDURE

**Note:** Do not use reagents that are contaminated or have exposed growth.

**Note:** hCG concentrations in serum are not corrected for the basal level.

### 10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of hCG for an unknown.

1. Record the RLUs obtained from the printout of the microplate luminometer as indicated in Example 1.
2. Plot the light intensity for each duplicate serum reference on a linear graph paper.
3. Draw the early dose curve through the plotted points.
4. To determine the concentration of hCG for an unknown, locate the value(s) in the low range that is closest to the unknown intersects the calibration curve at (216.8 RLU) hCG concentration (see Figure 1).

**Note:** Computer data reduction software designed designed for CLIA assays may also be used for the data reduction. If such software is utilized, the validated value of the software should be ascertained. Duplicates of the unknown may be averaged as indicated (see Figure 1).

### EXAMPLE 1

<table>
<thead>
<tr>
<th>Sample LD.</th>
<th>Well Number</th>
<th>RLU (A)</th>
<th>Mean RLU (B)</th>
<th>Value (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>A1</td>
<td>57</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>Cal B</td>
<td>C1</td>
<td>631</td>
<td>618</td>
<td>5</td>
</tr>
<tr>
<td>Cal C</td>
<td>E1</td>
<td>3771</td>
<td>3701</td>
<td>25</td>
</tr>
<tr>
<td>Cal D</td>
<td>G1</td>
<td>15763</td>
<td>15563</td>
<td>100</td>
</tr>
<tr>
<td>Cal E</td>
<td>A2</td>
<td>38334</td>
<td>38201</td>
<td>250</td>
</tr>
<tr>
<td>Cal F</td>
<td>C2</td>
<td>99441</td>
<td>100000</td>
<td>1000</td>
</tr>
<tr>
<td>Patient</td>
<td>E2</td>
<td>33537</td>
<td>F2</td>
<td>32850</td>
</tr>
</tbody>
</table>

**Note:** Test procedure should be performed by a skilled individual or trained professional.
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.

2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

3. Highly lipemic, hemolyzed or grossly contaminated specimens should not be tested.

4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.

5. Pipette reagents sequentially in triplicate to ensure that the signal reagent initiates a kinetic reaction, therefore the signal reagent should be added in the same sequence to eliminate any time-deviation during reaction.

6. Failure to remove adhering solution adequately in the washers and/or the automated instruments used may cause erroneous results. Heterophilic antibodies may produce false positive results.

7. False positive results can be seen when assays are performed on rare serum specimens and test reagents can cause erroneous results. Heterophile antibodies may cause these interactions and have been known to be problems for all kinds of immunoassays. (Bowes LM, Smith MC. Heterophile Antibodies: a problem for all immunoassays' Clin.Chem. 1988;34:27-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.

8. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

9. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

10. 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 tissue and length of gestation.

11. A hCG value alone is not of diagnostic value and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

12. A hCG value of 100,000 RLU's for the F calibrator is prepared with each assay. In addition, the RLU's of the calibrators should not be normalized to 100,000 RLU's for the F calibrator prepared with each assay. The data presented in Example 2 and Figure 2 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay. In addition, the RLU's of the calibrators have been normalized to 100,000 RLU's for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

13. The expected ranges for the hCG-XR AccuLite® CLIA test system with a population indigenous to the area in which the laboratory is located.

14.1 Precision

The hCG-XR AccuLite® CLIA test system was determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 3.

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Within Assay Precision (Values in mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>N</td>
</tr>
<tr>
<td>Level 1</td>
<td>24</td>
</tr>
<tr>
<td>Level 2</td>
<td>24</td>
</tr>
<tr>
<td>Level 3</td>
<td>24</td>
</tr>
</tbody>
</table>

14.2 Sensitivity

The hCG-XR AccuLite® CLIA test system has a specificity of 0.152mIU/ml, which is equivalent to 0.0054ml/l well. The sensitivity (detection limit) was ascertained by determining the variability of the 0ml/l serum calibrator and using the 2σ (95% certainty) standard to calculate the minimum dose.

14.3 Accuracy

The hCG-XR AccuLite® CLIA test system was compared with a reference enzyme immunoassay. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 68. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Method</th>
<th>Mean</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>This Method</td>
<td>Reference 30.2</td>
<td>y = -1.620 + 0.986 (x)</td>
<td>0.954</td>
<td></td>
</tr>
</tbody>
</table>

Only slight amounts of bias were found between this procedure 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.

14.4 Specificity

The hCG-XR AccuLite® CLIA test system with a population indigenous to the area in which the laboratory is located.

15.0 REFERENCES


