

 **Specific IgE  
BLAST on discs (RAST)**

<sup>125</sup>I-labelled Bio-Line Allergo-Sorbent Test for the  
quantitative determination of specific IgE in human serum.

040702 - Rev. 04

## Summary and background of the test:

Five different types of human antibodies have been characterized : IgA-IgD-IgE-IgM-IgG.

In-vitro techniques for allergy testing have improved since the specific immunoglobulin responsible for allergic hypersensitivity was discovered and identified as IgE (1-2).

Atopic allergy is a hypersensitive immunological condition mediated by IgE (3).

Immunocompetent B-lymphocytes, if stimulated by a specific allergen, produce antibodies to the allergen.

IgE antibodies bind, via their Fc portion, to receptors on the surface of mast cells and basophilic leucocytes. Upon further stimulation, these cell-bound IgE molecules bind via their Fab portion to the allergen. This combination triggers cell degranulation and the release of various substances, including vasoactive amines.

The most common clinical manifestations of this biological process are dermatitis, rhinitis, hay fever, asthma and anaphylactic shock.

IgE determinations are most valuable in the diagnosis assessment of patients with established or suspected allergic diseases (4).

IgE values are age-related.

Some parasitic infections may also lead to increased IgE levels (5). Immunological studies of IgE myelomatosis have also been performed (6-8).

The allergo-sorbent test on paper discs, developed in 1967 (9), is an in-vitro method for detecting IgE antibodies to specific allergens such as pollens, mites, house dusts, animal danders, foods, insect venoms, moulds and drugs.

## Principle of the test :

The patient serum is first incubated in a test tube with a specific allergen covalently bound to a paper disc. Any antibody against this allergen will bind to the disc, if present in the serum.

After the incubation period, the disc is washed, to remove unbound antibodies.

In a second step, <sup>125</sup>I-anti-h-IgE is added, to fix and radiolabel the allergen-specific IgE antibodies.

At the end of the test, the disc is washed again to remove the unbound tracer.

The radioactivity is measured in a gamma counter and is directly proportional to the amount of IgE in the sample.

The level of unknown IgE is then determined by comparing the radioactivity with data established using known standards in the same assay system.

## Precautions:

1. Radioactive material: Radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories, or hospitals for "In-Vitro" clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Compliance with these basic rules of radiation safety should provide adequate protection:

1. Do not eat, drink, smoke, or apply cosmetics in areas where radioactive material is used.
2. Do not pipet by mouth reagents containing radioactive materials.
3. Wear protective clothing; i.e., lab coats and disposable gloves, in order to avoid direct contact with radioactive reagents.
4. Work with radioactive materials should be performed in a designed area.
5. Radioactive materials should be stored in an acceptable location.
6. A log should be kept for receipt and disposal of radioactive materials.
7. Radioactive spills or accidents should be taken care of immediately according to established procedures.
8. Disposal of radioactive materials must comply with prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory.

2. Sodium azide: Sodium Azide, used as a bacteriostatic agent, is toxic in acid medium. In addition, it may form potentially explosive lead or copper azides. To avoid dangerous deposits, waste solutions should be flushed away with large volumes of water.

3. Hepatitis and Acquired Immune Deficiency Syndrome (HTLV-III): All Bio-Line reagents included in this kit have been tested and found to be non reactive for hepatitis B surface antigen. They have also been screened and determined to be non-reactive for HTLV-III antibody. However, human serum products should be handled as if potentially capable of transmitting hepatitis, Acquired Immune Deficiency Syndrome, or other infectious agents.

### Materials needed:

1. **Reference set:** 5 vials containing each 750 µl of ready-to-use sera for 5 duplicate curves, and 2 boxes containing each 25 reference discs (D1).  
Standards: Zero (0.17 RU/ml), D (0.35 RU/ml), C (0.7 RU/ml), B (3.5 RU/ml), A (17.5 RU/ml)
2. **High reference serum H (optional):** 1 vial of 750 µl (52.5 RU/ml).
3. **<sup>125</sup>I-anti-IgE tracer:** 1 vial (red solution) containing 5.2 ml of radiolabelled Mouse Monoclonal-anti-h-IgE. Activity per vial 6 µCi or 222 kBq. For 100 tests.
4. **Control sera (optional):** each vial containing 550 µl for 10 tests (3 types available).
5. **Wash solution concentrate:** 1 vial of 10.5 ml of concentrate, to be diluted into **1300 ml NaCl 9 ‰** and stored at 4°C (no more than 2 months).
6. **Allergen discs:** each box containing 25 specific or multiple allergen paper discs on a buffer substrate.

**All provided reagents be stored at 2° - 8° C.**

**Refer to the expiration date on the kit label for stability.**

### Materials required but not provided:

1. Pipets, micropipets, repeating syringes and repeating pipettors.
2. Plastic disposable tubes.
3. Gamma counter.
4. Logit log graph paper.
5. Test tube racks.
6. Rotator.

### Specimen collection and preparation:

Sera should be separated from blood cells immediately after collection. Sera are stable for at least 7 days at 4° C and for longer periods of time when stored frozen.

#### A) Assay procedure for the overnight technique in tubes:

Bring reagents to room temperature and mix before use. Label duplicate tubes for Zero,D,C,B,A (H optional) standards and Tc, and single tubes for control (optional) and patient samples. However, label 2 tubes per sample, when using specific allergen discs that must be used with HSA control discs.

1. Discs should be gently handled with tweezers and **blotted on absorbent paper**, to remove excess buffer solution. Place 1 reference disc into each standard tube, and 1 allergen disc into each sample tube. When allergen discs are provided with HSA control discs, place 1 allergen disc into one tube, and 1 HSA disc into a second tube.
2. Pipet 50 µl of standards, control and sample sera into their corresponding tubes (just above the disc), **and slightly hit the disc with the pipette tip against the tube bottom**, to eliminate any air bubble below the disc.
3. Cover with plastic film and incubate all the tubes for 3-4 hours at room temperature.
4. Aspirate, and wash all tubes, except Tc, three times with 2 ml of wash solution, **with 10 minutes incubation between each wash**. Aspirate and discard as much remaining liquid as possible.
5. Pipet 50 µl of tracer into each tube, including Tc, **using the same procedure as in step 2**.
6. Cover with plastic film and incubate all the tubes overnight (16-24 hours) at room temperature.
7. Repeat wash procedure as described in step 4.
8. Place tubes in gamma counter and count for one minute.

### Specific IgE Flow Chart (overnight technique in tubes):

	Tc	Stds. 0,D,C,B,A,H	samples, control
Discs should be blotted on absorbent paper.			
Refer. disc	-	1	-
Allergen disc	-	-	1
Stds., ctrl. & samples	-	50 µl	
Incubate at RT for 3-4 hours. Aspirate.			
Wash sol.	-	3 x 2ml (*)	
<b>(*) 10 minutes incubation between each wash!</b> Aspirate and discard as much remaining liquid as possible.			
Tracer	50 µl	50 µl	
Incubate at RT overnight (16-24h). Aspirate.			
Wash sol.	-	3 x 2 ml (*)	
<b>(*) 10 minutes incubation between each wash!</b> Count in a gamma counter for one minute.			

### Calculation of results:

Data need not be expressed as counts per minute (cpm) but the counting period must be the same for all tubes that are counted.

Determine the average counts for each set of duplicate tubes. Divide this value by the average net counts of Tc, and multiply by 100 to yield the % B/Tc.

$$\% \text{ B/Tc} = \frac{\text{cpm (Stds, Controls or sample)} \times 100}{\text{cpm (Tc)}}$$

Plot % B/Tc for each standard vs its concentration in RU/ml. The concentration of Specific IgE in the samples may then be read directly from the standard curve.

### Data Table (Example):

#	Duplicate cpm		Mean cpm	%B/Tc	Conc. RU/ml
Tc	67 166	66 123	66 645	100 %	
Std 0	716	891	804	1.2 %	0.17
Std D	1 730	1 611	1 671	2.5 %	0.35
Std C	2 498	2 464	2 481	3.7 %	0.70
Std B	7 652	8 216	7 934	11.9 %	3.50
Std A	23 746	25 608	24 677	37.0 %	17.5
Std H	39 733	39 157	39 445	59.2 %	52.5
Sample 1	2 257	-	2 257	3.4 %	0.59
Sample 2	8 898	-	8 898	13.4 %	3.88

## B) Assay procedure for 4h30 technique in tubes:

No shorter technique is recommended; however, possible alternatives between both techniques can be implemented by each laboratory, according to their needs. Our quality control is based on the overnight technique.

### Alternative Flow Chart (4h30 technique in tubes):

	Tc	Stds. 0,D,C,B,A,H	samples, control
Discs should be blotted on absorbent paper.			
Refer. disc	-	1	-
Allergen disc	-	-	1
Stds., ctrl. & samples	-	50 µl	
Incubate at RT for 2 hours on a rotator (± 250 rpm). Aspirate.			
Wash sol.	-	3 x 2ml (*)	
<b>(* 10 minutes incubation between each wash!</b> Aspirate and discard as much remaining liquid as possible.			
Tracer	50 µl	50 µl	
Incubate at RT for 2h30 on a rotator (± 250 rpm). Aspirate.			
Wash sol.	-	3 x 2 ml (*)	
<b>(* 10 minutes incubation between each wash!</b> Count in a gamma counter for one minute.			

## Analysis of results:

### 1. Specific Allergen Discs.

Results may be expressed either way: in classes (0, 1, 2..), or in RU/ml (Rast units/ml): 0 RU/ml < **class 0** < 0.35 < **class 1** < 0.7 < **class 2** < 3.5 < **class 3** < 17.5 < **class 4** < 52.5 < **class 5**.

In each case, the counts/min (cpm) of the samples have to be compared with the cpm of the reference standard curve.

Expected values:

- class 0: negative
- class 1: suspected
- class 2: low
- class 3: high
- class 4: very high

### 2. Multiple Allergen Discs.

Values equal to or higher than class 1 are considered as positive; they indicate that specific IgE antibodies to one or more allergens are present in the serum.

### 3. Allergen Discs with Control discs.

Some allergen discs are delivered with HSA (Human Serum Albumin) control discs. Due to the coupling of those allergens with HSA on discs, results are considered as positive when the cpm of the sample disc are higher than twice those of the HSA control disc.

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**Manufactured by *BIO - LINE* sa/nv**

**Rue André Fauchille, 17 - 1150 Brussels - BELGIUM**

**Tel: 32-2-736.62.18 Fax: 32-2-742.13.15**