

# Multidisc RAST: a reliable method for screening of specific IgE antibodies in serum?

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"Multidiscs" (md) are cellulose discs to which several allergens are coupled, allowing the simultaneous screening of several specific IgE antibodies. We compared RAST performed with md to RAST performed with the corresponding individual allergens coated on single discs (sd). One hundred and twenty-two RAST positive samples were analysed for four different allergen groups: moulds, cereals, weed pollens and tree pollens. We found lower RAST sensitivity when screening with mds than with the individual sds in all allergen groups except cereals. In 30 to 70% of the samples, the results were concordant. In 25.5 to 66.7%, md values were lower and in 1.3 to 26.3% higher than sd values. When only qualitative results were considered, the loss of sensitivity appeared acceptable: only 6.7 to 16.7% of the sd positive samples were not detected by md screening. A cost-benefit analysis, calculated on local data, confirmed economic advantages.

*Key words:* multidiscs; RAST; specific IgE.

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A complete allergy workup should be based on detailed clinical history, skin tests and determination of specific IgE levels in the serum. The test most widely used to identify allergen-specific IgE antibodies in the serum is the radio-allergosorbent test (RAST). Its routine use in clinical practice however, is controversial (1, 12, 13). It often provides confirmatory evidence of hypersensitivity and can be valuable in the management of patients in whom skin tests are not possible, for example, in cases of widespread dermatitis, dermographism or drug-induced suppression of the cutaneous reactivity (14). The major criticisms concern the RAST's lack of sensitivity and high cost, two drawbacks which limit the use of the test as a screening procedure in allergy (1). Nevertheless, an unfortunately increasing number of practitioners prefer the RAST for screening rather than devoting time in gathering the best information from patients' histories and skin tests.

Various methods have been suggested for lowering the costs of screening by RAST. Some authors recommend a screening battery based on the importance and frequency of allergens encountered in a given region (4, 5). Others have reduced the number of tests by fixing different antigens on the same disc (10) or on a fiber thread (3). These methods allow screening for multiple allergens in one single procedure.

Recently, so-called "multidiscs" (md) have been made commercially available and their clinical usefulness has already been examined in one study (7). They consist of cellulose discs to which a mixture of different allergens has been coupled. Simultaneous screening for several allergens in one single RAST determination is thus possible, but a positive result does not indicate which of the allergens tested is responsible for the positivity.

In the present study, we evaluated the reliability of mds as a diagnostic tool by comparing the results obtained with these discs to those obtained with the

corresponding single allergen coated discs (sd) and also calculated the saving in costs and time when using mds in routine laboratory work.

## MATERIAL AND METHODS

One hundred and twenty-two serum samples from patients known to be RAST positive were

Table 1  
Allergens used in the study

| Multidiscs | Corresponding individual allergens |    |     |     |     |
|------------|------------------------------------|----|-----|-----|-----|
| Mx1        | m1                                 | m2 | m3  | m6  |     |
| Fx3        | f4                                 | f7 | f8  | f10 | f11 |
| Wx3        | w6                                 | w9 | w10 | w12 |     |
| Tx5        | t2                                 | t4 | t8  | t12 | t14 |
| Tx6        | t1                                 | t3 | t8  | t7  | t10 |
| Tx9        | t2                                 | t3 | t4  | t7  | t12 |

### Abbreviations:

|                                   |                          |
|-----------------------------------|--------------------------|
| Mx1 : mixture of moulds           | Tx5,                     |
| m1 : <i>Penicillium notatum</i>   | Tx6,                     |
| m2 : <i>Cladosporium herbarum</i> | Tx9 : mixture of trees   |
| m3 : <i>Aspergillus fumigatus</i> | t1 : maple               |
| m6 : <i>Alternaria tenuis</i>     | t2 : grey alder          |
|                                   | t3 : common silver birch |
| Fx3 : mixture of cereals          | t4 : hazel               |
| f4 : wheat                        | t5 : american beech      |
| f7 : oat                          | t7 : white oak           |
| f8 : corn                         | t8 : white elm           |
| f10 : sesame seed                 | t10 : walnut             |
| f11 : buckwheat                   | t12 : willow             |
|                                   | t14 : cottonwood/poplar  |
| Wx3 : mixture of weeds            |                          |
| w6 : mugwort                      |                          |
| w9 : plantain                     |                          |
| w10 : goose foot                  |                          |
| w12 : golden rod                  |                          |

collected from 1984 to 1986 and kept at  $-70^{\circ}\text{C}$  until analysed. The samples consisted of four groups: 38 were positive for IgE specific to moulds, 40 for IgE to cereals, 29 for IgE to weed pollens and 15 to tree pollens. In the last group, three different mds were analysed. The samples were tested simultaneously with mds and sds. Determinations were repeated and evaluated separately for both types of discs. Ten samples of cord serum were used as a negative control and also tested with both mds and sds.

Mds are paper discs to which a mixture of allergens is covalently bound, whereas conventional sds are coated with one antigen. Both md and sd were provided by Pharmacia, AB, Uppsala, Sweden. Table 1 gives the list of allergens used in this study. Six mds were tested: one for moulds, one for cereals, one for weed pollens and three for tree pollens. Each sample was tested with one of the six mds and with the corresponding single antigens (sd).

A modified version of the Phadezym RAST was performed to determine specific IgE antibody levels, using the commercial Phadezym RAST kit from Pharmacia and a fluorescent reading (11). The paper discs were distributed on microtiter plates and 50  $\mu\text{l}$  of serum were added to each well. The plates were incubated for 60 min, washed 5 times and 50  $\mu\text{l}$  of enzyme-labeled anti-IgE were added to each well. The plates were incubated for 2 h and washed 5 times. After an incubation of 15 min with 200  $\mu\text{l}$  of the substrate, methyl-umbelliferyl- $\beta$ -D-galactoside, the reaction was terminated by adding 100  $\mu\text{l}$  of a stop solution. The

Table 2  
Reproducibility of RAST assays

| Antigen group | No. of sera | No. of tests | Number and % of RAST assays in which results moved from one class to another when test was repeated |      |                    |     |       |      |
|---------------|-------------|--------------|-----------------------------------------------------------------------------------------------------|------|--------------------|-----|-------|------|
|               |             |              | Changing 1 class                                                                                    |      | Changing 2 classes |     | Total |      |
|               |             |              | No.                                                                                                 | %    | No.                | %   | No.   | %    |
| Mx1           | 38          | 190          | 44                                                                                                  | 23.2 | 1                  | 0.5 | 45    | 23.7 |
| Fx3           | 40          | 240          | 52                                                                                                  | 21.7 | 6                  | 2.5 | 58    | 24.2 |
| Wx3           | 29          | 145          | 28                                                                                                  | 19.3 | 2                  | 1.4 | 30    | 20.7 |
| Tx5, 6, 9     | 15          | 195          | 33                                                                                                  | 16.9 | 4                  | 2.1 | 37    | 20.0 |
| Total         | 122         | 770          | 157                                                                                                 | 20.4 | 13                 | 1.7 | 170   | 22.1 |

fluorescence of 200 µl of the solution was measured in a fluorometer. All incubations were performed at room temperature. The samples were compared to various reference sera, and the levels of specific IgE expressed in Phadezym/RAST units (PRU) per ml or in Phadezym/RAST classes. A value below 0.35 PRU corresponded to RAST class 0, a value between 0.35 and 0.7 PRU to class 1, between 0.7 and 3.5 to class 2, between 3.5 and 17.5 to class 3 and above 17.5 to class 4. The quality control "Phadexact", provided by the manufacturer, was included in every assay, as a positive control.

Five hundred and fifty-three serum samples, which were sent to the Lausanne laboratory over a 12-month period for routine RAST determinations, served as a model for cost calculation. This material, with which 4289 allergen determinations had been made with sds, was used in the present study to calculate the number of discs necessary if samples had first

been screened by mds and then positive samples subsequently tested with all corresponding sds. The figure obtained was compared with the number of sds actually used. The list of the seven mds considered for this calculation is given in Table 5.

RESULTS

Table 2 documents the reproducibility of our RAST assays for each of the four antigen groups tested. In 22.1 % of the samples, a different result was obtained when the test was repeated. A difference of one class was obtained in 20.4 % and of two classes in 1.7 %. A change of three or four classes was not observed. The reproducibility obtained at the same time with sds was comparable. No batch-to-batch variations were observed when tests were repeated.

In Table 3, the results of RAST classes obtained with mds were compared with those found with sds for the corresponding individual

Table 3  
Comparison between RAST classes obtained with multidiscs (vertical) and the highest class obtained with corresponding single discs (horizontal). Figures indicate the number of tests performed

| RAST class<br>obtained with md | Rast class obtained with sd |   |   |    |    |    | Fcx3 |   |    |    |   |   |
|--------------------------------|-----------------------------|---|---|----|----|----|------|---|----|----|---|---|
|                                | Mx1                         | 0 | 1 | 2  | 3  | 4  | 0    | 1 | 2  | 3  | 4 |   |
|                                | 0                           | 2 | 3 | 5  |    |    | 0    | 4 | 7  |    |   |   |
|                                | 1                           |   | 4 | 26 |    |    | 1    | 4 | 14 | 8  |   |   |
|                                | 2                           |   | 1 | 20 | 11 |    | 2    |   | 5  | 18 | 5 |   |
|                                | 3                           |   |   |    | 4  |    | 3    |   | 1  | 10 | 2 |   |
|                                | 4                           |   |   |    |    |    | 4    |   |    |    | 1 | 1 |
|                                | Wx3                         |   |   |    |    |    | Tx5  |   |    |    |   |   |
|                                | 0                           | 1 | 2 | 3  | 4  |    | 0    | 1 | 2  | 3  | 4 |   |
|                                | 0                           | 2 | 3 | 2  |    |    | 0    | 7 | 2  |    |   |   |
|                                | 1                           | 1 | 6 | 3  |    |    | 1    | 1 |    |    |   |   |
|                                | 2                           |   | 2 | 19 | 4  |    | 2    |   | 9  | 1  |   |   |
|                                | 3                           |   |   |    | 3  | 13 | 3    |   |    | 2  |   | 5 |
|                                | 4                           |   |   |    |    |    | 4    |   |    |    |   | 3 |
|                                | Tx6                         |   |   |    |    |    | Tx9  |   |    |    |   |   |
|                                | 0                           | 1 | 2 | 3  | 4  |    | 0    | 1 | 2  | 3  | 4 |   |
|                                | 0                           |   | 3 |    |    |    | 0    |   | 3  | 2  |   |   |
|                                | 1                           |   | 2 | 3  | 1  |    | 1    |   | 2  | 3  |   |   |
|                                | 2                           |   |   | 1  | 6  |    | 2    |   |    |    | 3 |   |
|                                | 3                           |   |   |    | 1  | 2  | 3    |   |    |    | 6 | 7 |
|                                | 4                           |   |   |    |    | 4  | 4    |   |    |    |   | 4 |

allergens. Results obtained with mds were lower than those obtained with sds, with the exception of the cereal group (Fx3). Table 4 indicates the percentage of concordant and discordant results: In 30 to 70 % of the samples, the specific IgE level found with md was identical, in 25.5 to 66.7 % it was lower and in 1.3 to 26.3 % higher than those obtained with sd. The correlation md/sd differed substantially between the different antigen groups. The best correlation among tree pollens was found in Tx5 and the poorest in Tx6 group. Among the non-tree pollen groups, mds of the cereal group (Fx3) corresponded best and mds of the mould group (Mx1) worst to sd results.

Table 4 also indicates (last column) the number of samples found positive by screening for

individual antigens and negative in md RAST. This qualitative discordance was seen in 6.7 % (Tx5) to 16.7 % (Tx9) of the cases. In the mould group (Mx1) 10.5 % and in the cereal group (Fx3) 8.6 % of positive sd sera escaped detection by md RAST. On the other hand, positive md along with negative sd values were seen in only six cases: 4 (6.5 %) belonging to the Fx3 group, one (1.7 %) to the Wx3 and one (3.3 %) to the Tx5 group (Table 3), and, in all these cases, the sd result turned positive when the test was repeated. All md and sd RAST performed on cord serum samples were negative.

The number of discs effectively used to test the routine material of 1 year with only sds and the number which would have been used in

Table 4

Comparison between RAST classes found with multidiscs and the highest RAST classes obtained on single coated discs. Right-hand column indicates the number of tests where an sd positive escaped md screening

| Md (group of allergens) | Total No. tests | Concordant % | Discordant results |           | Tests with neg. md RAST and pos. sd results |      |
|-------------------------|-----------------|--------------|--------------------|-----------|---------------------------------------------|------|
|                         |                 |              | md > sd %          | md < sd % | n                                           | %    |
| Mx1                     | 76              | 39.5         | 1.3                | 59.2      | 8                                           | 10.5 |
| Fx3                     | 80              | 48.8         | 26.3               | 25.5      | 7                                           | 8.8  |
| Wx3                     | 58              | 51.7         | 5.2                | 43.1      | 5                                           | 8.6  |
| Tx5                     | 30              | 70.0         | 3.3                | 26.7      | 2                                           | 6.7  |
| Tx6                     | 30              | 30.0         | 3.3                | 66.7      | 3                                           | 10.0 |
| Tx9                     | 30              | 40.0         | 0                  | 60.0      | 5                                           | 16.7 |

Table 5

Number of discs used if mds had been substituted by sds on our routine material over a 12-month period. Calculation is made on the assumption that the seven mds listed in the first column had been substituted by sds wherever possible

| Allergen tested                  | No. of tests with sds | No. of tests with mds | Reduction in % | Reduction in % of whole RAST screening |
|----------------------------------|-----------------------|-----------------------|----------------|----------------------------------------|
| d1 d2 h1 h3                      | 1216                  | 768                   | 36.8           | 10.45                                  |
| m1 to m6                         | 594                   | 255                   | 57.1           | 7.91                                   |
| f1 to f3                         | 87                    | 50                    | 42.5           | 0.86                                   |
| f4 to f8                         | 165                   | 88                    | 46.7           | 1.80                                   |
| f12, 13, 17, 20, 25, 26, 31, 33  | 96                    | 44                    | 54.2           | 1.21                                   |
| w6 to w10                        | 135                   | 92                    | 31.9           | 1.00                                   |
| t3, 4, 5, 7                      | 108                   | 58                    | 46.3           | 1.17                                   |
| Other allergens not on these mds | 1894                  | 1894                  | 0              | 0                                      |
| Total                            | 4295                  | 3249                  |                | 24.40                                  |

d1 and d2 = *Dermatophagoides pteronyssinus* and *farinae*. h1 and h2 = house dust extracts. For other allergens see Table 1.

screening with mds are given in Table 5. An overall reduction of 4295 to 3249 discs (-24.4%) would have been obtained.

## DISCUSSION

The simultaneous screening of several IgE antibodies by RAST, using a disc coated with several allergens, is a more rapid and less expensive procedure than conventional RAST screening. It is justified if the already low sensitivity of RAST is not further decreased by multi-coating of the solid phase.

We found that simultaneous screening of reactivity to several allergens by using mds was an acceptable procedure when only qualitative results (positive or negative) were considered: 90.5% of the sd positivities were also detected by mds. The highest percentage of sd-positive samples missed by md testing was obtained with Tx9 (16.7%) and the lowest with Tx5 (6.7%). The risk of false positive reactions with md testing appeared to be negligible. All cord blood samples were also negative, as expected (1, 7, 9). It is certainly more relevant for clinical screening to distinguish between positive and negative, rather than to quantitate the *in vitro* reaction. The correlation between specific IgE antibody levels and clinical symptoms is anyway poor (9). In this respect, md RAST, which is intended for screening, appears fairly reliable.

When the different RAST classes were considered, however, testing with mds appeared less sensitive than testing with sds (Table 3). This applied particularly for the groups Mx1, Wx3 and the tree pollen discs Tx5, 6, 9, whereas the Fx3 group showed a better sensitivity since the number of samples md neg./sd pos. was equal to that of md pos./sd neg. The high interassay variability (Table 2) shows how careful one must be when interpreting RAST quantitatively, even if a quality control is included. It also shows that the risk of false negative md RAST results can be reduced by repeating the test.

The use of mds is also justified if cost-benefit analyses calculated on local data confirm economic advantages. Indeed, if samples have

been selected in such a way that many are positive, and if the individual specificities are to be determined in a second step, the procedure might eventually become more expensive. Subsequent determination of individual specificities might be desirable, for instance, prior to hypo-sensitization. On the other hand, if samples to be tested are predominantly negative the advantage of md screening is obvious.

The cost-benefit relationship calculated on the samples entered over a 12-month period confirmed that testing with mds might be beneficial, even if md positive samples are subsequently tested with the corresponding sds. The 24.4% reduction in the number of discs will give a similar reduction in costs only if the prices of mds and sds are the same.

We conclude that screening for specific IgE by using RAST multidiscs instead of the conventional discs coated with one antigen is time- and cost-saving and constitutes a reasonably sensitive procedure when only qualitative results are required. The loss of sensitivity, quite moderate when only positive/negative results are considered, was more evident when results were expressed semi-quantitatively in RAST classes.

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