EnzAb(p) - AgLH - BtnAb( m) suitable signal to produce light. A reaction with a conjugate by aspiration or decantation. The activity of the enzyme

Enzyme Immunoassay, Chemiluminescence

Intended Use: The Quantitative Determination of Luteinizing Hypothalamic - pituitary - gonadal axis has been well

B. LH Tracer Reagent — 13 ml/vial - Icon C. Kit and component stability are identified on the

4.0 REAGENTS

Materials Provided

- LH Calibrators – 1ml/vial - Icons A-F
- LH Calibrators – 1ml/vial - Icons A-F
- Cal A
- Cal B
- Cal C
- Cal D
- Cal E
- Cal F

LH Calibrators for LH are given at levels of 0(A), 25(B), 50(C), 250(D), 1000(E) and 20000(F) mIU/ml. Store at 2-30 °C.

A preservative has been added.

Note: LH calibrators, serum controls, or other serum based were calibrated using a reference preparation, which was assayed against the WHO 2nd IS (80/552).

1. Wash Buffer
2. Working Signal Reagent Solution - Store at 2 - 30 °C
3. Dilute contents of Wash Concentrate to 1000ml with distilled or
4. Dilute contents of Wash Concentrate to 1000ml with distilled or
5. Dilute contents of Wash Concentrate to 1000ml with distilled or

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 Surface Antigen, HIV 1&2 and HCV Antibodies. According to FDA guidelines, 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 Guide to Good Laboratory Practice of the 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 requirements.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual regulatory and statutory requirements. Safe Disposal of kit components must be according to local regulatory and statutory requirements.

DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION

8. Incubate at room temperature in the dark for five (5) minutes.

9. Read the relative light units in each well, minimum 0.5 – 1 second reproducibility of the specimens.

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

10. CALCULATION OF RESULTS

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

1. Plot the RLUs for each duplicate serum reference versus the corresponding LH concentration in mIU/ml on linear graph paper.

2. To determine the presence of LH in an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph.

In the following example, the reference (99818) should be used to calculate the concentration of the unknown (49871), its concentration should be calculated as 67.5 mIU/ml (See Figure 1).

Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8 °C.

2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of LH Tracer Reagent solution to all

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate the microplate in darkness for 4-6 additional hours.

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 (tap and blot) or aspirate. Repeat four (4) additional times with a well plate washer or a squeeze bottle. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Discard the wash and repeat the four (4) additional times.

8. Add 0.100 ml (100µl) of working signal reagent to all wells (see Reagent Preparation Section).

Add all reagents in the same order to minimize reaction time differences between wells.

Lab Reagent

Sample I.D. Well Number RLU (A) Mean RLU (B) Value (mIU/ml)

Cal A
Cal B
Cal C
Cal D
Cal E
Cal F

Ctrl A
Ctrl B

Patient

Example 1

Note: Use of 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. Pipette capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.

2. Dispense(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.

3. Microplate washer or a squeeze bottle (optional).

4. Vacuum aspirator (optional) for wash steps.

5. Time.
11.0 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.

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. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly hemolyzed, hemoglobin or grossly contaminated sample(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 signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the analyzer and decantation of wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential.
9. Any deviation from Monobind’s IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compatibility and proper device usage.
11. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results 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. The reagents for the test system 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 NC. "Heterophilic antibody and a problem for all immunoassays" Clin. Chem. 1988;34:273-33). For diagnostic purposes, the results from this assay should be 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. Exceptional eating 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 not sufficient 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 AccuLite® CLIA test system. The expected values are presented in Table 1.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Follicular phase</th>
<th>Midcycle</th>
<th>Luteal phase</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>0.5-10.5</td>
<td>14.5-36</td>
<td>16.4-10.5</td>
<td>8.2-40.8</td>
</tr>
<tr>
<td>Men</td>
<td>0.7-4.7</td>
<td></td>
<td></td>
<td></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 develop their own range.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the LH AccuLite® CLIA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (s) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>19.48</td>
<td>0.87</td>
<td>4.5%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>55.30</td>
<td>2.63</td>
<td>4.8%</td>
</tr>
</tbody>
</table>

15.0 REFERENCES


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

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>0.90</td>
<td>0.09</td>
<td>9.5%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>21.28</td>
<td>2.16</td>
<td>10.1%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>56.53</td>
<td>4.80</td>
<td>8.2%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>0.90</td>
<td>0.09</td>
<td>9.5%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>21.28</td>
<td>2.16</td>
<td>10.1%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0mIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. It was determined to be 0.010mIU/ml.

14.3 Accuracy

The LH AccuLite® CLIA test system was compared with a reference method. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 80. The least square regression equation and correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (x)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monobind Reference</td>
<td>13.8</td>
<td>y=0.081x+0.0175 (e)</td>
<td>0.991</td>
</tr>
</tbody>
</table>

Only slight amounts of bias between the LH AccuLite® CLIA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the LH AccuLite® CLIA 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 dose of interfering substance to dose of Luteinizing Hormone needed to produce the same light intensity.