Assessment of commercial specific IgE assays for detection of allergens in allergic patients

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ABSTRACT
Allergy is a serious health problem throughout the world, affecting people of all ages. Allergic diseases such as asthma, rhinitis and atopic dermatitis are becoming epidemic in all countries. The cost of investigating these diseases is increasing and becoming very expensive. There are many ways to explore allergenic antibodies to assess the presence and the amount of specific IgE. These are: Skin test (Prick), Specific IgE (ELISA), RAST Sp.IgE and Elimination Challenge methods. Skin test produces pain, local or anaphylactic reaction and patient discomfort. Other procedures are expensive to the patient. So, a modified procedure, based on the same principle of previous tests, was studied in Allergy and Immunology laboratory of Ain Shams University. The procedure has suitable cost for all patients; it is very simple, accurate, cheap and does not produce any problems for patients. It depends on ELISA technique and measures the quantitative amount of the following different allergens: Food and Drug allergens such as, Milk, Eggs, Banana, Maize, Fish, Chocolate, Wheat, Nuts, Strawberry, Shrimps, Spices and Aspirin as a drug allergen. Inhalants, as House dust, Mite, Mixed Pollens, Mixed Moulds, Hay dust, Wool, Latex and Cat Hair. The results of this test for 150 allergic patients were compared with those of national specific IgE kits (ELISA), Sp.IgE (RAST), Skin test and elimination challenge test. Statistical results of sensitivity showed respectively: 88.9%, 89.6%, 91.2%, 71.4%, 93.1%. As regards specificity, the results were 93.1%, 94.7%, 95.3%, 65.5%, 91.6%, respectively. These results conclude that the test is in line with all other standard tests. It can also be noted that it is not only the cheapest and most commercial technique using the immediately available, locally prepared reagents, plates and other requirements found in any standard laboratory, but, additionally, probably it can be unique in using foods, drug and inhalants allergens at the same time. Now, it is applied successfully in Allergy and Immunology unit in Ain Shams university hospitals in Egypt. The test has also been recently introduced at the Center for Advanced Bio Medical Research and Innovation (CABRI) at the Gulf Medical University, Ajman, UAE, using a commercial system from Phadia. About two hundred different IgE levels against specific antigens are tested using the Immunocap 100. The allergen of interest, covalently linked to the Immunocap is incubated with the serum being tested. The unbound IgE is washed away and the bound specific IgE is detected using a fluorescent reader. The concentration is calculated using a calibration curve.

Keywords: Specific IgE; ELISA; RAST; Elimination Test
INTRODUCTION

The prevalence of Allergy appears to be increasing. Hypersensitivity reactions to different allergens account for significant morbidity and mortality\(^1\). The accurate diagnosis of Allergic disease using allergen-specific IgE can be detected by skin prick testing and by blood specific IgE testing (e.g., serum RAST)\(^2-4\). However, a food elimination/challenge trial is a reliable way to confirm a food allergy\(^3\). Skin testing remains an essential diagnostic tool in modern Allergy practice. A significant variability has been reported regarding technical procedures in preparing extracts of foods and Inhalant allergens (1:10 wt/vol), in interpretation of reactions, in pain produced and in the documentation used \(^1,5\). Several reports indicate a number of analytical measurements used to promote more accurate diagnosis and better management of allergic individuals. These measurements detect different subsets of the IgE antibody response\(^6\).

The aim of this study was to evaluate the sensitivity, specificity and validity of a modification ELISA specific IgE test, for which all reagents and plates are made locally in the Allergy and Immunology unit in Ain Shams University hospitals in Egypt. Results were compared with other national tests as: Skin test, Specific IgE, RAST test and elimination challenge test.

MATERIALS AND METHODS

- In the present study, 150 patients were selected from different sectors of ages and gender, suffering from allergy using the Allergy and Immunology clinical sheet of Ain Shams University hospital, from Feb. 2009 to April 2012.
- Discussion with the patients as regards the benefits of the study and the informed consents was performed.
- For each patient, the following investigations were conducted:
  1. Clinical examination and Routine investigations to exclude any disease simulating Allergy.
  2. Skin prick test: Epicutaneous prick method was done using all allergen extracts. The latter were prepared in Ain Shams Allergy and Immunology Extract Unit by aqueous vaccine. Positive (histamine) and negative (coca solution) controls were included in the test and patients with dermographism were excluded\(^7\).
  3. Specific IgE test (National kits) for allergens was done by AlaSTAT Microplate Allergen specific IgE (RIDASCREEN Spezifisches IgE, Germany)\(^8,9\).
  4. Specific IgE by RAST technique\(^3,9\).

Commercial Specific IgE Assay

This was done according to the procedure described by El Shami et al 1998\(^10\) and modified by Mohammed et al (2012) as follows:

Extraction of Allergens are used in Coating Microplate according to the protocol followed in Allergy & Immunology Unit of Ain Shams University Hospital.

Many allergens as those of the methods of Slavin et al\(^7\) and Krishman et al\(^8\) are used. These allergens are food allergens (Milk, Eggs, Banana, Maize, Fish, Chocolate, Wheat, Nuts,
Strawberry, Shrimps, Spices, and drug as Aspirin) and inhalant allergens (House dust, Mite, Mixed Pollens, Mixed Moulds, Hay dust, Wool, Latex and Cat Hair.

Ligand-Coated Microplate

This is a 96-well polystyrene microplate consisting of twelve removable strips mounted in a frame. Each strip includes eight ligand-coated flat-bottom wells. Well positions are indexed by a system of letters and numbers (A through H, 1 through 12) embossed on the left and top edges of the frame. The Antigen Coated Procedure start by Pipetting 300 μL of each specific antigen in labeled wells (Foods: Milk, Eggs, Banana, Maize, Fish, Chocolate, Wheat, Nuts, Strawberry, Shrimps, Spices, Aspirin & Inhalants: House dust, Mite, Mixed Pollens, Mixed Moulds, Hay dust, Wool, Latex and Cat Hair) and 100 μL of human albumin for fixation of antigen in the wall and bottom of wells and Left for 72 hours to be completely coated in the wells. The plate is washed in washer three times using 400 ml. distilled water. The plate is put in incubator at 37˚C to dry, packaged in a zip-lock foil bag with desiccant. It is stored refrigerated and protected from moisture to be stable at 2˚C - 8˚C until the expiration date marked on the bag is reached.

Materials Provided

1) Microplate coated with different labelled allergens.
2) Enzyme Conjugate: The allergen/IgE complexes thus linked to the microplate wells are reacted with horse- radish peroxidase-labeled monoclonal anti-IgE during a third 1-hour incubation, after which excess enzyme label is washed away.
3) Buffered Wash Solution Concentrate: One vial containing 85 mL of a concentrated (10%) buffered saline solution, with surfactants and preservative. Using a transfer container, the contents of the vial are diluted with distilled water for a total volume of 850 mL and stored at 2˚C - 8˚C.
4) TMB Substrate Solution: One brown vial containing 55 mL of 3,3',5,5'-tetramethyl benzidine (TMB) in buffered hydrogen peroxide solution, ready to use. It is stored at 2˚C - 8˚C.
5) IgE Calibrators: One set of six vials, labeled A through F, each containing 1.6 mL, with preservative. The calibrators contain, respectively, 0.35, 0.7, 3.5, 17.5, 52.5, and 100 kilo units of IgE per liter (KU/L). It is stored at 2˚C - 8˚C.
6) Stop Solution: Vial containing Hydrochloric acid (1 ml of HCL).

Specimen Collection

The patient need not be fasting, and no special preparations are necessary. Blood is collected by venipuncture into plain tubes, avoiding hemolysis and the serum is separated from the cells. Samples are stored refrigerated at 2˚C - 8˚C for 2 days or up to 2 months frozen at −20˚C. Samples are allowed to come to room temperature before assayed.

Procedure

All components must be at room temperature (15˚C - 28˚C) before use.
1) 50 mL of each calibrator is added into the wells prepared.
2) 50 mL of the individual Serum is pipetted to the specified wells.
3) 50 mL of the conjugate is pipetted into all wells of calibrators and serum wells.
4) The plate is rotated on the micromix for 45 min. and incubated at 37˚C.
5) Decant, then the plate is washed 4 times with the microwash, each time with 300 mL buffered wash solution. Before TMB substrate is added, the plate is wrapped up with absorbent paper to stroke off all residual droplets.
6) 100 mL of TMB substrate solution is added to all wells.
7) The plate is incubated for 15 min.
8) 50 mL of stopping solution is added to all wells.
9) The microplate is read, immediately for 5 minutes in the microplate reader at 450 nm.

Calculation
A standard curve is plotted using log-log graph paper. The average mOD/min. of each calibrator on the vertical axis is plotted against concentration on the horizontal axis. A straight line segments connected adjacent plotted values, then the allergen-specific IgE concentration for the patient samples is estimated by interpolation.

Expected Values
Quantitative values and interpretation of class results are provided in the table 1 below:

<table>
<thead>
<tr>
<th>Class</th>
<th>KU/l</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;0.35</td>
<td>Negative for individual component</td>
</tr>
<tr>
<td>I</td>
<td>0.35-0.69</td>
<td>allergen(s)</td>
</tr>
<tr>
<td>II</td>
<td>0.70-3.49</td>
<td>Positive</td>
</tr>
<tr>
<td>III</td>
<td>3.50-17.49</td>
<td>Strongly positive</td>
</tr>
<tr>
<td>IV</td>
<td>17-50</td>
<td>Strongly positive</td>
</tr>
<tr>
<td>V</td>
<td>More 50</td>
<td>Strongly positive</td>
</tr>
</tbody>
</table>

Sensitivity
Sensitivity is unique as a result of the high IgE-binding capacity of the system, and also because the soluble matrix is able to support allergens which are carbohydrates, in addition to proteins and nucleic acids.
Specificity

The system is not subject to interference from high total IgE level and other nonspecific binding problems which affect solid-phase systems. See Tables 2-5.

Table 2. Comparison between the value of National sp.IgE, Egyptian commercial sp.IgE, Skin test and RAST in diagnosis of allergens in allergic patients.

<table>
<thead>
<tr>
<th></th>
<th>National sp.IgE</th>
<th>Eg.Com.sp.IgE</th>
<th>Skin Test</th>
<th>RAST Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>94.7</td>
<td>93.1</td>
<td>65.5</td>
<td>95.3</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>89.6</td>
<td>88.9</td>
<td>71.4</td>
<td>91.2</td>
</tr>
<tr>
<td>Predictive −veTest</td>
<td>97.3</td>
<td>96.2</td>
<td>68.6</td>
<td>97.8</td>
</tr>
<tr>
<td>Predictive +veTest</td>
<td>95.8</td>
<td>93.7</td>
<td>81.2</td>
<td>96.4</td>
</tr>
</tbody>
</table>

Table 3. The validity of Egyptian commercial sp.IgE test in comparison to elimination-challenge test (confirmatory).

<table>
<thead>
<tr>
<th>Screen test</th>
<th>Confirmatory test</th>
<th>Confirmatory test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 +ve</td>
<td>77 true +ve</td>
<td>3 false +ve</td>
<td>80 patients</td>
</tr>
<tr>
<td>70 −ve</td>
<td>66 true −ve</td>
<td>4 false −ve</td>
<td>70 patients</td>
</tr>
</tbody>
</table>

Table 4. Validity of Egyptian commercial sp.IgE test in comparison to elimination-challenge test in foods, aspirin & inhalants

<table>
<thead>
<tr>
<th></th>
<th>Foods</th>
<th>Aspirin</th>
<th>Inhalants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>90.8%</td>
<td>94.2%</td>
<td>94.7%</td>
</tr>
<tr>
<td>Specificity</td>
<td>89.5%</td>
<td>91.9%</td>
<td>93.4%</td>
</tr>
<tr>
<td>Predictive −ve test</td>
<td>95.6%</td>
<td>98.1%</td>
<td>97.3%</td>
</tr>
<tr>
<td>Predictive +ve test</td>
<td>93.4%</td>
<td>97.6%</td>
<td>91.8%</td>
</tr>
</tbody>
</table>
Table 5. Comparison between National Sp.IgE and Egyptian commercial Sp.IgE as regard many variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>commercial Sp.IgE</th>
<th>National Sp.IgE Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergens</td>
<td>All allergens including Aspirin</td>
<td>Not Including Aspirin</td>
</tr>
<tr>
<td>Materials</td>
<td>Very simple prepared procedures made in our lab.</td>
<td>Complicated procedures and need a factory for preparation</td>
</tr>
<tr>
<td>Time</td>
<td>Less time about 1.2 hours needed for complete the test</td>
<td>More time 2.5 hours needed for complete the test</td>
</tr>
<tr>
<td>Washing</td>
<td>2 times</td>
<td>4 times</td>
</tr>
<tr>
<td>Laboratory description</td>
<td>The test is proceeded in any standard laboratory</td>
<td>The test is proceeded in special lab. had special facilities</td>
</tr>
<tr>
<td>Cost</td>
<td>The Cheapest Cost</td>
<td>Highly Expensive Cost</td>
</tr>
<tr>
<td>Application</td>
<td>It is applied successfully in Allergy and Immunology unit in Shams University hospitals in Egypt and poor patients benefit from it.</td>
<td>Applied in private laboratory and rich patients benefit from it.</td>
</tr>
</tbody>
</table>

RESULTS

**Statistical analysis:** Data were analyzed using the SPSS program Version 15 & results were as follows:

As regards sensitivity results for modified test, Specific IgE national kits (ELISA), Sp.IgE (RAST), Skin test and elimination challenge test were respectively: 88.9%, 89.6%, 91.2%, 71.4%, 93.1%, while for specificity, results were: 93.1%, 94.7%, 95.3%, 65.5%, 91.6% respectively. These results showed that our test is in line with all tests.

DISCUSSION

There are many ways to explore allergenic antibodies to assess the presence and the amount of specific IgEas: Skin test (Prick), Specific IgE (ELISA), RAST Sp.IgE and Elimination Challenge method\(^3,10-14\). Skin test produces pain, local and anaphylactic reaction and patient discomfort, other procedures are costly to the patient. This motivated the team of Allergy and Immunology unit of Ain Shams University to use a modified test. The test is based on the principle of other tests but it is very simple, accurate, cheap, so, having a suitable cost and does not produce any problem for all patients. This test depends on ELISA technique, it measures the quantitative amount of the following different allergens: food allergens including Milk, Eggs, Banana, Maize, Fish, Chocolate, Wheat, Nuts, Strawberry, Shrimps, Spices, with drug as Aspirin and inhalant allergens as House dust, Mite, Mixed Pollens, Mixed Moulds, Hay dust, Wool, Latex and Cat Hair. 150 allergic patient results of our test were compared with specific IgE national kits (ELISA), Sp.IgE (RAST), Skin test and elimination challenge test. The statistical evaluation results as regards sensitivity were respectively: 88.9%, 89.6%, 91.2%, 71.4%, 93.1%. As regards
specificity the results were: 93.1%, 94.7%, 95.3%, 65.5, 91.6% respectively. These results show that this test is in line with all other tests. In conclusion, this test in addition to being probably using the common food, drug and inhalant allergens, it is the cheapest of the most commercial techniques. So, we recommend it to be used and to be available immediately in any standard laboratory as it depends on its own reagents, plates and any other requirements needed to be prepared locally in the laboratory. Now, it is applied successfully in Allergy and Immunology Unit of Ain Shams University hospitals in Egypt. The test has also been recently introduced at the Center for Advanced Bio Medical Research and Innovation (CABRI) at Gulf Medical University, Ajman, UAE, using a commercial system from Phadia™. About two hundred different IgE levels against specific antigens are tested using the Immunocap 100™. The allergen of interest, covalently linked to the Immunocap is incubated with the serum being tested. The unbound IgE is washed away and the bound specific IgE is detected using a fluorescent reader. The concentration is calculated using a calibration curve.

REFERENCES

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