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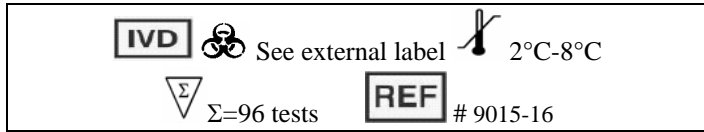
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## **CHEMILUMINESCENCE ENZYME IMMUNOASSAY (CLIA) HUMAN CHORIONIC GONADOTROPIN (HCG)**

### **INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY**

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the *MPL2* microplate luminometer from Berthold Detection System, *Lus2* microplate luminometer from Anthos, *Centro LB960* microplate luminometer from Berthold Technologies, and *Platelumino* from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

### **Introduction of hCG Immunoassay**

Human chorionic gonadotropin (hCG) is a glycoprotein hormone normally produced by the placenta during pregnancy. The hCG molecule consists of two combined, dissimilar subunits designated alpha and beta. The beta subunit, with a molecular weight of approximately 30,000 daltons, confers biological and immunological specificity to the entire hCG molecule by virtue of its unique amino acid sequence and content. The alpha subunit, with a molecular weight of approximately 18,000 daltons, is essentially identical to the alpha subunit of the pituitary glycoprotein hormones; luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH).

The appearance of hCG in urine or serum soon after conception and its rapid rise in concentration makes it an ideal indicator for the detection and confirmation of pregnancy. However, elevated hCG levels are also frequently associated with trophoblastic and non trophoblastic neoplasmas; these conditions should be considered before a diagnosis of pregnancy can be made.

Immunoassays utilizing antibodies specific to the beta subunit of hCG provide a sensitive and specific technique allowing early detection of pregnancy around the time of the first missed menstrual period.

In women with a multiple pregnancy (twins, triplets, etc.), levels of hCG have been reported to be higher than those expected during a normal single pregnancy. This is probably the result of the increased placental mass necessary to sustain multiple fetuses. Also, as one might suspect, cases of placental insufficiency show levels of hCG lower than those expected during normal pregnancy. Decreased values have also been associated with threatened abortion and ectopic pregnancy.

The hCG EIA test provides a rapid, sensitive and reliable assay. The antibodies developed for the test will determine a minimal concentration of 2 mIU/ml.

### **Principle of the test**

The hCG Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-hCG antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-hCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the hCG antibody coated microtiterwells and incubated. If hCG is present in the specimen, it will combine with the antibody on the well, and then hCG antibody labeled with horseradish peroxidase (conjugate) are added. The conjugate will bind immunologically to the hCG on the well, resulting in the hCG molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a incubation at room temperature, the wells are washed with wash buffer to remove unbound labeled antibodies.

A solution of chemiluminescent substrate is added and then read relative light units (RLU) in the appropriate

Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of hCG in the sample. By reference to a series of hCG standards assayed in the same way, the concentration of hCG in the unknown sample is quantified.

**Materials and Components**

**Materials provided with the test kit:**

1. Antibody-coated microtiter wells. 96 wells per bag.
2. Reference standard set, contains 0, 5.0 , 20, 50, 150, and 300 mIU/ml hCG, liquid.
3. Zero buffer, 12 ml
4. Enzyme Conjugate Reagent, 18 ml.
5. 20x Wash Buffer, 30 ml
6. Chemiluminescence Reagent A, 6.0 ml.
7. Chemiluminescence Reagent B, 6.0 ml.

**Materials required but not provided:**

- Precision pipettes: 50µl~200µl.
- Disposable pipette tips.
- Distilled water.
- Glass tubes or flasks to mix Chemiluminescence Reagent A and Chemiluminescence Reagent B.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate Luminometer

**Specimen Collection and Preparation**

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

**Storage of Test Kit and Instrumentation**

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

**Reagent Preparation**

1. All reagents should be allowed to reach room temperature (18-25°C ) before use.
2. To prepare substrate solution, make an 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
3. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

**Assay procedures**

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of hCG Zero Buffer into each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 30 minutes.

6. Remove the incubation mixture by flicking plate content into a sink.
7. Rinse and flick the microtiter wells 5 times with running tap or distilled water.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 30 minutes.
11. Remove the incubation mixture by flicking plate contents into sink.
12. Rinse and flick the microtiter wells 5 times with running tap or distilled water.
13. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
15. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

**Important Note:**

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

**Calculation of Results**

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend use proper software to calculate the results. The best curve fitting used in the assays are quadratic regression or 4-parameter regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against hCG concentration in mIU/ml on linear graph paper, with RLU on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of hCG in mIU/ml from the standard curve.

**Example of Standard Curve**

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

hCG (mIU/ml)	RLU (10 <sup>4</sup> )
0	0.0
5	1.0
20	7.5
50	20.0
150	59.3
300	98.0

**Sensitivity**

Each laboratory must establish its own normal ranges based on patient population. hCG is not normally detected in the serum of healthy men or healthy non-pregnant women. The concentration of hCG in the serum of pregnant women

increases to 5-50 mIU/ml one week after implantation and continues increasing exponentially during the first ten weeks, reaching a maximum of 100,000-200,000 mIU/ml at the end of the first trimester. The minimum detectable concentration of hCG by this assay is estimated to be 2.0 mIU/ml.

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Revision Date: 3/14/06