

Assay of Allergen Preparations Using Direct RAST Titration and Skin Tests

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We have studied the feasibility of the direct RAST titration method for determining allergen concentrations (3) and schemes for converting results into meaningful biological histamine-equivalent prick (HEP) units (1). We chose the direct RAST method rather than RAST inhibition, since the inhibition method requires the availability of a good allergen reference preparation coupled to paper discs for each allergen (e.g. cat dander or birch pollen) to be assayed. Obtaining suitable reference preparations appeared difficult.

MATERIALS AND METHODS

For direct RAST titrations serum pools were collected for each allergen by pooling equal volumes from approximately 20 patients with IgE specific to the allergen in question at a concentration corresponding to RAST score 3-4. In the case of mite, score 2 was accepted as well.

In skin prick testing, reference tests were made with a solution containing 10 g/l histamine acid phosphate. It should be noted that the choice of reference compound and its concentration will affect the HEP scale (actually its accuracy=bias). Otherwise HEP units were as defined by Aas & Belin (1).

Prick tests were performed on groups of 10-26 patients considered sensitive to the allergen in question on the basis of history and previous tests. When two or more preparations of the same allergen were being compared, tests were performed simultaneously with the preparations on the same patients. In a second part of the study, prick tests were performed on 708 unselected 9th grade students in Imatra schools. Of these 99% were 15-17 years old (2).

RESULTS AND DISCUSSIONS

The location of the titration curve and consequently the observed allergen concentration as expressed in direct RAST titration units (3) was affected by a number of variables. When cat allergen was assayed using pools collected from 2, 4, 8, 16 or 32 patients, the concentration values varied over a 30-fold range. When 16 sera or more were pooled, reasonable stability apparently was approached. When two different lots of cyanogen-

bromide activated paper discs were tried, a 2-fold difference was observed. When the same allergen preparation was assayed with the same reagents on 7 different days, the coefficient of variation was 25%. Non-typical form of titration curves caused occasional problems.

Allergen concentrations in direct RAST titration units can be converted to HEP units by multiplication with a factor, which is allergen specific. Factors can be obtained by the simultaneous assay of preparations with the direct RAST and with skin prick tests, and a comparison of the results.

To study the dose-response relationship, we made skin tests with 10 different allergen stock solutions and 10-fold dilutions of these, altogether 128 pairs of tests on patients. For pairs in which the larger weal was 3-10 mm in diameter, we found that the ratio of the diameters corresponding to the two dilutions was approximately constant and equal to 1.58. From this and the definition of the HEP unit we derived the equation

$$\frac{d}{h} = c^{\log x} \quad (1)$$

Solving for x

$$x = \text{antilog} \left(\frac{\log d - \log h}{\log c} \right) \quad (2)$$

where d and h are diameters of the weals obtained with allergen and histamine solution, respectively, c is the constant 1.58, and x is the allergen concentration (biological activity) in HEP units.

Using equation (2) and measured values for d and h , an "individual HEP" value can be calculated for each patient. The individual HEP value is a function of the allergen concentration in the preparation used and the patient's sensitivity to the allergen in question. Individual HEPs could be used to register skin test results instead of diameters, plusses, etc.

Patient sensitivity varies greatly, and the distribution of individual HEPs is very skewed (Table 1). The "median HEP", that is the median of the individual HEPs in a suitable population group, can be taken as the HEP value indicating the concentration of the

Table 1

Determination of individual, mean and median HEP values with a timothy pollen preparation in a population of 20 timothy-sensitive patients

Patient	Weal diameter (mm)		Individual HEP
	Timothy	Histamine	
1	5	8	0.1
2	5	6	0.4
3	5	6	0.4
4	5	6	0.4
5	5	6	0.4
6	5	5	1.0
7	6	6	1.0
8	4	4	1.0
9	6	5	2.8
10	6	5	2.8
11	8	6	3.6
12	8	6	3.6
13	8	6	3.6
14	8	5	10.0
15	8	5	10.0
16	8	5	10.0
17	10	6	11.8
18	10	5	32.8
19	10	4	187.5
20	20	5	912.2
Mean HEP			59.8
Median HEP			3.2

allergen preparation. Aas & Belin (1) originally suggested a calculation procedure based on means rather than medians, but we find means poorly suited due to the skewness mentioned.

Even with median HEPs, though less than with mean HEPs, the result will be affected by the choice of population. If, for instance, the five most sensitive patients had been omitted from the population group of Table 1, the median HEP would have been reduced from 3.2 to 1.0. The mean would have fallen from 59.8 to 2.7. As might be expected, we obtained higher median HEPs when an allergen was assayed using hospital out-patients than using teenagers selected only for the presence of a weal with a diameter of at least 3 mm. The difference was roughly 4-fold.

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Standardization of the RAST Inhibition Assay

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The radioallergosorbent test (RAST) is basically a technique for the determination of specific IgE antibodies (2). The allergens are coupled to an activated solid support. Serum from the atopic patient is added and the antibodies are bound to the solid phase allergens. The bound IgE is detected by ¹²⁵I labelled anti-IgE. The basic method is easily modified to be used for the assay of allergens or for the determination of

allergen extract potency. A RAST based direct titration and a RAST inhibition assay (3) have been used in research and routine analysis in our laboratories for several years.

The IgE antibody, i.e. the serum from the allergic patient, is the critical reagent of both methods. There is a considerable variation in the specificity of the antibodies of individual sera. This polymorphism can be

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