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 Goat Anti-hCG antibody 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:

$$\text{Ab}_{hCG} + \text{MoAb} + \frac{K_a}{K_a-a} \frac{\text{Ab}_{hCG} \cdot \text{MoAb}}{\text{Ab}_{hCG} \cdot \text{MoAb}}$$

Ab$_{hCG}$ = Anti-hCG (MoAb) (On the Microwells in Excess Quantity)
Ab$_{hCG}$ = Native Antigen (Variable Quantity)
K$_a$ = Rate Constant of Association
K$_a$-a = 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 levels, 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 through a microplate spectrophotometer to obtain semi-quantitative results.

4.0 REAGENTS

Materials Provided:
- hCG Calibrators – 1ml/vial - Icons A-E (Lyophilized) (A-E) Five (5) vials, of references for hCG Antigen at levels of 0(A), 25(B), 50(C), 100(D) and 250(E) mIU/mL. Store at 2-8°C. Reconstitute each vial with 1.0ml of distilled or deionized water.

Note: The calibrators, human serum based, were calibrated and the calibrator reference assay was the assay targeted by the WHO 3rd IS (75/037).

- Anti-hCG Enzyme Conjugate – 13 ml/vial
- Anti-hCG Coated Microplate – 96 wells.
- Wash Solution Concentrate – 20 ml - Icon
- Wash Solution Concentrate – 20ml - Icon
- Wash Solution Concentrate – 20ml - Icon

Water:
- hCG Assay Buffer, pH 7.2
- Water
- Distilled or Deionized Water

4.1 Required But Not Provided:
- A precision better than 1.5%.
- Plastic wrap or microplate cover for incubation steps.
- Microplate Reader with 450nm and 620nm wavelength absorbance capability. (For Semi-Semi-quantitative interpretation Only)
- Absorbent Paper for blotting the microplate wells.
- Timer.
- Quality control materials.
- Urine collection containers.

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 Virus, 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 practice and the handling of 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) 88-6385.

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

6.0 SPECIMEN COLLECTION AND PREPARATION

Serum Sample:
- The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. 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 qualitative results it is advisable to collect first morning urine sample.

Samples may be refrigerated at 2-8°C for a maximum period of 7 days. Do not freeze. If stored frozen, let thaw slowly at room temperature before use. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05 ml of the specimen is required.

6.1 INTERPRETATION OF RESULTS

Semi-quantitative results can be obtained by visual inspection or by photometric reading of the test. For Semi-quantitative results go to step 11 below.

For quantitative results based on the blue color reading before stopping the reaction with stop solution. See ‘Interpretation of Results’.

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

10.0 INTERPRETATION OF RESULTS

Qualitative Results:
- 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 the stop solution.
- Do not change the blue color, in the sample well, to the color in the calibrator A well. Calibrator A is used to determine the results.
- Be sure to add the stop solution to all wells.

Note 1: Do not use reagents that are contaminated or have bacteria growth.
10. For semi-quantitative determination the patient specimens with hCG concentrations above 250 mIU/ml may be diluted with normal male urine (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.

11. Accurate and precise pipetting, as well as following the exact protocol as outlined in Example 1.

12. Plot the absorbance for each serum reference versus the corresponding hCG concentration in mIU/ml on linear graph paper.