IMMUNOLAB GmbH

Specific IgE RIA (RAST)

Radio Immunoassay for the Semi-Quantitative Determination of

Allergen-Specific IgE Antibodies in Human Serum or Plasma

Incubation Time : 20 (7) Hours

Cat. No : ILR-E01

September 2007

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Summary and Explanation of the Test

The existence of IgE in man as a unique class of immunoglobulins which are important in the mediation of the allergic response has been known for over twenty years. The mechanism of action involves an initial antigenic stimulation of immunocompetent B lymphocytes by a specific antigen, a process which induces the lymphocyte to respond by producing specific antibody of several classes.

One class, reaginic or IgE antibody, becomes partially bound via its Fc portion to receptors on the surface of mast cells and basophilic leukocytes. Upon further stimulation by specific allergens, these cell-bound IgE molecules bind via their Fab portion to the allergen. This combination triggers the mast cells and basophilic leukocytes to release various vasoactive amines into the blood and the surrounding tissue. These substances cause smooth muscle constriction and lead ultimately to allergic conditions such as wheal and flare reactions, hives, dermatitis, rhinitis, hay fever, asthma and anaphylactic shock.

IgE determinations are most valuable in the diagnostic assessment of patients with established or suspected allergic disease. In normal subjects, IgE values are related to age, with normal values peaking around 10-14 years. Infants and children with family history of atopic allergy are at increased risk of developing disease and constitute a prime population for screening. Studies have shown that conditions such as asthma, rhinitis, eczema, urticaria, dermatitis and some parasitic infections lead to increased IgE levels. Asthma, hay fever and atopic eczema patients may produce levels 3-10 times those of normal patients.

Circulating levels of allergen-specific IgE can be determined by the use of specific allergens attached to a solid phase carrier. This approach uses a radiolabeled antibody to IgE and is known as the radioallergosorbent test (RAST).

Principle of Procedure

The radioimmunoassay for Specific IgE is performed by the following procedure. An allergen disc is incubated three hours with allergen-specific IgE from the patient's serum. The disc is washed, incubated 16-24 hours with radio(I125)-labeled anti-IgE and then washed again, yielding a solid-phase matrix with iodine-125 proportional to the amount of allergen-specific IgE in the patient sample. The concentration of allergen-specific IgE is directly proportional to the measured counts per minute.

Reagents

1. Reagent Unit

Tracer: One vial containing 5.2 ml anti-human IgE (goat) conjugated to iodine-125 in a buffered protein solution with 0.05% sodium azide as a preservative.

Wash Solution Concentrate (50x): Four bottles containing 10 ml each of concentrated PBS solution with a detergent.

2. Reference Unit (not included, has to be ordered extra)

Reference Discs: One vial containing 40 discs in a storage solution.

Reference Sera A,B,C,D: Four vials, each containing 0.55 ml A,B,C and D sera for use in preparing a calibration reference.

Serum A is prepared from a human serum which contains a high concentration of IgE specific for the reference allergen (timothy). Sera B, C and D are dilutions of A respectively, with horse serum free of IgE specific for the reference allergen.

3. Allergen Discs (not included, has to be ordered extra)

Each cassette contains 25 specific allergen discs with storage buffer. Discs available include specific allergens from the following groups: grasses, weeds, epithelia, molds, foods, dust, dust mites, chemicals and drugs.

Warnings or Precautions for Users

For In Vitro Diagnostic Use

Warning: Potential Biohazardous Material

Each donor unit used in the preparation of the human serum base reagents was tested by an approved method for presence of the antibody to human T-lymphotropic virus type III/lymphadenopathy associated virus (HTLV III/LAV), as well as for Hepatitis B surface antigen (HBsAg) and found to be negative (not repeatedly reactive).

Because no test method can offer complete assurance that HTLV III/LAV, HBsAg, or other infectious agents are absent, human serum base materials should be handled at Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen.

Disposal of Solutions Containing Sodium Azide

Warning: Contains sodium azide, which may react with lead and copper plumbing to form potentially explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Instructions for Reagent Preparation

Wash Solution (50x)

Into a graduated cylinder of at least 1000 ml capacity, dispense 10 ml Wash Solution Concentrate. Add sufficient distilled water to the cylinder to bring the total volume to 500 ml. Transfer the contents of the cylinder to a bottle for storage. This quantity is sufficient for a 30-tube assay.

Wash Solution must be freshly prepared for each assay. Working Wash Solution is stable for 48 hours at room temperature.

Storage Conditions

Store all reagents at 2-8 °C.
Use all reagents before expiration date on vial labels.

Instrumentation

Performance of the Specific IgE RAST assay requires the use of a gamma-counter.

The instrument should be calibrated routinely to ensure proper performance.

Specimen Collection and Preparation

Serum should be used in this allergen-specific IgE RIA procedure.

It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is processed in an individual immunoassay.

Perform phlebotomy using a non-traumatic venipuncture technique with either a sterile syringe or a vacuum tube. If a syringe is used, remove the needle and carefully transfer the blood to a tube. Allow blood to clot at least 20-30 minutes, until the clot just begins to retract. Before centrifugation, ream the collection tube gently with a wooden applicator stick.

Immediately following centrifugation, transfer the cell-free serum to a tube and cap tightly. Store at 2-8 °C, or frozen if assay is not performed the same day.

1. Precautions

As with any sample that may contain pathogens, care must be taken to prevent contact with open wounds.

2. Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen.

3. Storage, Handling and Shipping

Specimens should be capped, stored at 2-8 °C and assayed within 24 hours after collection. If the assay cannot be performed within 24 hours, or if the specimen is to be shipped, it should be frozen. As in the case of most proteinaceous material, repeated freezing and thawing should be avoided. The use of hemolyzed or lipemic specimens is not recommended.

Specimens should be allowed to come to room temperature and mixed thoroughly by gentle inversion before assaying.

Performance of the Assay

Materials Provided

Reagent Unit

Tracer	1 vial	5.2 ml
Wash Solution Concentrate	4 bottles	10 ml

Reference Unit

Reference Discs	1 vial	40 discs
Reference Sera	4 vials	0.55 ml each

Specific Allergen Discs 25 discs per cassette.

Materials Required but not Provided

1. Test tube rack
2. Precision pipet - 0.05 ml
3. Disposable serological pipet - 10 ml
4. Repipettor - 2.0 ml
5. Disposable pipet tips
6. Vortex Mixer
7. Gamma-Counter
8. Graduated cylinder, 1000 ml capacity
9. Polystyrene test tubes, 12 mm x 75 mm
10. Plastic film
11. Aspirator
12. Forceps
13. Water bath, 37 °C

Assay Procedure

Allow all reagents to come to room temperature and gently mix before use.

1. Label tube 1 to be used for total counts. Starting with tube number 2, label duplicate tubes for calibrators (A, B, C and D) and controls. Label appropriate patient tubes for each allergen to be tested.

   Note: All discs should be blotted on absorbent paper to remove excess storage buffer before being placed in tubes. Handle all discs with forceps.

2. Using forceps, add a reference disc to the bottom of each reference tube (2 - 9). Add each specific allergen disc to the bottom of the appropriate tube. Do not add discs to total counts tubes.

3. Pipette 50 µl (0.05 ml) of reference sera A - D directly onto the discs in the appropriate tubes. Pipette 50 µl (0.05 ml) of control or patient sample directly onto the discs in the appropriate tubes.

4. Cover tubes with plastic film and allow to incubate 3 hours at room temperature (20 - 25 °C).

5. Pipette 2 ml of freshly prepared wash solution to each tube except the total counts. Let set for 10 minutes. Aspirate wash solution completely. Repeat this wash procedure two more times for a total of three washes. After last aspiration, let the tubes stand for one minute. Aspirate again to remove any residual wash solution. Wash solution must be freshly prepared for each assay. Working wash solution is stable for 48 hours at room temperature.

6. Pipette 50 µl (0.05 ml) of I-125 tracer directly onto the disc in each tube. Do not add tracer to total counts tubes. Cover tubes with plastic film to prevent evaporation and let react overnight (20 hours) at room temperature (20 - 25 °C) or 2 hours at 37 °C.

7. Wash three times according to step 5.

8. Wipe the outside of each tube and vortex before loading tube into the gamma-counter.

Calculation of Results

1. Calculate the average counts for A, B, C and D reference tubes.

2. Calculate the count level of an additional reference point E, by dividing the average counts of Reference D by two.

3. Calculate the results of the controls and patient samples on all discs by the following method.

Compare the counts of each control and patient sample with the counts for the reference sera A, B, C and D, plus calculated reference E. Assign the class value to the knowns as follows:

	Specific IgE	
Class	Count Rate	Concentration
4	> A	Very High
3	B - A	High
2	C - B	Moderate
1	D - C	Low
1/0	E - D	Very Low
0	< E	Non-Detectable

Alternatively a semi-quantitative assessment can be done by assigning A, B, C, D and E the values of 17.5, 3.5, 0.7, 0.35 and 0.1 U/ml respectively. Plot the counts vs. concentration of the reference points on log-log graph paper. Read values of controls or patients directly from the graph. Values below 0.1 U/ml are non-detectable. Values increase with increasing concentration of allergen-specific IgE.

Quality Control

Good laboratory practice requires that quality control specimens be run to check on assay performance. Any material used should be assayed repeatedly to establish mean values and acceptable ranges.

Expected Values

The determination of allergen-specific IgE levels is a semi-quantitative measurement. Also, similar responses between different types of allergen discs do not necessarily imply similar specific IgE concentrations or similar clinical conditions. Expected levels for the allergen-specific IgE concentrations are, therefore, not entirely applicable.

In general, the greater the response as interpreted by a higher classification, the greater is the concentration of allergen-specific IgE for any allergen disc.

Frequently, multiple assays are done at varying times in order to monitor a patient's response at different seaons of the year or to monitor therapy. A reliable quality control system with known positive and negative control sera should be established to ensure continuity of reported values or classifications.

Limitations of the Procedure

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a
   complete understanding of the package insert instructions and with adherence to good laboratory practices.

2. Clinical diagnosis should not be made on the findings of a single test result, but should integrate all clinical and laboratory findings.

3. The determination of circulating levels of allergen-specific IgE is a semiquantitative assay. It has no absolute standard and is arbitrarily assigned units of value or classification.

4. Similar response or classification between different types of allergen discs does not necessarily imply clinical equivalence.

5. When total IgE values are very high (> 500 U/ml), low level allergen-specific IgE response should be interpreted with caution.

References

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