Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the equilibrium equation:

\[
\frac{k_a}{k_a} = \text{Rate Constant of Association} \quad \frac{k_a}{k_a} = \text{Rate Constant of Dissociation} \quad K = \frac{k_a}{k_a} = \text{Equilibrium Constant}
\]

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effect separates the antibody-bound fraction after decantation or aspiration.

\[
\text{AbBtn} = \text{Biotinylated Antibody (Constant Quantity)}
\]

\[
\text{Ag} = \text{Native Antigen (Variable Quantity)}
\]

\[
\text{EnzAgAbBtn} = \text{Enzyme-antigen Conjugate (Constant Quantity)}
\]

\[
\text{EnzAgAbBtn} + \text{Streptavidin-dv} \rightarrow \text{immobilized complex}
\]

\[
\text{Streptavidin-dv} = \text{Streptavidin immobilized on well}
\]

\[
\text{immobilized complex} + \text{sandwich complex bound to the solid surface}
\]

The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Progesterone Calibrators – 1ml/vial - Icon A-G

Seven (7) vials of serum reference for progesterone at concentrations of 0.01 (A), 0.3 (B), 2.0 (C), 5.0 (D), 15.0 (E), 30.0 (F) and 60.0 (G) ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nmol) by multiplying by 3.18. For example: 1ng/ml x 3.18 = 3.18 nmol.

B. Progesterone Enzyme Reagent – 6.0 ml/vial

One (1) vial of Progesterone (Analog)-horseradish peroxidase (HRP) conjugate in a protein stabilizing matrix with red dye. Store at 2-8°C.

C. Progesterone Biotin Reagent – 6.0 ml - Icon

One (1) vial containing anti-Progesterone biotinylated purified rabbit IgG conjugate in buffer, yellow dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells –Icon

One 96-well microwell coated with 1.0 µg/ml streptavidin and preserved in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml – Icon

One (1) bottle contains a surfactant in buffered saline. A preservative is added. The reagent is stable for twenty (20) days. Store at 2-8°C.

F. Substrate Reagent – 12ml/vial - Icon S

One (1) bottle contains tetramethylbenzidine (TMB) and hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon S

One (1) vial contains a strong acid (H2SO4). Store at 2-30°C.

Product Instructions

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

Note 2: Avoid external 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 microwell. The specimens shall be blood, serum or heparinised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to the dose response curve, an unknown specimen’s activity can be correlated with progesterone concentration.

5.0 PRECAUTIONS

For In Vivo Diagnostic Use

Note: For in vitro use, follow the in vitro use instructions in the product information.

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 required tests. Since no known test can offer complete assurance that some rare disease is absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products are outlined in the Centers for Disease Control / National Institute of Health, “Biosafety in Microbiological and Biomedical Laboratories,” 2nd Edition, 1988, HHS Publication No. (CDC) 88-8385. Always add reagents in the same order and in the same quantity.

Saliva of the patient may contaminate the specimen. If saliva is observed, the specimen should be discarded and a new specimen collected.

6.0 SPECIMEN COLLECTION AND PREPARATION

Note: Dilute the samples suspected of concentrations higher than 60.0 ng/ml with 1:10 with progesterone 0.01 ng/ml calibrator or male patient serum pools with a known low value for progesterone.

10.0 CALCULATION OF RESULTS

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

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference value versus the corresponding progesterone concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Connect the points with a best-fit curve.

4. To determine the concentration of progesterone for an unknown, locate the average absorbance of the duplicates for each unknown on the y-axis of the graph, find the corresponding dose response curve, and read the absorbance value from the absorbance graph for the corresponding unknown.

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.
TABLE 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>10</td>
<td>0.70</td>
<td>8.9%</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
<td>11.1</td>
<td>3.44</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>60.5</td>
<td>1.155</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The progesterone AccuBind™ Microplate EIA Test System has a sensitivity of 0.105 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The progesterone AccuBind™ Microplate ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and high progesterone level populations were used (The values ranged from <0.15 ng/ml – 128 ng/ml). The total number of such samples was 60. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

14.59 References


11.0. Q.C. PARAMETERS

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

1. The absorbance (O.D.) of calibrator 0 ng/ml 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 upon 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. Pippetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolized or grossly contaminated specimens should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose reconstruction.

12.2 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 not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges 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 incorrectly interpreted, Monobind shall have no liability.
5. 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 with 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.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a “normal” adult population and females during gestation the expected ranges for the progesterone AccuBind™ ELISA Test System are detailed in Table 1. During pregnancy the progesterone serum levels rise quickly till the end of third trimester(11).

10.59 Table 2

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>100.0</td>
</tr>
<tr>
<td>17OH-Progesterone</td>
<td>0.375</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.158</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.014</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>0.347</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.005</td>
</tr>
<tr>
<td>Danazol</td>
<td>0.003</td>
</tr>
<tr>
<td>DHEA sulfate</td>
<td>0.007</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.004</td>
</tr>
<tr>
<td>Estriol</td>
<td>0.003</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.002</td>
</tr>
<tr>
<td>Prednisone</td>
<td>0.023</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.015</td>
</tr>
</tbody>
</table>

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the progesterone AccuBind™ Microplate EIA System were determined by analyses on three different levels within 72 control sera presented in Table 2 and Table 3.

15.0 REFERENCES