

Optimization of skin testing

I. Choosing allergen concentrations and cutoff values by factorial design

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cutoff values by factorial design.
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Standardized extracts of *Phleum pratensis* (grass) and *Dermatophagoides pteronyssinus* (house-dust mite) were used as test allergens for multiple regression in order to determine optimum concentrations and cutoff values with regard to diagnostic capacity and safety. If a RAST value of class 1 or more was taken as an indication of sensitization, it was found that the optimum concentrations for the detection of sensitization are in the range 10–100 BU/ml and 1500–10 000 BU/ml for intracutaneous tests (ICT) and skin prick tests (SPT), respectively. The skin test results were expressed as histamine ratios. Using allergen concentrations of 30 and 3000 BU/ml, we found cutoff values of 0.87 and 0.53 and predictive values of 87.1% and 79.1% for ICT and SPT, respectively. The maximum wheal size (mean wheal size + 2 SD) to be expected in 95% of the population was 26.6 mm (ICT) and 10.9 mm (SPT), sizes regarded as safe by most clinicians. In conclusion, by using this method with a limited number of patients, one can probably improve the diagnostic precision and safety of the skin test. In the second part of this study, these hypotheses were prospectively tested in a multicenter study.

Clinical aspects

A multiple regression method is presented to determine optimum allergen concentrations and cutoff values for skin testing. *Phleum pratensis* (grass) and *Dermatophagoides pteronyssinus* (house-dust mite) were used as test allergens. It was found that the optimum concentrations for the detection of sensitization are in the range 10–100 BU/ml and 1500–10 000 BU/ml, for intracutaneous tests (ICT) and skin prick tests (SPT), respectively. From these ranges, 30 BU/ml (ICT) was chosen in order to limit the maximal expected wheal size. For the SPT, a concentration of 3000 (SPT) BU/ml was chosen. These test concentrations corresponded to cutoff values of 0.87 (ICT) and 0.53 (SPT), resulting in predictive values of 87.1% (ICT) and 79.1% (SPT), as compared with RAST. By this method with a limited number of patients and well-standardized allergens, one may be able to improve the diagnostic capacity and safety of the skin test.

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Skin prick tests (SPT) and intracutaneous tests (ICT) are widely used to detect IgE-mediated allergy (2, 4, 8, 10–12, 14, 15, 17, 23–25, 27). In the skin test guidelines (9) published in 1989, the following recommendations were made: 1) use a well-documented method with a documented reproducibility; 2) use standardized allergens with a potency whose diagnostic effect has been documented.

The predictive value of SPT, using standardized

allergen preparations, has been studied by several authors (8, 10, 14, 25, 27), but little recent information is available about the diagnostic capacity of ICT. The ICT was found to have a good concordance with RAST (2), but false positive results of the ICT have been reported (4, 26).

The data that have been published on the safety of skin tests indicate that systemic side-effects occur in 0.03–0.48% of the tested patients (6) and that

minor adverse reactions occur in a maximum of 0.49% of the tested patients (29). However, fatalities have also been reported to follow skin tests (16).

Only a few studies deal with the determination of optimum concentrations and cutoff values of SPT and ICT in a systematic way. Petersson et al. (25) recommend 100 000 BU/ml of birch and timothy allergen preparations for diagnosis of reagenic allergy by SPT, while Aas & Belin (1) recommend 10 000 BU/ml of the same product. Little is known about the optimum concentration and cutoff value of ICT.

The cutoff limit of a skin test is very important, since, at a given concentration, it determines the predictive value. For SPT, cutoff limits of > 3 mm (5, 8, 13, 19, 31) are reported, and Aas & Belin (1) proposed a grading system in which the wheal size is divided by the histamine wheal. Norman (21) suggested a 2+ reaction (wheal size 5–10 mm) of the ICT as a criterion for positivity.

In the past, large batch-to-batch variations of allergenic extracts limited the implications of studies on optimal potency and cutoff limits for skin tests. However, since standardized allergen extracts have become available, a good batch-to-batch consistency can be achieved.

In this study *Phleum pratensis* (grass) and *Dermatophagoides pteronyssinus* (house-dust mite) were used as test allergens in a multivariate design in order to determine the optimum sensitivity and specificity of the SPT and ICT in relation to the concentration of the allergen preparations, and the cutoff limit of the skin tests. For this purpose, a common method to select variables for multiple regression was chosen (3, 7). These last references also contain further details and examples of proper method selection, multivariate designs, and multiple regression.

Material and methods

Patients and study design

A total of 41 patients who visited the allergy department of the University Hospital of Groningen were selected for both ICT and SPT, after giving informed consent to take part in this study. There were 19 men and 22 women, and the mean age was 31.6 years (range 19–54).

Patients were not allowed to use antihistamines or oral corticosteroids during the 48 h before skin testing, in accordance with the skin test standardization report (20).

Specific IgE determinations were performed for *D. pteronyssinus* and *P. pratensis* by Phadebas RAST (Pharmacia AB, Uppsala, Sweden). The results were divided into five RAST classes; RAST class ≥ 1 was regarded as an indication of sensitization.

Allergen preparations

Standardized extracts (ALK, Hørsholm, Denmark; obtained through Laboratorium Diephuis BV, Groningen, the Netherlands) of *D. pteronyssinus* (1000 BU corresponds to 0.15 μ g *Der p* I) and *P. pratensis* (1000 BU corresponds to 0.46 μ g *Phl p* V) were used with concentrations of 50, 500, 5000, and 50 000 BU/ml (SPT), and 0.05, 0.5, 5, 50, and 500 BU/ml (ICT).

All allergen extracts were dissolved in phosphate-buffered saline pH 7.4, and they contained 0.5% phenol and 0.03% human serum albumin (ICT), or 50% glycerol (SPT). The solutions were kept at 4–8°C for a maximum of 2 weeks and used at ambient temperature. The positive control was histamine phosphate (base) 0.01 mg/ml (ICT) and histamine dihydrochloride (base) 3 mg/ml (SPT); the diluent was used as a negative control.

Skin prick test

The SPT was performed according to Dreborg's guidelines for skin testing (9) by directing an ALK prick test lancet (ALK, Hørsholm, Denmark) through the allergen droplet at an angle of 90° to the skin. The droplet was removed 30–60 s after the prick. The results were recorded after 15 min by circumscribing the wheal in ink and transferring the imprint of the wheal to adhesive tape.

Intracutaneous test

Intracutaneous testing was performed in accordance with Dreborg's guidelines for skin testing (9). Briefly, 0.03 ml allergen extract was injected into the skin of both forearms at ventral sites at least 5 cm apart, with a 0.5-ml syringe (Monoject® no. 8881-500501, Sherwood Medical, St Louis, MO, USA). The results were recorded after 15 min by circumscribing the wheal in ink and transferring the imprint of the wheal to adhesive tape.

The mean wheal diameter was calculated by adding the longest diameter to the orthogonal diameter measured at one-half the longest diameter, and dividing by two. All allergen wheal sizes were divided

Table 1. Variables used in the STATGRAPHICS computer program

Independent variables	X=log (concentration) (BU/ml) Y ₁ =cutoff value of the skin test (mm) Y ₂ =RAST class of patients
Dependent variables	Z ₁ =specificity or sensitivity(%) Z ₂ =wheal size of skin test (mm)
First order	X; Y
Second order	X*Y; X*X; Y*Y
Third order	X*X*Y; Y*Y*X

by the histamine wheal sizes to obtain a histamine-equivalent wheal size (HEWS).

Statistical analysis

Mean wheal sizes, their standard deviations, sensitivity, and specificity were calculated by an SPSS/PC + computer program (22).

Optimization by means of a multivariate design

In a 5×4 (ICT) or 4×6 (SPT) factorial design, the influence of the cutoff value and concentration on the sensitivity and specificity of the skin tests (as compared with RAST) was determined. The concentrations used were 0.05, 0.5, 5, 50, and 500 BU/ml (ICT) and 50, 500, 5000, and 50000 BU/ml (SPT); the cutoff values used of the HEWS were 0.5–1.2 (ICT) and 0.3–1.2 (SPT). In the same factorial design, the influence of the concentration and the sensitivity of the patient (RAST class) on the wheal size of the skin test was determined.

The best fitting regression models were calculated by the STATGRAPHICS computer program (ver-

sion 2.0; STSC, Inc., Rockville, MD, USA) with the option (stepwise) forward selection of variables. A set of seven variables was used for the calculations (Table 1). One a variable has been entered, all variables in the equation are again examined for removal. This process continues until the best fit is obtained.

Calculation of optimal cutoff value and concentration

The optimum predictive value of a skin test was obtained when both the sensitivity and specificity were balanced; this balance was achieved at equal sensitivity and specificity. Therefore the line of intersection of the planes defined by the equations of the sensitivity and specificity was calculated by equating both equations. The obtained equation related the cutoff value to the concentration at optimum sensitivity and specificity.

The last equations were entered in equations 1–8 of the sensitivity or specificity. Equations were obtained that related the concentration to the optimum value of the sensitivity and specificity.

The optimum combination of the cutoff value and

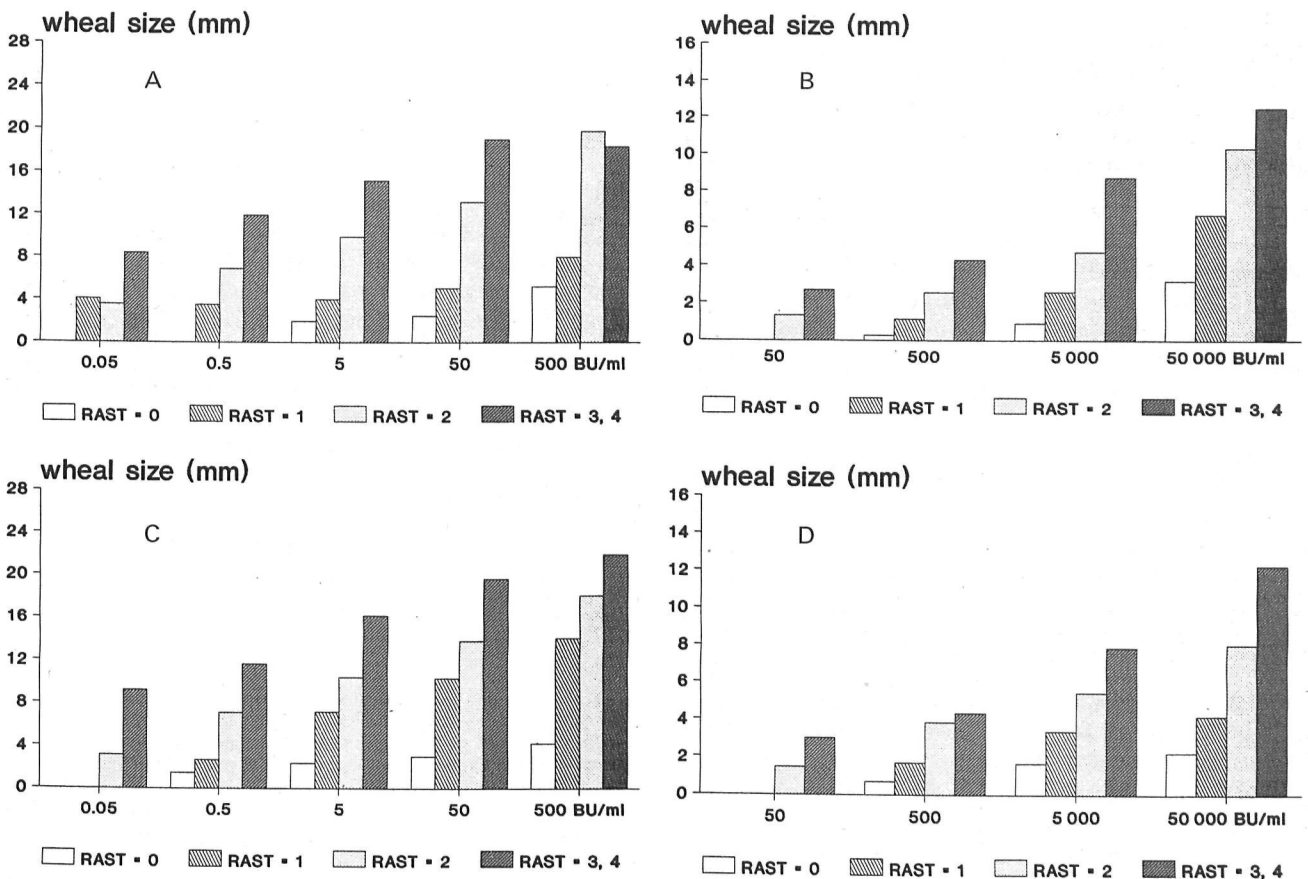


Fig. 1. Mean wheal size for different allergen concentrations and RAST classes of patients sensitive to *P. pratensis* by ICT (A) and SPT (B), and to *D. pteronyssinus* by ICT (C) and SPT (D). For *P. pratensis*, 11, 4, 10, and 16 patients were of RAST classes 0, 1, and 2 and 3 and 4, respectively. For *D. pteronyssinus*, 10, 6, 11, and 14 patients were of RAST classes 0, 1, 2, and 3 and 4, respectively.

concentrations was found at the highest sensitivity and specificity.

The safety of the skin test plays a very important role in the optimization of the concentration of the allergenic preparations. The maximum acceptable wheal size in 95% of the population was arbitrarily set at 30 mm (ICT) and 15 mm (SPT); these are regarded as safe by most clinicians. From the calculated optimum concentrations, the expected mean wheal size was calculated by equations 9–12. Two times the standard deviation was added to this mean wheal size. The obtained maximum wheal size should not be larger than the maxima described above.

Results

Histamine

The reproducibility of both the ICT and SPT was measured by the histamine control. The mean his-

tamine wheals were 10.4 mm (ICT) and 5.06 mm (SPT); the coefficients of variation within patients were 5.66% (ICT) and 5.96% (SPT), and between patients 11.6% (ICT) and 21.2% (SPT).

Allergens

The mean wheal sizes of *P. pratensis* and *D. pteronyssinus* for the different allergen concentrations and RAST classes of patients are depicted in Fig. 1. With these data, the sensitivity and specificity of both skin tests, as compared with RAST, were calculated for every combination of concentration and cutoff value.

Table 2 shows the results of the sensitivity and specificity of the multivariate design for ICT and SPT of both allergens. In Fig. 2 the sensitivity and specificity are plotted as a function of the concentration and cutoff value for the SPT and *D. pteronyssinus*.

Table 2. Results of the factorial designs, expressed as percentage of sensitivity (upper) and specificity (lower)

P. pratensis

Cutoff value	concentration (BU/ml) <i>P. pratensis</i>									
	SPT					ICT				
	50	500	5000	50 000		0.05	0.5	5	50	500
0.3	69.6	80.0	93.3	96.7						
	100	90.9	72.7	36.4						
0.4	69.6	80.0	86.7	96.7						
	100	90.9	85.4	36.4						
0.5	47.8	63.3	83.3	96.7	74.1	83.3	93.3	93.1	95.7	
	100	90.9	81.8	36.4	100	100	72.7	72.7	54.5	
0.7	13.0	36.7	76.7	90.0	51.9	70.0	86.7	89.7	95.7	
	100	100	90.9	81.8	100	100	81.9	72.7	63.6	
1.0	0.0	20.0	56.7	83.3	14.8	40.0	66.7	82.8	91.3	
	100	100	100	90.9	100	100	100	90.9	81.8	
1.2	0.0	6.7	46.7	80.0	0.0	23.3	40.0	72.4	91.3	
	100	100	100	90.9	100	100	100	100	81.8	

D. pteronyssinus

Cutoff value	Concentration (BU/ml) <i>D. pteronyssinus</i>									
	SPT					ICT				
	50	500	5000	50 000		0.05	0.5	5	50	500
0.3	65.0	87.1	93.5	93.3						
	100	70.0	50.0	40.0						
0.4	65.0	83.9	93.5	93.3						
	100	80.0	60.0	40.0	100	75.0	70.0	70.0	60.0	
0.5	65.0	53.7	93.5	93.3	63.0	80.6	93.5	93.5	91.3	
	100	90.0	70.0	40.0	100	87.5	70.0	70.0	60.0	
0.7	15.0	45.2	77.4	86.7	51.9	71.0	87.1	93.5	91.3	
	100	100	70.0	70.0	100	100	80.0	70.0	60.0	
1.0	0.0	22.6	51.6	80.0	11.1	38.7	71.0	83.9	87.0	
	100	100	100	90.0	100	100	100	90.0	80.0	
1.2	0.0	6.5	45.2	73.3	0.0	22.6	54.8	74.2	87.0	
	100	100	100	90.0	100	100	100	100	90.0	

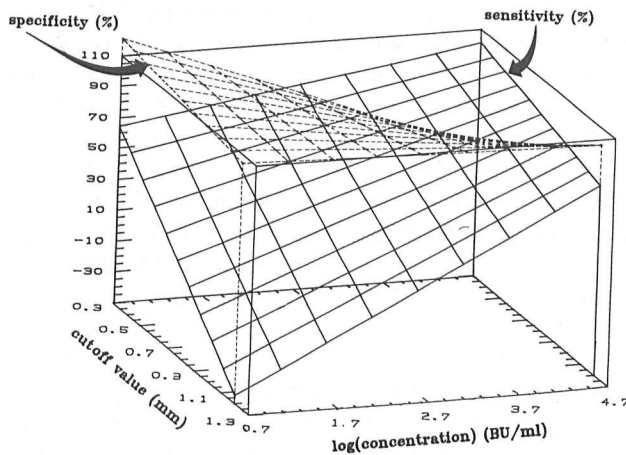


Fig. 2. Sensitivity and specificity plotted as a function of the concentration and cutoff value for *D. pteronyssinus* by SPT.

The calculated regression equations are shown in Table 3. From the regression equations of the sensitivity and the specificity for each skin test and each allergen, an equation of the intersecting line was calculated at which sensitivity equals specificity (equations not shown). these lines are depicted in Fig. 3.

By entering the equations obtained in equations 1, 3, 5, and 7, respectively, we obtained the values of the optimum sensitivity and specificity at different concentrations. The results of these calculations are depicted in Fig. 4. Table 4 shows the calculated regression equations in which wheal size is a function of the concentration of the allergen preparation and the RAST class of the patients tested.

From Figs. 3 and 4 it was concluded that the optimum concentrations are in the range 1–100 BU/ml and 1500–10 000 BU/ml for ICT and SPT, respectively. From these ranges, concentrations of 30 and 3000 BU/ml were chosen, corresponding to optimum cutoff values of 0.87 and 0.53 for ICT and SPT, respectively.

The sensitivity/specificity were 83.6% and 76.5%, and the predictive values were 87.1% and 79.1% for ICT and SPT, respectively.

Table 3. Calculated regression equations for sensitivity and specificity, *P. pratensis* (PP) and *D. pteronyssinus* (DP), by ICT and SPT

Equation	Z=	R-sq (adj) ¹
Sens DP ² /SPT (1)	98.67+2.59 X-136.96 Y+22.41 XY	0.9296
Spec DP/SPT (2)	146.86-28.70 X-34.13 Y+22.70 XY	0.9290
Sens PP ³ /SPT (3)	93.99+3.17 X-138.62 Y+24.04 XY	0.9526
Spec PP/SPT (4)	109.19+6.21 X-47.75 Y+23.96 XY	0.8910
Sens DP/ICT (5)	123.34-0.25 X-71.54 Y+23.79 XY-3.67 X ²	0.9799
Spec DP/ICT (6)	73.82-17.53 X+22.24 Y+12.46 XY	0.8985
Sens PP/ICT (7)	127.97-5.07 X-79.75 Y+26.14 XY-1.76 X ²	0.9821
Spec PP/ICT (8)	82.05-16.26 X+16.52 Y+12.71 XY-1.47 X ²	0.8957

¹ R-square adjusted.

² *D. pteronyssinus*.

³ *P. pratensis*.

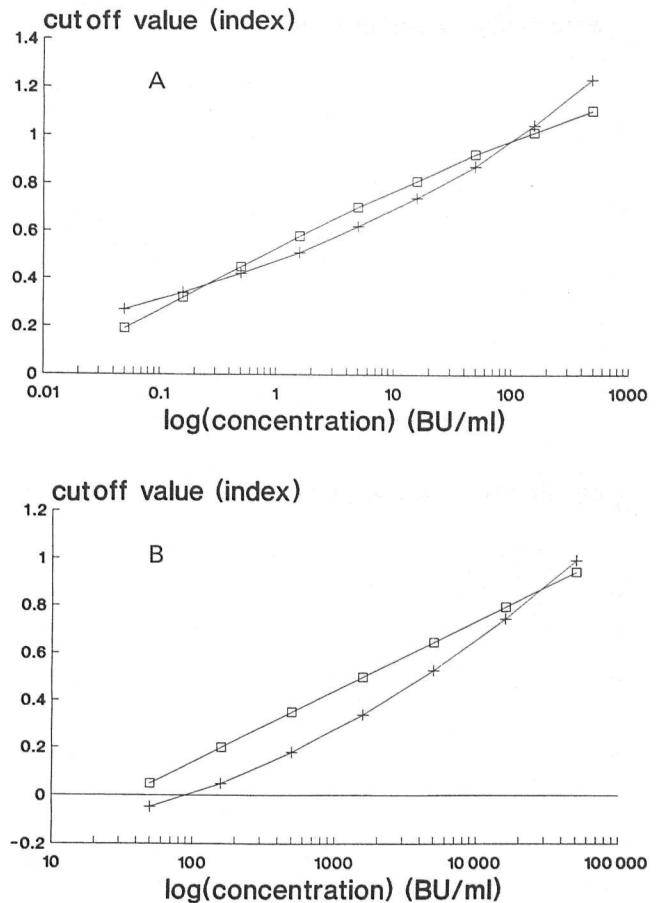


Fig. 3. The cutoff value plotted as a function of the log(concentration) at optimum sensitivity and specificity for ICT (A) and SPT (B), with *P. pratensis* (+) and *D. pteronyssinus* (□).

Safety

The maximum wheal size which could be expected in sensitive patients (RAST classes 3 and 4) was calculated by equations 9–12. The maximum wheal sizes (mean $\pm$ 2 SD) were 26.6 mm (ICT) and 10.9 mm (SPT).

Discussion

In two multivariate designs the sensitivity and specificity for two allergens in the two types of skin tests were determined at various concentrations and cutoff values. The optimum combinations of concentrations and cutoff values were defined by maximal sensitivity and specificity.

The results of the relation between the log(concentration) and sensitivity/specificity indicated that for a high sensitivity/specificity the concentration of the allergen should be as low as possible. This is also relevant to safety. The value of the concentration is limited by the practical usefulness of the related cutoff value. In practice, a cutoff limit of 0.5–1.0 (ICT) and 0.4–1.0 (SPT) is most suitable for a good interpretation of a skin test. Smaller cutoff limits cause

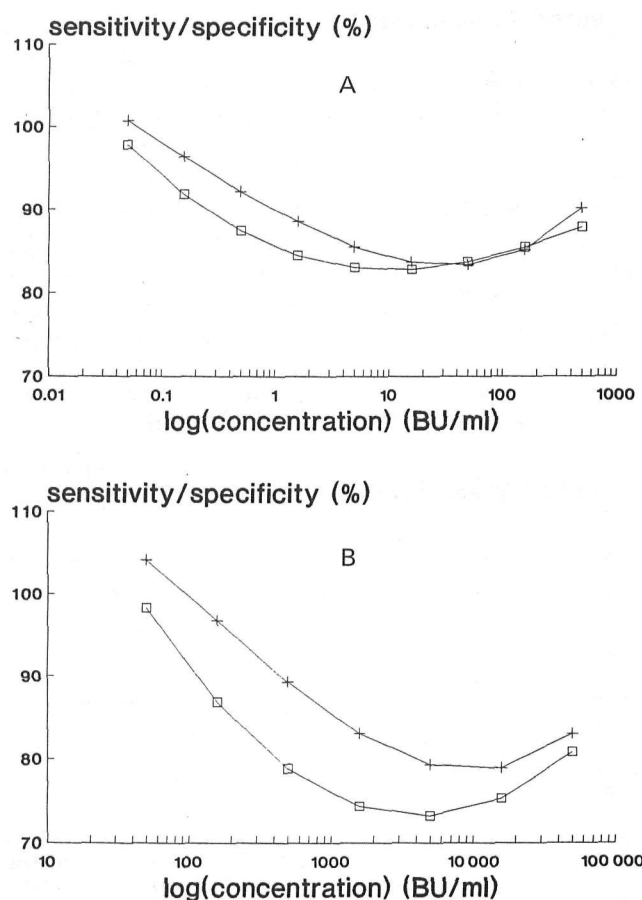


Fig. 4. Optimum sensitivity or specificity plotted as a function of log(concentration) for ICT (A) and SPT (B), with *P. pratensis* (+) and *D. pteronyssinus* (□).

interpretation problems, because of the skin bleb caused by the injection volume (ICT), and because of the difficulty of measuring very small wheal sizes.

The optimum concentrations for SPT (1500–10000 BU/ml) were lower than those reported by Petersson et al. (25), but agreed with the findings of others (1, 8). The optimum concentrations of ICT (10–100 BU/ml) could not be compared, because no results have been published for comparable allergens. It is remarkable that the optimum concentrations, expressed in BU/ml, for two completely dif-

ferent allergens are so close numerically. This may mean that the optimum concentrations are also valid for other standardized allergen extracts whose potency is also expressed in the Nordic Biologic Unit system. In the second part of this study we tested this hypothesis. It should be noted, however, that the optimum concentrations that were found are characteristic of these preparations and may not be applicable to the preparations of other manufacturers.

The cutoff limit of SPT (0.52 ≈ 3 mm) agrees with the findings of others (10, 13, 14, 25). The cutoff limit of ICT (0.83 ≈ 7.5 mm) is in the range of a positive ICT (5–10 mm) of the grading system as proposed by Norman (21). However, the cutoff limit of our preparation is more precise, making a more detailed analysis possible.

The mean predictive values of both skin tests of 87.1% and 79.1% for ICT and SPT, respectively, are comparable with, or better than, those reported by others (10, 24, 25, 30).

In all calculations, HEWS was used rather than wheal size, since other studies performed in our group have shown that wheal indices allow a better comparison between skin tests results, especially in multicenter studies.

In carefully selected patients, it is theoretically possible to use lower allergen concentrations (in both skin tests) in combination with a lower cutoff value, but, as these skin tests were intended for use in clinical practice, a higher cutoff value and concentration was preferred, in order to avoid interpretation problems. The maximum wheal sizes to be expected in 95% of a population of sensitive patients were well below the preset limits, indicating that these concentrations are well tolerated, even in highly sensitized patients. The reproducibility within patients was comparable to the findings of others (8, 18, 19, 28), indicating that the injection technique used in this study is acceptable.

In conclusion, by using this method in a limited number of patients, we optimized the allergen concentration and cutoff value for skin testing, which were strikingly similar for the two allergens tested. This method is applicable only to well-standardized

Table 4. The calculated regression equations relating the concentration of the allergens *P. pratensis* (PP) and *D. pteronyssinus* (DP), and the RAST class of patients to wheal size

Equations	Z=	R-sq (adj) ¹
DP ² SPT (9)	$1.45 - 1.24 X - 0.44 Y + 0.64 XY + 0.32 X^2$	0.9060
PP ³ SPT (10)	$5.46 - 4.65 X - 0.80 Y + 0.89 XY + 0.89 X^2$	0.9716
DP ICT (11)	$1.16 + 1.38 X + 3.96 Y + 1.73 XY + 0.009 X^2 - 0.019 X^2 Y - 0.31 Y^2 X$	0.9672
PP ICT (12)	$-0.54 + 0.068 X + 3.90 Y + 2.60 XY + 1.05 X^2 + 0.16 Y^2 - 0.48 X^2 Y - 0.43 Y^2 X$	0.9672
		0.9581

¹ R-square adjusted.

² *D. pteronyssinus*.

³ *P. pratensis*.

allergens that have been produced with minimum batch-to-batch differences to guarantee a constant optimum predictive value and safety at the same concentration of the preparation.

In part II of this study, we prospectively tested the obtained concentrations and cutoff values and the safety for the same allergen preparations as well as for two other standardized allergens used in the same concentrations.

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