**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 tailoring 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 parts of an immune response to allergic stimuli. The IgE molecules (MW 220,000) bind to the surface of the mast cells and basophilic granulocytes. Subsequently, the binding of antigen to IgE on these cells results in histamine release and other vasoactive substances. The release of histamines in the body results in the symptoms of an allergic reaction.

Before making any therapeutic determination it is important, however, to know whether the allergic reaction is IgE mediated or non-IgE mediated. Measurement of total IgE levels in serum samples, along with other supporting diagnostic information, can help to make that determination. Measurement of total circulating IgE may also be of value in the early detection of allergy in children and as a means of predicting future atopic manifestations. Before deciding on any therapy it is important to take into consideration all the relevant clinical information as well as information supplied by specific allergy testing.

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 allergies a person has and the number of times of exposure to the relevant allergens. Significant elevations may be seen in the sensitized individuals, but also in cases of myeloma, pulmonary aspergillosis, and during the active stages of parasitic infections.

**PRINCIPLE**

Immunoenzymometric sequential assay:

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-IgE antibody. Upon exposure to monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an Antibody-Antigen complex. The interaction is illustrated by the following equation:

\[
\frac{A_{igE}}{k_a} + \frac{B_{tnAb(m)}}{k_b} \rightarrow \frac{A_{igE}B_{tnAb(m)}}{k_{a-b}}
\]

\[
A_{igE}B_{tnAb(m)} \rightarrow \text{Immobilized complex (IC)}
\]

After a suitable incubation period, the antibody-antigen bound fraction is separated by washing the excess antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce light measurable with the use of a microplate fluorometer. The enzyme activity on the well is directly proportional to the native antigen concentration. By utilizing several light source references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

**REAGENTS**

Provided:

A. Human Serum References -- 1.0 ml/vial - Icons A-F

Six (6) vials of human serum based reference calibrators at concentrations of 0 (A), 5 (B), 25 (C), 50 (D), 150 (E) and 400 (F) IU/ml. Store at 2-8°C. A preservative has been added. (The calibrators are stabilized against the WHO’s 2ndIRP 75/502 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 2-8°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 2-8°C.

**PRODUCT CODE:**

2575-300

**PRODUCT INFORMATION**

Immunoglobulin E (IgE) Concentration in Human Serum by a Microplate Chemiluminescence Immunoassay.

**TEST PROCEDURE**

Before proceeding with the assay, bring all reagents, serum references/controls to room temperature (20 - 27°C).

1. Format the microplates’ 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 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of the IgE Biotin Reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.

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

5. Incubate 30 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

7. Add 350µl of wash buffer (see Reagent Preparation Section) (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 four (4) additional times.

8. Add 0.100 ml (100µl) of the IgE Tracer Reagent to each well. DO NOT SHAKE THE PLATE AFTER TRACER ADDITION.

9. Incubate 30 minutes at room temperature.

10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

11. Add 350µl of wash buffer (see Reagent Preparation Section) (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 four (4) additional times.

12. Add 0.100 ml (100µl) of working signal reagent to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time.

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

14. Read the Relative Light Units (RLU) in each well for 0.2 – 1.0 seconds. The results should be read within thirty (30) minutes of adding the stop solution.

15. Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at room temperature 20-27°C.

16. Working Signal Reagent Solution - Store at 2 - 8°C. Determine the amount of reagent needed and prepare by mixing equal volumes of A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of signal reagent can be used if not used within 36 hours after mixing). If complete utilization of the reagents is anticipated, within the above time frame, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.
QUALITY CONTROL

Each laboratory should assay controls at levels in the low, mid and high range for monitoring assay performance. These controls should 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. Pertinent 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. Fresh reagents should be used to determine the reason for the variations.

RESULTS

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 unknown versus the corresponding IgE concentration in IU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of IgE for an unknown, locate the average RLU's for each unknown on the vertical axis of the graph (the duplicates of the unknown may be averaged and plotted). In the following example, the average RLU’s (50945) of the unknown intersects the calibration curve at (177 IU/ml) IgE concentration (See Figure 1)*.

O.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.

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 step(s) may result in poor replication and spurious results.
5. Use components from the same lot. No intermixing of reagents from different batches.
6. Multichannel pipettes are recommended for addition of reagents.
7. Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or hemolyzed specimen(s) should not be used.

B. Clinical Interpretation

1. 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. Many clinical findings especially specific allergy testing should be taken into consideration while determining the clinical status of the patient.
2. Since all atopic reactions are 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.

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>
<thead>
<tr>
<th>Age (Yrs)</th>
<th>Number (n)</th>
<th>Median</th>
<th>Absolute Range</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 – 280</td>
</tr>
<tr>
<td>Adult</td>
<td>145</td>
<td>43</td>
<td>0 - 200</td>
</tr>
</tbody>
</table>

This is important to know 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 as understood by 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 with a population indigenous to the area in which the laboratory is located.

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. Biological specimens (e.g., IgE) obtained from the patient are utilized in this method. The concentration of IgE is determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

REFERENCES


A. Precision

The within and between 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 (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

B. 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).

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.

E. High Dose Effect

Since the assay is sequential in design, high concentrations of IgE do not modify the hook effect. The Monobind IgE patient samples with concentrations over 8 million IU/ml demonstrated extremely high levels of light intensity.

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* 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 new lot. 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.

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EXAMPLE 1

<table>
<thead>
<tr>
<th>L.D.</th>
<th>Raw Result</th>
<th>Absorption</th>
<th>Mean Concentration (RLU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>187</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal B</td>
<td>1914</td>
<td>1847</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>1760</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal C</td>
<td>8019</td>
<td>8170</td>
<td>25</td>
</tr>
<tr>
<td>F</td>
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</tr>
<tr>
<td>Cal D</td>
<td>18351</td>
<td>15330</td>
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<td>H</td>
<td>19755</td>
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<tr>
<td>Cal E</td>
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<td>150</td>
</tr>
<tr>
<td>A</td>
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</tr>
<tr>
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<td>100000</td>
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<td>50945</td>
<td>177</td>
</tr>
<tr>
<td>B3</td>
<td>51144</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**TABLE 2**

**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 3**

**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 4**

**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.