1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Chorionic Gonadotropin (hCG) Concentration in Human Serum by a Microplate Immunoenzymometric assay

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 hCG similar glycoproteins can also be produced by a wide variety of trophoblastic and non-trophoblastic 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.

According to the literature, hCG is detectable as early as 10 days after ovulation, reaching 100 mIU/mL by the first missed period. At the time for the next ovulation, the hCG level is 200 mIU/mL (approximately 28 days after conception) (1). A peak of 50,000 or even 100,000 mIU/mL will occur by the third month, then a gradual decline is observed (2, 3).

In this method, hCG calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of hCG) are added and the reaction is mixed. Interaction between the various hCG antibodies and native hCG forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-chlorionic gonadotropin antibody bound conjugate is separated from the unbound enzyme-chlorionic gonadotropin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known chorionic gonadotropin levels helps to establish standards that are suitable for the ELISA curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with chorionic gonadotropin concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-hCG antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and the antigen, the reaction results between the native antigen and the antibodies or competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:

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

where:

- EnzAb is Biotinylated Monoclonal Antibody (Excess Quantity)
- AghCG is Native Antigen (Variable Quantity)
- BtnAb is Enzyme labeled Antibody (Excess Quantity)

4.0 REAGENTS

Materials Provided:

A. hCG Calibrators – 1 ml-vials - Iicons A-F
   Six (6) vials of references for hCG Antigen at levels of (A), (B), (C), (D), (E), and (F).
   Store at 2-8°C.

B. hCG Enzyme Reagent – 13 ml-vial - Icon (C)
   One (1) vial containing a surfactant in buffered saline. A preservative has been added.
   Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells - Icon (D)
   One (1) vial containing tetramethylbenzidine (TMB) in buffered saline.
   Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml - Icon (E)
   One (1) vial containing tetramethylbenzidine (TMB) in buffered saline.
   Store at 2-8°C.

E. Streptavidin Coated Plate – 96 wells - Icon (F)
   One (1) bottle containing tetramethylbenzidine (TMB) in buffer.
   Store at 2-8°C.

F. Substrate B – 8ml-vial - Icon (G)
   One (1) bottle containing hydrogen peroxide (H2O2) in buffer.
   Store at 2-8°C.

G. Stop Solution – 8ml-vial - Icon (H)
   One (1) bottle containing a strong acid (1N HCl).
   Store at 2-30°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light.
Note 3: Stable for sixty (60) days when stored at 2-8°C and 25°C.

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 should 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, “ Biosafety in Microbiological and Biomedical Laboratories.” 2nd Edition, 1988, HHS Publication no. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values and fastings may be necessary.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. Calibration of the controls is based on regulatory and statutory requirement.

8.0 REAGENT PREPARATION:

1. Wash Buffer
   Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container.

2. Working Substrate Solution
   Pour the contents of the amber vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly.

9.0 TEST PROCEDURE

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

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

1. Prepare the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

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

3. Add 0.100 ml (100µl) of hCG-Enzyme Reagent to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

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

7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.

8. Discard all wash buffer and gently mix for 20-30 seconds.

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050 ml (50µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well curvature of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with chorionic gonadotropin concentration.

12. Qualitative results should be read within thirty (30) minutes of adding the stop solution.

13. Quantitative results should be read with the plate at room temperature.

14. Stop all reactions by adding 1N HCl to each well.

15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well curvature of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with chorionic gonadotropin concentration.

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

17. Reference Materials:

- Human chorionic gonadotropin (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.
- The measurement of hCG by various assay systems with suitable sensitivity and specificity has proven great value in the detection of 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 missed period. At the time for the next ovulation, the hCG level is 200 mIU/mL (approximately 28 days after conception) (1). A peak of 50,000 or even 100,000 mIU/mL will occur by the third month, then a gradual decline is observed (2, 3).
- In this method, hCG calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of hCG) are added and the reaction is mixed. Interaction between the various hCG antibodies and native hCG forms a sandwich complex that binds with the streptavidin coated to the well.
- After the completion of the required incubation period, the enzyme-chlorionic gonadotropin antibody bound conjugate is separated from the unbound enzyme-chlorionic gonadotropin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.
- The employment of several serum references of known chorionic gonadotropin levels helps to establish standards that are suitable for the ELISA curve of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with chorionic gonadotropin concentration.

18. Measurement of hCG Concentration:

- The results should be recorded within 30 minutes of adding the stop solution.

19. Note 1: Do not use the working substrate if it looks blue.
- Note 2: Do not use reagents that are contaminated or have bacterial growth.
10.1 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 ‘F’ should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

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

12.1 Assay Performance

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 specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

12.2 Interpretation
1. Measurement and interpretation of results must be performed by a skilled individual or trained professional. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
2. For valid test results, adequate controls and other parameters must be within the listed ranges and assay reagents from different batches.
3. 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, the assay will have no validity.
4. 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.
5. 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 neoplasm should be eliminated prior to diagnosing pregnancy.
6. Also, false positive results may be seen when assessing specimens from individuals taking the drugs Pergonal® and Clomid®. Additionally, Pergonal will often be followed with an injection of hCG.
7. Spontaneous microabsorbtions 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 (5, 6, 7).
8. 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).
9. A hCG value alone is not of diagnostic value which is determined by the clinical state of the patient.
10. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the hCG AccuBind™ ELISA were determined by analyses on three different levels of control sera. The number (n), mean (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 3 and Table 4.

14.2 Sensitivity

The hCG AccuBind™ ELISA test system has a sensitivity of 0.003 mIU/Well. This is equivalent to a sample containing 0.102 mIU/ml hCG concentration. The analytical sensitivity (detection limit) was ascertained by determining the variability of the ‘0 level’ calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This hCG AccuBind™ ELISA test system was compared with a reference radioimmunoassay. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 110. 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 below.

15.0 REFERENCES


Revision: 3 Date: 061112 DCO: 0640