between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. This interaction is illustrated by the following equation:

\[
\text{EnzAb} \cdot \text{Ab(hCG)} = \text{Enzyme labeled Antibody} \cdot \text{Antigen (Excess Quantity)}
\]

**Additional Materials (Not Provided):**
- Pipette capable of delivering 50µl volumes with a precision of better than 1.5%.
- Microplate washer can be used. Follow the manufacturer's instructions for proper usage. If a squeeze bottle is used, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.
- A dose response curve is used to ascertain the concentration of hCG in unknown specimens.

**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. "Biologicals in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC)88-8395.

**SPECIMEN COLLECTION AND PREPARATION**

The specimens shall be blood, serum in tube and 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 be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

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 samples may be stored at temperatures of -20°C for up to 30 days.

A dose response curve is used to determine the reason for the variations.

**RESULTS**

A dose response curve is used to ascertain the concentration of hCG in unknown specimens.

1. Record the RLUs obtained from the printout of the microplate luminometer as outlined in Example 1.
2. Plot the light intensity for each duplicate serum referenceversus the corresponding hCG concentration in mIU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. Determine the concentration of hCG for an unknown, locate the average RLU’s of the unknown on the horizontal axis of the graph and read the corresponding hCG concentration in mIU/ml from the vertical axis of the graph. (See Figure 1).

**QUALITY CONTROL**

Each laboratory should assay controls at levels in the low, medium and high levels for monitoring assay performance. These controls should not 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. Pertain 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.

**REAGENT PREPARATION**

A. hCG Calibrators -- 1 ml/vial - Icons A-F

Six (6) vials of references for hCG Antigen at levels of 0(A), 5(B), 25(C), 50(D), 100(E) and 250(F) mIU/ml. Store at 2-8°C. A preservative has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd (75/537).

B. hCG Tracer Reagent -- 13 mlvial - Icons A

One (1) vial containing enzyme labeled affinity purified antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Light Reaction Wells -- 96 wells - Icon

One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C. (Note: if not used within 36 hours after opening, mix reagents freshly and freeze until next use.)

D. Wash Solution Concentrate -- 20 ml - Icon

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at -20°C.

E. Signal Reagent A --7mlvial - Icon S4

One (1) bottle containing luminol in buffer. Store at 2-8°C.

F. Signal Reagent B -- 7mlvial - Icon S4

One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at -20°C.

**PRODUCT INSTRUCTIONS**

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

Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.

Note 3: Above reagents are for a single 96-well microplate assay.
LIMITATIONS OF PROCEDURE

A. Assay Performance

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Failure to remove adhering solution adequately in the aspiration or decantation wash steps may result in poor reproducibility and spurious results.
5. Use components from the same lot. No intermixing of reagents from different batches.

Substance Cross Reactivity Concentration
Follitropin (hFSH) < 0.0001 1000ng/ml Lutropin Hormone (hLH) < 0.0001 1000ng/ml Thyrotropin (hTSH) < 0.0001 1000ng/ml

B. Interpretation

1. 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.
2. 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 neoplasia should be eliminated prior to diagnosing pregnancy.
3. 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.
4. Spontaneous microabortions 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 (4, 5, 6).
5. 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).

*Pelogal is a registered trademark of Serono Laboratories, Inc.
**Clomid is a registered trademark of Merrell-National Laboratories

EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the hCG AccuLite™ CLIA method. The mean (X) values, standard deviations (σ) and expected ranges (±2σ D) are presented in Table 1.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Expected Values for the hCG AccuLite™ CLIA (in mIU/ml) (3rd IS 75/537)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>50</td>
</tr>
<tr>
<td>Mean (X)</td>
<td>2.3</td>
</tr>
<tr>
<td>Standard Deviation (σ)</td>
<td>1.6</td>
</tr>
<tr>
<td>Expected Ranges (±2σ)</td>
<td>0.01 - 5.5</td>
</tr>
</tbody>
</table>

Only slight amounts of bias between this procedure and the reference method were found, the least square regression equation and correlation coefficient indicates excellent method agreement.

C. Sensitivity

The sensitivity (detection limit) was ascertained by determining the within and between assay precision of the hCG AccuLite™ CLIA assay were 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 and Table 4.

TABLE 3 | Within Assay Precision (Values in mIU/ml) |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>N</td>
</tr>
<tr>
<td>Level 1</td>
<td>20</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

TABLE 4 | Between Assay Precision (Values in mIU/ml) |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>N</td>
</tr>
<tr>
<td>Level 1</td>
<td>10</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
</tr>
</tbody>
</table>

REFERENCES


FIGURE 1

The data presented in Example 1 and Figure 1 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 calibration (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the hCG AccuLite™ CLIA method. The mean (X) values, standard deviations (σ) and expected ranges (±2σ D) are presented in Table 1.

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</tr>
</tbody>
</table>

Expected levels for hCG during normal pregnancy (3) are listed in Table 2.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Expected Values for hCG levels (3rd IS 75/537) during normal pregnancy (in mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td>30 - 30</td>
</tr>
<tr>
<td>2nd week</td>
<td>30 - 100</td>
</tr>
<tr>
<td>3rd week</td>
<td>100 - 1000</td>
</tr>
<tr>
<td>4th week</td>
<td>1,000 - 1,000</td>
</tr>
<tr>
<td>5th &amp; 6th month</td>
<td>30,000 - 100,000</td>
</tr>
<tr>
<td>1st trimester</td>
<td>10,000 - 30,000</td>
</tr>
<tr>
<td>2nd trimester</td>
<td>5,000 - 15,000</td>
</tr>
</tbody>
</table>

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 is dependent 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 by the analyst using the method with a population indigenous to the area in which the laboratory is located.

D. Specificity

The cross-reactivity of this method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriv- ing a ratio between dose of interfering substance to dose of Chorionic Gonadotropin needed to produce the same light intensity.

Substance Cross Reactivity Concentration
Chorionic Gonadotropin (hCG) 1.0000 100,000ng/ml Follitropin (FSH) < 0.0001 100,000ng/ml Lutropin Hormone (LH) < 0.0001 100,000ng/ml Thyrotropin (hTSH) < 0.0001 100,000ng/ml

REFERENCES