3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immunized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place in the assay surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-LH antibody. Upon mixing microplate biotinylated antibody, the enzyme labeled antigen antibody and a second antibody, the native antigen and antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
\text{EnzAb(p)-AgLH-BtnAb(m)} = \text{Antigen-Antibodies Sandwich Complex}
\]

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

\[
\text{EnzAb(p)} + \text{AgLH} + \text{BtnAb(m)} \rightarrow \text{EnzAb(p)-AgLH-BtnAb(m)}
\]

Enzyme Immunoassay, Colorimetric

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s), for repetitive deliveries of 0.100 and 0.350ml (100 and 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Wash solution, ethanol or isopropanol, 95% v/v.
5. Microplate reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Vacuum aspirator (optional) for wash steps.
8. Microplate cover for incubation steps.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

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 WHO 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 practices 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, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without anticoagulants or gel additives. Blood collected in tube should be centrifuged 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 sample(s) may be stored at temperatures of 20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 ml (150 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied controls. Each laboratory should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit contents. Each laboratory should use the results of the controls to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.

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. Store at 2-8°C.

Note 1: Do not use the working substrate if it looks blue. Note 2: Do not use reagents that are contaminated or have bacteria growth.

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. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips into the aluminum bag, seal and store at 2-8°C
2. Pipette 0.050ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of LH-Enzyme Reagent to all wells.
4. Seal 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 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) decant (lap and blot) and aspirate. Repeat this (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add 0.100 ml (100µl) 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. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. 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 imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of luteinizing hormone (LH) in unknown specimens. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

1. Plot the absorbance for each duplicate serum reference versus the corresponding LH concentration in µml/l on linear graph paper (do not average the duplicates of the serum references before plotting).
2. Draw a smooth curve through the plotted points.
3. To determine the concentration of LH for an unknown, locate the absorbance value closest to the concentration of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µml/l) from the horizontal axis. The concentration of the unknown may be averaged as indicated. In the following example, the average absorbance (1.005) intersects the dose response curve at 42.7 µml/l LH concentration (See Figure 1).

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.
5. The addition of substrate solution initiates a kinetic reaction.
4. If more than one plate is used, it is recommended to
3. Highly lipemic, hemolyzed or grossly contaminated

12.1 Assay Performance
*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.

11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of the 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.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may incur inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC

2. Four out of six quality control pools should be within the

Patient

11.2 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 determinations.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. Heterocholic antibodies: a problem for all immunoassays’ Clin Chem. 1998:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. 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.
6. 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.
7. LH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive diabetes and weight loss may lead to low gonadotropin concentrations.
8. Luteinizing hormone is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is insufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the LH AccuBind® ELISA Test System. The expected values are presented in Table 1.

Table 2: Within Assay Precision (Values in mIU/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>1.4</td>
<td>0.10</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>21.6</td>
<td>0.85</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>58.3</td>
<td>2.10</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the LH AccuBind® ELISA Test System were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

Table 3: Between Assay Precision* (Values in mIU/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>30</td>
<td>1.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Level 2</td>
<td>30</td>
<td>21.5</td>
<td>2.32</td>
</tr>
<tr>
<td>Level 3</td>
<td>30</td>
<td>55.4</td>
<td>3.34</td>
</tr>
</tbody>
</table>

The within and between assay precision of the LH AccuBind® ELISA Test System was determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

Table 4: Between Assay Precision* (Values in mIU/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
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<tr>
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<td>30</td>
<td>21.5</td>
<td>2.32</td>
</tr>
<tr>
<td>Level 3</td>
<td>30</td>
<td>55.4</td>
<td>3.34</td>
</tr>
</tbody>
</table>

14.2 Sensitivity

The LH AccuBind® ELISA Test System has a sensitivity of 0.030mIU/mL. This is equivalent to a sample containing 0.647 mIU/mL LH concentration. The analytical sensitivity (detection limit) was measured by determining the variability of the 0 mIU/mL calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This LH 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 LH ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

14.4 Specificity

The cross-reactivity of the LH AccuBind® ELISA Test System to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between the value of interfering substance to dose of Luteinizing Hormone needed to produce the same absorbance.

15.0 REFERENCES