

Tests for allergy to housedust

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Summary

In an attempt to find a satisfactory combination of method and antigen extract for tests of hypersensitivity to housedust, variations of skin test, RAST, histamine release and basophil degranulation tests have been used to test efficacy of five commonly used extracts of housedust or its components.

The Lincoln Multitest for skin prick tests in its present form induced too much trauma for type-I hypersensitivity tests, though it has advantages for comparison of several extracts. Histamine release and two versions of basophil degranulation tests all gave good results but not consistently. RAST gave the best correlation between all extracts, but neither the *in vivo* nor *in vitro* tests gave perfect concordance with sensitivity in all patients and all extracts.

Introduction

Probably the most difficult allergen for which it is necessary to make diagnosis of type-I hypersensitivity in man is housedust. Though the various mites which breed in dust are potent sensitizing allergens there are many other components, such as toxic factors, polysaccharides, skin, animal danders, moulds, pollens and fibres (Brighton & Topping, 1977) which make it difficult to specify the nature and mechanisms of the responses obtained in tests with different commercial extracts which may vary greatly both in composition and potency. The advent of new tests and variations of older techniques led us to make a comparison of the activity of the five most commonly used extracts in France, by both *in vitro* and *in vivo* methods, to investigate whether there is a

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satisfactory combination of method and extract that will give reliable information with this most difficult allergen.

Materials and methods

Patient groups

Allergy-positive group. A group of nineteen asthmatic subjects with positive acetyl-choline provocation tests. Allergy to dust was diagnosed by clinical history and preliminary positive skin tests with housedust extract and *Dermatophgoides pteronysinus* from the Pasteur Institute, Paris.

Allergy-negative group. A group of sixty-seven male volunteers, aged between 20 and 40 years, who were without known clinical history of allergy. For ethical reasons, as well as to conserve tests, only ten of this group were bled for the *in vitro* tests, though all were prick-tested.

Allergen extracts

A. Stallergenes housedust. Batch No. 90557. 1/10 w/v in 50% glycerol-saline.

B. Stallergenes dust and domestic moulds. No. 90536. 1/10 in 50% glycerol-saline.

C. Stallergenes D. pteronyssinus No. U80302. 1/10 w/v in 50% glycerol-saline.

D. Bencard housedust. No. 51 170. 150% w/v in 50% glycerol-saline.

E. Institut Pasteur housedust. Fractionated. 1×10^{-3} g/ml.

TN. Negative control of 50% glycerol-saline.

TP. Positive control of 50% glycerol-saline, 5% codeine phosphate.

TH. Histamine reference. 50% glycerol-saline, 1 mg/ml histamine HCl.

The tests used

In vivo. Each subject had two series of eight prick tests by means of the Lincoln 'Multitest' (Knicker, Anderson & Roumiantzeff, 1979), applied on each side of the spine in the latero-vertebral region, after the skin was cleaned with alcohol. Weals were outlined and transferred by means of 'Micropore' tape to card as a permanent record.

In vitro

RAST. Substrate discs from each of the extracts were made in advance by the method of Ceska, Eriksson and Varga (1972). Duplicate titrations were done by the usual laboratory technique. The results, expressed in relative IgE titres, were considered to be positive if the ratio:

$$\frac{\text{cpm test serum}}{\text{cpm negative standard (5\% human serum albumin)}}$$

was equal to or greater than 1.7 (Brighton & Topping, 1978).

Histamine release. The modification of Lebel (1982) was used, where the test is considered to be positive if the titre of histamine released is greater than 20% of the total.

Human basophil degranulation test on microplates (HBDT M). This is a modification by the Laboratoire des Stallergenes of that of Benveniste (1981). Briefly, a homogenous blood white-cell concentrate, suspended in either the subject's own plasma, or after washing in Tyrode's buffer, was incubated in the presence of five serial decimal dilutions of allergen extract. After 10 min the cells were fixed and stained, then 10 mm³ samples diluted 1/10 were examined in a Nageotte haemocytometer.

Non-degranulated basophils, stained with toluidine blue were counted, and this count was compared with that from a standard. The results were expressed as a percentage of degranulation.

$$\frac{\text{No. of basophils in standard} - \text{No. of basophils in test}}{\text{No. of basophils in standard}} \times 100.$$

The result was considered to be positive for values of 30% or over when the basophils counted in the control sample was sixty or greater, for a 5% challenge. For forty basophils counted the threshold of degranulation was 40%, and with less than this number of basophils the test was inconclusive.

Human basophil degranulation test on slides (HBDT S). The method of Leynadier, Luce and Dry (1979) now automated was used.

Scoring of cell techniques. The sliding scale method of Siraganian (1977) was used, adapted to the needs of the present work, shown in Table 1.

Table 1. Sliding-scale system, devised by seraganian (adapted to present need)

Histamine release (%)	Allergen concentration		
	A	B	C
5.0-9.9	3	2	1
10.0-14.9	4	3	2
15.0-19.9	5	4	3
20.0-29.9	6	5	4
30.0-39.9	7	6	5
40.0-49.9	8	7	6
> 50.0	9	8	7

Add scores from A, B and C, then +3 if histamine release is positive at two allergen concentrations: +6 if histamine release is positive at all three allergen concentrations. Divide total score by 3, range of possible scores 0-10.

Allergen concentrations A < B < C log dilutions.

Results

Skin tests

Allergen control group. With all extracts, fifty-three gave negative skin prick tests, (80% of the population studied). Nine (31.5% of this same population) gave positive skin tests, but four (6%) of these, or relatives had a history of previous allergy. The remaining five (9.4%) with positive skin tests had no clinical history, and of these, three (5.7%) also had negative RAST. Five (9.4%) of those with negative skin tests gave positive RAST with one of the extracts.

The complete results are shown in Table 2.

Allergen-positive group. The subjects in this group gave variable results to the different dust extracts, but they always gave strong positives to two or three allergen extracts, not always the same.

Table 2. Agreement and non-agreement between results of skin test and RAST

	Skin tests	RAST	No. of subjects	%	Observations
I	-	-	53	80.0	
	+	+	4	6.0	Previous personal or familial allergy discovery later
	+	+	2	3.0	Positive only for dust D. The other <i>in vivo</i> and <i>in vitro</i> tests were negative for the other extracts
II	+	-	3	4.5	Dermographism?
	-	+	5	7.5	4 RAST positives with extract D, 1 RAST positive with extracts D and E

I: tests in which there is agreement; II: tests in which there is disagreement.

All the 'negative reference' solutions gave weals greater than 2 mm, so it seems that no comparison was possible with the negative control group. This may be a defect of the 'Multitest' device in the 9-point-head form, in showing mediator release by trauma in patients with an already reduced histamine threshold.

Table 3. Correlation between RAST and PRIST for the different allergen extracts

	RAST Bencard (D)	RAST <i>D.pt</i> (C)	RAST PMD(B)	RAST PM(A)	RAST Pasteur (E)	PRIST
RAST Bencard (D)	1.0					
RAST <i>D.pt</i> (C)	0.93	1.0				
RAST PMD(B)	0.97	0.92	1.0			
RAST PM (A)	0.96	0.92	0.96	1.0		
RAST Pasteur (E)	0.86	0.77	0.89	0.82	1.0	
PRIST	0.26	0.28	0.19	0.28	0.38	1.0

Threshold of significance $P < 0.05$, $r > 0.41$.

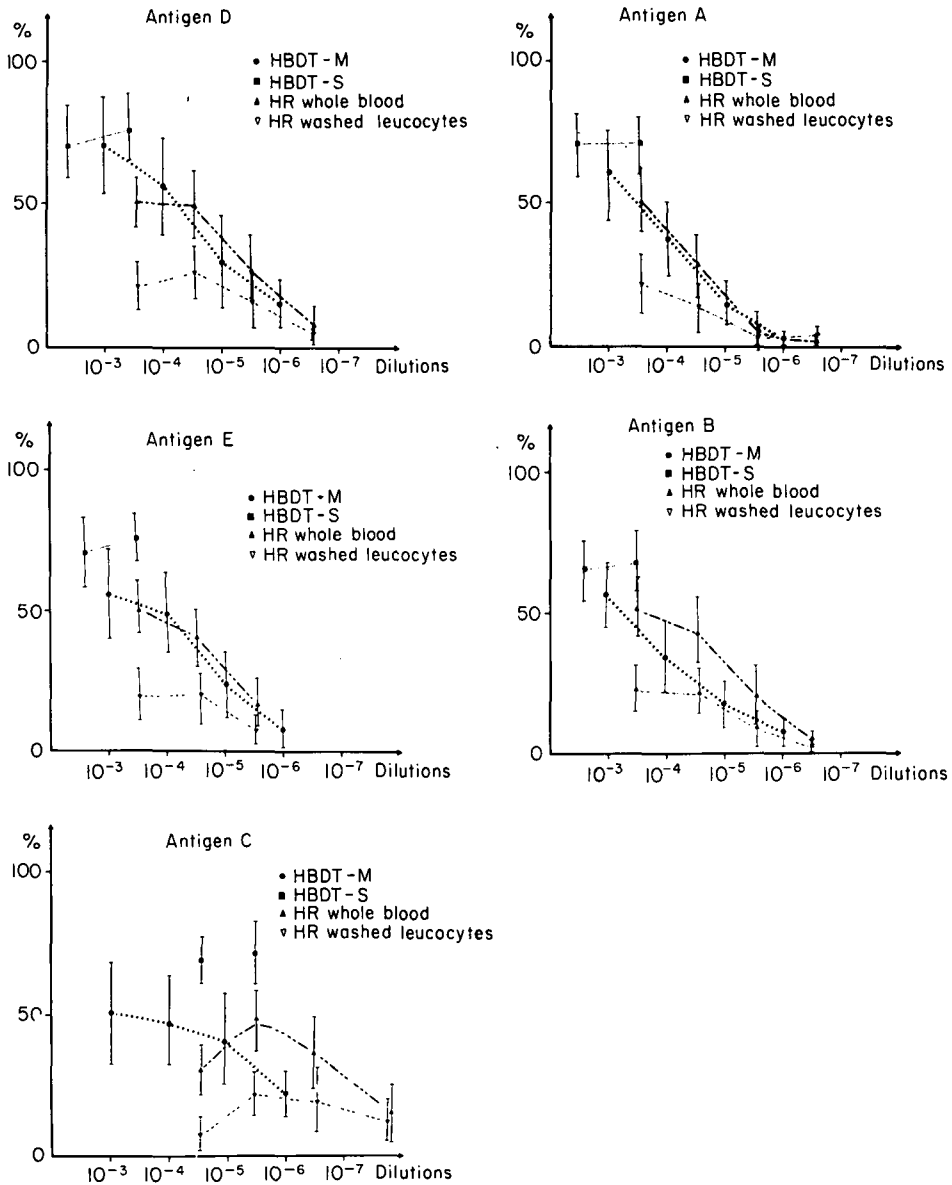


Fig. 1 Mean results from nineteen subjects. A comparison of different antigenic preparations used in human basophil degranulation test on microplates (HBDT M) and as slides (HBDT S), histamine release (HR) on whole blood and on washed leucocytes.

*In vitro tests**Rast*

Allergen-positive group. An excellent, significant correlation was found between RAST to the different dust extracts, but PRIST results were not significant. The results are in Table 3.

Allergen-negative group. No positive results were found.

HBDT and histamine release. The curves in Fig. 1 correspond to the mean results on nineteen subjects, obtained with each technique for each of the different allergen extracts. Each point on the curve has a vertical bar that shows the standard deviation.

Presentation in this way gives a good indication of general tendencies but it cloaks the scatter of individual results, and in some cases the scatter of results makes interpretation of a single test very difficult.

The results show:

- 1 Discrepancies in the results, depending on the methods used, leading to the opposing interpretations between, on the one hand HBDT S and histamine release and HBDT M on the other, with antigen D and antigen A, but showing good concordance with the mite extract, for which all the results are obviously positive, whatever the thresholds and dilutions considered.
- 2 In tests and methods done in parallel though with alterations according to the allergen. Degranulation is always superior to histamine release in whole blood or washed cells. HBDT S gives in this case a level of degranulation inferior to that of HBDT M, contrary to the usual pattern.

Discussion

Though the 'Multitest' device appears to be useful for allergenic challenge to dust constituents, or extracts from different manufacturers, it does not seem to permit in its present form determination of a scale of sensitivity, as it provokes strong skin reactions in allergic subjects and the allergy-negative group gave weals that by other prick-test methods would be considered to be positive. However, the simultaneous test with several extracts, together with different reference preparations avoids the risk of false negatives with extracts of variable antigenic composition.

It is not helpful to look for quantitative correlations between RAST and scores of different cellular methods, when the two types of techniques explore different immunological mechanisms. Non-allergic subjects may easily show very high specific IgE titres without their basophils responding with degranulation to antigenic provocation whilst conversely, atopic subjects with low titres of circulating IgE may carry on their basophils enough specific IgE to cause degranulation.

Patients 16 and 17 in this context are very significant since, although they are negative by RAST for several allergens and only weakly positive for the others, their degranulation curves are definitely positive with all the allergens. This observation is in agreement with the relatively poor correlations that have been reported in the literature between skin tests *in vivo* and *in vitro* RAST. It should also be remembered that IgG (S-TS), another class of anaphylactic antibodies, can give positive results in cellular techniques, where the physiological properties are assayed, but not in IgE-mediated RAST. (Parish, 1973).

In the present work, histamine release was perhaps the reference method for the following reasons:

- 1 Because it is done with whole blood it eliminates the statistical problems associated with the need to count a sufficient number of cells, and also the artefacts which arise from the concentration procedures for clean basophils in each of the HBDT techniques.
- 2 It is the only technique by which, regularly and systematically, in every case studied with mite extract, a typical bell-shaped curve can be obtained, which indicates an immunological reaction and antigen-antibody equilibrium.

Nevertheless, in the special case of housedust and mite extracts, the histamine release also sets a problem since it is much less characteristic than that found with pollen extracts in pollen patients (Stahl Skov, Norn & Weeke, 1977) and also because the type of mechanism responsible for histamine release is uncertain for the strongest concentration of allergen extracts studied, and thus for the response which allows in some cases the formation of a bell-shaped curve.

The possible intervention of a direct toxicity, or at least a non-physiological mechanism is excluded by the HBDT M results, which always include a control tube without calcium; a difference in response indicates intervention of a non-immunological mechanism.

More than 50% of the subjects showed large discrepancies in response with washed cells and whole blood, in that the level of response with washed cells, in patients who were ill, was less than 50% of that found with whole blood, and the values were very low, almost nil.

Spontaneous liberation of histamine during washing may be postulated, but the percentage spontaneously released in this study is almost the same for the group of subjects who showed disagreement of whole blood and washed cells, and this was under conditions where the histamine-release curves are very close.

However, this discrepancy does not invalidate the HBDT S results, which are almost always superior to those of the histamine release from whole blood, when the leucocytes are also washed and resuspended in Hepes buffer.

All of this reinforces the hypothesis of a plasma activation factor, which formed one of our initial hypotheses, involving the alternative pathway for activation of complement, possibly from endotoxins and cell-wall polysaccharides of contaminating bacteria and dust moulds, more especially as the mite extracts, which are much less contaminated than the dusts show the same discrepancies.

It is also possible that some loss of the outer layers of the cell surface, weakens the receptor- immunoglobulin bond, which in some subjects leads to detachment of these immunoglobulins which are lost in the washing liquid. In other subjects it only weakens the cells, which may be the explanation of these instances where release of histamine from washed cells is greater than that from whole blood. It is also possible that the disagreement can be explained by a competition in whole blood of free serum IgE and that which is fixed to target cells. (Okudaira *et al.*, 1980).

In theory, from a statistical interpretation of the results, HBDT M, which is done in a liquid medium without procedures that might modify the fundamental properties of the samples, is more satisfactory than HBDT S, which needs a step of drying on the slide. In practice, HBDT M results do raise problems because:

- 1 The total absence of degranulation recorded with *D. pteronyssinus* extract in the first seven patients studied is in complete disagreement with the results from the other techniques. This finding led us to discover a technique artefact which was corrected for the subsequent tests.

- 2 It rarely showed true bell-shaped curves, especially when provocation was by the strongest concentrations of mite extract, and response at that point was always high. This cannot be considered to be a simple artefact, since the absence of degranulation in the reference tube without calcium shows that it is probably not due to toxicity.

With the extract antigen E the curves are more often (7/19) bell-shaped and this may be explained by the particular composition of this fractionated extract, being less complex than the other dust extracts.

With these reservations HBDT M produced positive results that correlate well with histamine release and the clinical history of patients, and in no case has it given false positive or negative results

However, HBDT M is a rather long and laborious procedure, and it cannot be simplified for example by reducing the number of tubes, or omitting the no-calcium controls, without the risk of producing aberrant results.

The HBDT S method developed by Leynadier *et al.* (1979) is a treatment of target cells with antigen directly on the same slide that is subsequently used for observation of degranulation. This has both advantages and disadvantages.

The advantages are: (i) indisputable identification of basophils by direct microscopical reading; (ii) systematic stepping of the automated reading, that permits constant optical control; (iii) simultaneous treatment of each of the dilutions and standards under study, and this guards against the problems of staining that have been found by others; and (iv) prevention of the possible problems of cell adherence on the tube walls, as can happen with the automated reading of HBDT M.

The disadvantages are: (i) the necessity to concentrate basophils from the blood samples by a treatment which is theoretically little damaging to the cells, but which seems in practice to make them more fragile. It is possible to reduce this damage which does not seem to happen with simple, uncomplicated allergens, but which can cause problems in tests with housedust. It is interesting to note that the discrepancies, whatever the allergen, appear for the same patient and this raises the question of a possible technique artefact, or a special sensitivity of the subject's cells; and (ii) the deposition and drying of the cells on the slide which is not strictly appropriate to preparation of a film, and therefore introduces a systematic error.

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