

Proposed guidelines for quantitative skin prick test procedure to determine the biological activity of allergenic extracts using parallel line assay

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Guidelines are proposed for determining the potency of allergenic extracts in relation to a reference extract using parallel line bio-assay. The practical performance, limitations, and advantages of skin prick test are discussed.

Key words: allergenic extracts; biological standardization; parallel line bio-assay; skin prick test.

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For many years biological standardization of allergenic extracts intended for diagnostic purposes has been recommended in order to increase safety and efficacy (1, 2, 3, 11, 14). Furthermore it is required by registration authorities (16). Skin test has been recommended as convenient for biological standardization (1, 11). Biological standardization is essential from two aspects. Firstly, to evaluate the potency of reference extracts and here standardization is in relation to a histamine reference (1, 11, 16). Secondly, to estimate the relative potency of new production batches in relation to an inhouse standard (4, 12); this has become even more important with the introduction of international allergen references compiled by the Allergen Standardization Subcommittee working under the International Union of Immunological Societies (IUIS). The present proposal of guidelines using the skin prick test (SPT) is adapted from the proposal by Turkeltaub et al. using intradermal tests (13).

Quantitative SPT procedure to determine the relative potency of allergenic extracts

1 Selection of test subjects

1.1 Subjects should have an unequivocal history of allergic disease related to exposure to the test allergen

- 1.2 Subjects should not have received anti-histamines, steroids and other medications interfering with skin testing within 72 h of the test.
- 1.3 Subjects should not have received or be receiving immunotherapy with the test allergen.
- 1.4 Subjects should differ in sensitivity to the allergen in question by at least a factor 100.

2 Materials

- 2.1 Disposable 1 mm precision needles designed for skin prick testing (SPT).
- 2.2 Transparent tape.
- 2.3 Fine-tip rolling pen.
- 2.4 Reference allergenic extracts.
- 2.5 Test allergenic extract.
- 2.6 Saline diluent with 0.03 % human serum albumin (HSA). Other diluents can be used if proved not to interfere with the allergenic activity and composition and do not elicit non-specific skin reactions.
- 2.7 Skin test template. The test should be performed with a vertical and horizontal distance of 3 cm.

3 Dilution of allergens

- 3.1 Start with undiluted or reconstituted lyophilized extract.

- 3.2 Prepare serial 10-fold dilution using sterile technique.
- 3.3 The serial 10-fold dilutions from the undiluted extract (#0) are labelled #-1, #-2, #-3, etc. (log allergen concentration).
- 3.4 From the undiluted extract (#0) a half log dilution is prepared (1 ml extract + 2 ml diluent) and from this one further 10-fold dilution labelled #-0.5, #-1.5, #-2.5, etc. (log allergen concentration).
- 3.5 Use a new syringe and needle for each 10-fold dilution.
- 3.6 Dilution should be kept continuously refrigerated until use and discarded after 1 day of use.

4 Skin testing

- 4.1 Skin prick testing is performed by gently jabbing the needle at right angles to the skin through the allergen extract drop. The force should be moderate and bleeding avoided. The drop is wiped off immediately.
- 4.2 A preliminary test is performed on the volar aspect of the forearm with a 10-fold dilution starting with #-2.
- 4.3 The lowest dilution eliciting a wheal larger than 10 mm² is estimated (e.g. #-4.0).
- 4.4 The lowest dilution and the next four lesser dilutions (e.g. #-4.0, #-3.5, #-3.0, #-2.5, #-2.0) are applied in quadruplicate in random order on patient's back. This is done for both reference and test extract.
- 4.5 The skin wheal area of both reference and test extract on the back should be in the range of 10–100 mm², otherwise a more concentrated extract must be used.
- 4.6 The randomized scheme of SPT sites should differ for each subject.

5 Recording of skin test

- 5.1 Fifteen minutes following skin test, the wheal contour is outlined using the skin marking pen.
- 5.2 The test results are transferred to a record sheet by means of tape.
- 5.3 The wheal areas are measured in mm² by a digitizer following the inner contour of the wheal outline.

Log wheal area

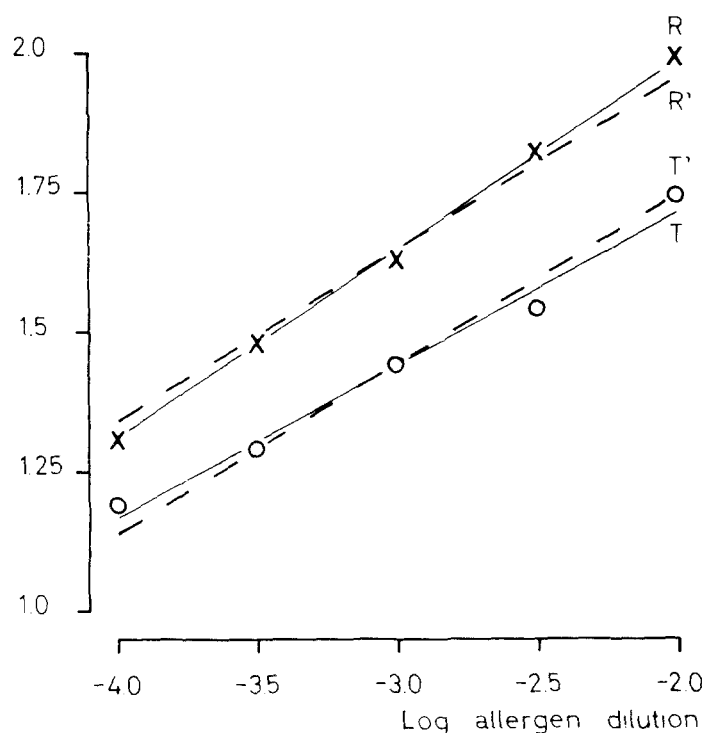


Fig. 1. Dose response curve (wheal area versus allergen dilution) of reference (R) and test (T) extract. The fully drawn line represents the best-fit linear regression line of the mean wheal area. The dotted lines are the calculated best-fit parallel lines of reference (R') and test (T') extract. The horizontal distance between the dotted lines is the log of the relative potency of the test sample as compared to the reference.

- 5.4 The reproducibility of the four-double determinations of each allergen concentration is calculated. The coefficient of variation ($SD\% = \text{standard deviation}/\text{mean area} \times 100\%$) should be less than 40%; tests with a higher $SD\%$ are discarded as invalid.
- 5.5 The mean wheal area of the four-double determinations is plotted in a log-log dose-response curve as a function of allergen concentration (Fig. 1).
- 5.6 linear regression is performed using the least square method: $\text{Log area} = \text{log intercept} + \text{log slope} \times \text{log allergen concentration}$.
- 5.7 For both reference and test extract the correlation coefficient (r) for the best-fit line should be greater than 0.9. If r is less than 0.9, the assay is considered invalid and the test is discarded.

6 Calculation of relative potency

- 6.1 Using a statistical test, check for the parallelism of the regression lines of reference and test extract. If the lines are not parallel at $P < 0.01$, the assay is considered invalid and no further computations are performed.
- 6.2 For each sample using the combined data of reference and test extract, calculate the best-fit parallel lines, i.e. compute the common slope and the two new intercepts (Fig. 1).
- 6.3 Using the slope and the intercept of the parallel line, calculate the horizontal distance between the two lines by subtracting the intercept of the reference from the intercept of the test sample and dividing by the common slope. This distance is the log of the relative potency of the test sample as compared to the reference.
- 6.4 Perform valid assay on 10 different subjects and calculate the median relative potency.
- 6.5 The test material can be considered equal in potency to the reference extract if the median relative potency of the test extract is within 50–200% of the potency of the reference extract.

DISCUSSION

Compared to the intradermal test (IT), SPT is simple and quick and, with the reservations suggested concerning reproducibility and unequivocal dose-response curve, IT has no advantage over SPT (8). However, a minimal wheal area of 10 mm² and a correlation coefficient > 0.9 is essential (7, 8, 9). Another advantage of SPT is the steeper dose-response curve (8), except when measuring the erythema area (12, 13) which seems less reproducible (8). SPT uses more concentrated extracts (8), thus eliminating the problem with the extensive dilution sequences needed in IT (8, 13).

Estimation of the relative potency of allergenic extracts by parallel line bio-assay (5) has been proposed by many authors (4, 12, 15), not only for skin testing but also for measuring potency by RAST-inhibition (6). The use of five half-log dilutions and quadruplicate deter-

minations enables exact determinations of the dose-response curve. Comparison of extracts without parallel dose-response curves is still an unsolved problem, probably mainly caused by differences in the composition of allergenic determinants (a qualitative difference) and, to a minor extent, differences in potency.

The possibility to relate extracts from various manufacturers to WHO reference preparations and the use of international units in labelling are essential for improving communication of scientific and clinical information to allergologists throughout the world (2, 10, 14, 15). And finally, it is the only means of allowing a direct comparison of international publications.

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