1.0 INTRODUCTION

Intended Use: The Visual Qualitative or Spectrophotometric determination of hCG in Human Serum or Urine by a Microplate Immunoassay method for early detection of pregnancy.

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 placental tissue, beginning with the primitive trophoblast, almost from the time of implantation, and serves to support the corpus luteum during the early weeks of pregnancy. hCG or similar glycoproteins can also be produced by a wide variety of trophoblastic and nontrophoblastic 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.

3.0 PRINCIPLE

Sandwich Equilibrium ELISA Method (Type 2): 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 between the two antibodies. Excess enzyme and sample is removed via a wash step. The interaction is illustrated by the following equation:

$$\text{EnzAb(p)} + \text{AghCG} + \text{Ab(m)} \rightarrow \text{EnzAb(hCG)} \rightarrow \text{EnzAb(p)} \rightarrow \text{EnzAb(hCG)} \rightarrow \text{EnzAb(p)}$$

Ab(p) = Anti-hCG (MoAb) (On the Microwells in Excess Quantity)

Ab = Native Antigen (Variable Quantity)

EnzAb(hCG) = Enzyme labeled Goat α hCG (P) (Excess Quantity)

ka = Rate Constant of Association

k-d = 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 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.

4.0 REAGENTS

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

B. Anti-hCG Enzyme Conjugate – 13 ml/vial
One (1) vial, containing enzyme labeled affinity purified Goat Anti-hCG (IgG) in buffer, dye, and preservative. Store at 2-8°C.

C. Anti-hCG Coated Microplate – 96 wells
One 96-well microplate coated with Anti-hCG (MoAb-IgG) and enzyme present on the surface of the well is quantitated by a spectrophotometer (Exposure wavelength of 620-630 nm to minimize well haze) followed by a wash step. 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.

5.0 PRECAUTIONS

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 human serum products must be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biohazard in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

6.0 SPECIMEN COLLECTION AND PREPARATION

Serum Sample:
The specimens shall be blood, serum, and urine 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 should not be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. For serum samples centrifuge the specimen to separate the serum or plasma from the cells.

Urine Sample:
Collect urine 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 2-8°C for up to 15 days. Allow them to warm to room temperature before assay. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05 ml of the specimen is required.

7.0 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. Quality control charts should be maintained to follow the performance of the supplied reagents. Performance statistical methods should be employed to ascertain trends. Significant deviation from 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.

8.0 REAGENT PREPARATION AND STORAGE:

Wash Buffer:
Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

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

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.

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

9.0 TEST PROCEDURE

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

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

1. Format the microplate wells for each serum reference, control and 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 gently 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 (tap and blot) or aspirate. Repeat step (2) 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.

7. Add 100µl of substrate solution to all the wells.

8. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for 5 minutes.

10. For Qualitative results see Interpretation of Results Section.

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

12. The essential control solution to each well and gently mix.

13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader.

Note: The results should be read within thirty (30) minutes of adding the stop solution.

10.0 INTERPRETATION OF RESULTS

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 to an appropriate control, B (25mIU/ml).

3. If the color in the sample well is less than the color in the Calibrator B well the sample should be considered Negative because it falls within the normal range.

4. If the color in the sample well is more than the color in the Calibrator B well the sample should be considered Positive because it has hCG concentration > 25 mIU/ml at the time the sample was taken.

5. If the color in the sample well is more than the color in the Calibrator B well the sample should be considered Positive because it has hCG concentration > 25 mIU/ml at the time the sample was taken.
It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons depends upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined. The analysis using the method with a population indigenous to the area in which the laboratory is located.

### 12.0 RISK ANALYSIS

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

### 12.1 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should be interpreted in the context of the patient's medical picture.
3. For valid test results, adequate controls and other parameters must be followed till the test results and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are not interpreted correctly, Monobind shall have no liability.
5. Control data is an essential tool to follow the results of the test. It is imperative that the predicted values for the calibrators fall within ±10% of the assigned or expected values.
6. False positive results may occur in the presence of a wide variety of trophoblastic and non-trophoblastic tumors that secrete hCG. Therefore, the possibility of an hCG secreting neoplasm should be eliminated prior to diagnosing pregnancy.
7. 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.
8. Spontaneous micro abruptions and ectopic pregnancies will tend to interfere less than expected during pregnancy while somewhat higher values are often seen in multiple pregnancies (4, 5, 6).
9. 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).

### 12.2 Sensitivity

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

### 12.3 Accuracy

The Accubind™ Rapid hCG ELISA Microplate Test System was compared with a predicate Elisa immunoassay. Biological specimens from normal and pregnant populations 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.

### 12.4 Specificity

The Accubind™ Rapid hCG ELISA Microplate Test System's specificity is 100%.

### 12.5 Cross-reactivity

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 determining a ratio between dose of interfering substance to dose of Chorionic Gonadotropin needed to produce the same absorbance.

#### Table 3

Within Assay Precision (Values in mIU/ml)

<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>
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</table>

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

#### Table 4

Between Assay Precision* (Values in mIU/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
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<td>10</td>
<td>6.1</td>
<td>0.52</td>
<td>8.6%</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>22.3</td>
<td>1.63</td>
<td>7.3%</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>85.1</td>
<td>6.17</td>
<td>7.3%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

#### Table 2

Expected Values for hCG levels (3rd trimester 57/537) during normal pregnancy (in mIU/ml)

<table>
<thead>
<tr>
<th>Week</th>
<th>Range</th>
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<tbody>
<tr>
<td>1st week</td>
<td>10</td>
</tr>
<tr>
<td>2nd week</td>
<td>30</td>
</tr>
<tr>
<td>3rd week</td>
<td>100</td>
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<tr>
<td>4th week</td>
<td>1000</td>
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<tr>
<td>2nd &amp; 3rd month</td>
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### 15.0 REFERENCES


### Revision: 3

Date: 860712  DCO & 0653  Cat #: 3225-30

<table>
<thead>
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<th>Method</th>
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<th>Correlation Coefficient</th>
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<tr>
<td>Accubind</td>
<td>25.67 y = 0.1031x+0.9474x</td>
<td>0.956</td>
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</tr>
</tbody>
</table>

**For Orders and Inquiries, please contact**

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