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4.0 REAGENTS

Material Provided: A. Human Serum References – 1.0 ml/vial - Icon A-F

Six (6) vials of human serum based reference calibrators at concentrations of 0.0 (A), 5.0 (B), 25.0 (C), 125.0 (D), 500 (E) and 4000 (F) IU/ml. Store at -20°C. A preservative has been added. (The calibration curve is standardized against the WHO’s 2nd RP 75/052 for IgE).

B. IgE Biotin Reagent – 13ml/vial - Icon

One (1) vial of biotinylated mouse anti-human IgE reagent in a protein-stabilized matrix. A preservative has been added. Store at -20°C.

C. IgE Tracer Reagent – 13 ml/vial - Icon

One (1) vial of Anti-Human IgE-HRP conjugate in a protein-stabilized matrix. A preservative has been added. Store at -20°C.

D. Streptavidin 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.

E. Wash Solution Concentrate – 20ml - Icon

One (1) vial containing surfactant in buffer saline. A preservative has been added. Store at 2-8°C (see Reagent Preparation Section).

F. Signal Reagent A – 7ml/vial - Icon

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

G. Signal Reagent B – 7ml/vial - Icon

One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C (see Reagent Preparation Section).

H. Product Insert

1. INTRODUCTION

Intended Use: The Quantitative Determination of Immunoglobulin E (IgE) Concentration in Human Serum by a Microplate Chemiluminescence Immunoassay.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Allergic reactions, which are becoming more widespread, are usually diagnosed on the basis of medical history and clinical symptoms. In vitro and in vivo testing, however, play a key role in confirming clinical suspicions and tailored treatment. The measurement of immunoglobulin E (IgE) in serum is widely used in the diagnosis of allergic reactions and parasitic infections. Many allergies are caused by the immunoglobulins of subclass IgE acting as point of contact between the allergen and specialized cells. The IgE molecule is known to have 6 distinct epitope recognition. Subsequently the binding of allergens in cell-bound IgE causes, among the immune system to secrete various other vasoactive substances. The release of histamine by the body results initiates what is commonly known as an allergic reaction.

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IgE levels show a slow increase during childhood, reaching adult levels in the second decade of life. In general, the total IgE levels increase with the allergic process, being highest during the acute phase of the disease.

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

1. Record the RLU’s (Relative Light Units) obtained from the printout of the luminometer as outlined in Example 1.
2. Plot the RLU’s for each duplicate serum reference versus the corresponding IgE concentration in IU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.

To determine the concentration of IgE for 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 IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (50045) of the unknown intersects the calibration curve at (177 IU/ml) IgE concentration (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.

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 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 signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-depence during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation 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. 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.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
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. The laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, especially for results in agreement with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay results must be acceptable.
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. 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.
6. Serum IgE concentration is dependent upon a multiplicity of factors: including if the patient is sensitized, how many times the patient has been exposed to a specific allergen etc. Total IgE concentration alone is not sufficient to assess the clinical status. All the clinical findings especially specific allergy tests should be taken into consideration while determining the clinical status of the patient.
7. The report is not IgE mediated. All relevant clinical information should be taken into consideration before making any determination for patients who may be in the normal range.

13.0 EXPECTED RANGES OF VALUES

A study of population from different age groups was conducted to evaluate the Monobind IgE AccuLite™ CLIA procedure. The results are presented in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Age (Yrs)</th>
<th>Median Absolute Range</th>
<th>Lower Limit (IU/ml)</th>
<th>Upper Limit (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>31</td>
<td>6.4</td>
<td>ND* - 46</td>
</tr>
<tr>
<td>3-16</td>
<td>43</td>
<td>25.0</td>
<td>ND* - 80</td>
</tr>
<tr>
<td>Adult</td>
<td>145</td>
<td>43</td>
<td>0 - 290</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 analysts using the method within a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

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

**TABLE 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Level</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl 1</td>
<td>Level 1</td>
<td>78.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Ctrl 2</td>
<td>Level 2</td>
<td>189.1</td>
<td>6.6</td>
</tr>
<tr>
<td>Patient</td>
<td>Level 3</td>
<td>394.2</td>
<td>11.6</td>
</tr>
</tbody>
</table>

As measured in twelve experiments in duplicate.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the (mIU/ml) serum calibrator and using the 20% (95% certainty) statistic to calculate the minimum dose. It was determined to be 0.023 IU/ml.

14.3 Accuracy

The Monobind the IgE AccuLite™ CLIA method was compared with a predicate microplate Elisa method. Biological specimens with IgE levels in the low, medium and high ranges were used (The values ranged from 1 to 4500 IU/ml). The total number of such specimens was 156. The least square regression equation and the correlation coefficient were computed for this procedure in comparison with the predicate method (Table 4).

**TABLE 4**

<table>
<thead>
<tr>
<th>Method</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl 2</td>
<td>y = 0.87x + 0.987(x)</td>
<td>0.992</td>
</tr>
</tbody>
</table>

14.4 Specificity:

The specificity of IgE AccuLite™ CLIA method, to closely related immunoglobulins was evaluated by adding those at twice the physiological concentrations to a serum matrix. No cross-reaction between the antibodies used and the related molecules was detected.

15.0 REFERENCES