

Quantification of Contact Allergic Inflammation: A Comparison of Existing Methods with a Scanning Laser Doppler Velocimeter

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Responses to a range of doses of common contact dermatitis-producing allergens were measured using a novel scanning laser Doppler velocimeter and three commonly used conventional measurement techniques. The techniques were compared in terms of sensitivity, measurement error, range of the linear portion of the dose-response curve and ease of use. The detection thresholds of the objective methods did not differ significantly and did not detect responses at concentrations less than those required to produce a visible response. Of the objective methods the range of linearity was greatest when reactions were measured using change in skin fold thickness, erythema or area of inflammation. Measurement error was greatest with measurements made using the conventional laser Doppler velocimeter. Present instrumental methods are no more sensitive than visual assessment in the reading of patch test reactions. The conventional laser Doppler velocimeter was least suited for measurement of allergic contact hypersensitivity reactions as readings are time-consuming, show detectable changes over a more limited range of allergen concentration, and have a larger measurement error than the other methods. There is no single best method for measuring allergic contact hypersensitivity reactions. Useful data over a wide range of allergen concentrations can best be obtained by measurement of skin fold thickness, erythema or area of reaction using the scanning laser Doppler velocimeter. The scanning laser Doppler velocimeter has the added advantages of being able to measure area of reaction without contact with the skin surface and to measure reactions at all skin sites. *Key words:* Dose response; Erythema meter; Skin fold thickness.

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Patch testing is widely used by dermatologists to identify individuals with eczema caused or aggravated by delayed type hypersensitivity reactions to contact sensitizers. In routine clinical practice, patch test responses are evaluated subjectively and graded using a clinical rating scale based on the degree of erythema, induration and the presence or absence of vesicles (1). Although this grading is useful as an indicator of the likely clinical significance of any given reaction, it lacks objectivity, grades responses on an ordinal scale and it is likely that the increments between the commonly used clinical grades are unequal. A number of instrumental methods have been used to quantify patch test responses in an attempt to introduce objectivity (2–8). These different techniques mea-

sure specific components of the allergic patch test reaction such as oedema and vasodilatation and produce data on a continuous scale which is suitable for dose-response analysis.

The recent application of objective measurement methods and dose-response analysis to the study of patch test responses in humans has highlighted the potential usefulness of this approach in providing important but simple quantitative explanations for commonly recognised phenomena, such as the negative patch test response in patients with apparent contact hypersensitivity (9). Preliminary studies suggest that a similar approach may be used to study the effect of pharmacological agents on allergic contact hypersensitivity (ACH) reactions in vivo (10).

Previous studies comparing instrumental methods of patch test reading with conventional visual grading have mainly relied on responses to single concentrations of multiple allergens in a number of subjects attending for routine patch testing (2, 3, 5, 7). Although these methods have clearly shown that some patients may have two or more responses of the same grade which show marked differences in the degree of erythema or induration, the instrumental method most suited for dose-response analysis of ACH reactions has not been established.

We have used a range of concentrations of common contact dermatitis-producing allergens in sensitized patients to produce a series of graded responses, comparing a number of instrumental methods which measure different aspects of the allergic response with a scanning laser Doppler velocimeter (LDV) that permits measurement of both intensity and area of inflammation (11).

MATERIALS AND METHODS

Patients

Ten patients who were known to have positive reactions to at least one common antigen were challenged with a series of dilutions of the appropriate antigen as described previously (9). Four patients were sensitive to nickel, 3 to thiuram mix, 1 to wool alcohols, 1 to fragrance mix and 1 to potassium dichromate. Stock solutions of the different allergens were prepared in either water (nickel) or chloroform (thiuram mix, wool alcohols, fragrance mix and potassium dichromate {as supplied for routine patch testing by Trolab, Hermal, Hamburg}). The concentration of the different allergens in their respective stock solutions was one fifth of that used in the European standard patch test series. Successive dilutions (range 1 in 5 to 1 in 5×10^6) of the allergen stock solutions were prepared in water or chloroform as appropriate and 20 μ l of each dilution pipetted onto an Al-test (Imeco) and allowed to evaporate. Patches were applied to the volar aspect of the forearm and secured with adhesive tape. The patches were removed after 48 h.

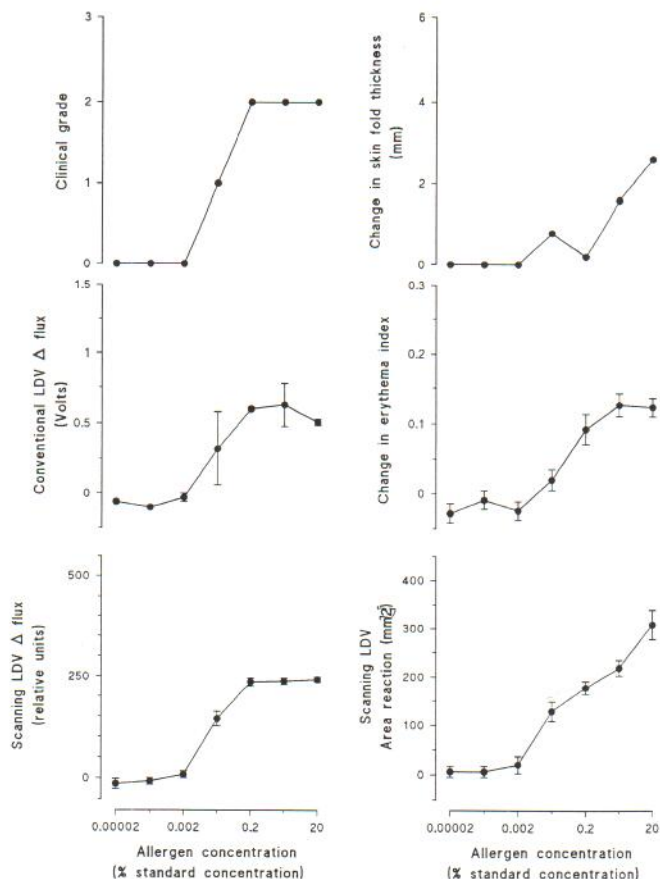


Fig. 1. Dose-response curves obtained in one patient using the different methods to measure allergic contact hypersensitivity reactions. Error bars show the standard deviation of readings made in triplicate.

Measurement method

The inflammatory reactions in each subject were measured using the following methods:

1) *Clinical grading*: Reactions were assessed clinically 72 h after application using the International Contact Dermatitis (ICD) grading scale, which is as follows: 0, negative reaction; ?+, doubtful reaction, faint erythema only; +, weak positive reaction, erythema, infiltration, possibly papules; 2+, strong positive reaction, erythema, infiltration, papules and vesicles; 3+, extreme reaction (bullae or ulceration).

2) *Skin fold thickness*: Measurements were made before and 72 h after application of allergen using Harpenden skin calipers from which one spring was removed; the increase in thickness was calculated by subtraction (12). All measurements were made by the same observer.

3) *Erythema*: Erythema was measured using a reflectance instrument which compares the amount of reflected red and green light to produce an "erythema index" which depends mainly on the blood content of the superficial dermis (13). The effective area of erythema measurement for this instrument is 24 mm². Measurements were made in triplicate before and 72 h after application of the allergen. The increase in erythema was calculated as the difference between the mean erythema index before and after allergen application (14).

4) *Conventional laser Doppler velocimetry*: Blood flux was measured using a commercially available laser Doppler velocimeter (Periflux Pf2, Perimed, Sweden). Measurements of the different inflammatory reactions were made at each site in triplicate, the probe holder and probe being re-positioned between each measurement. Randomly selected, adjacent, untreated sites were used as controls. The reading from the instrument was relayed to a chart-recorder and the mean

deflection estimated visually from the recording produced at each site over a period of at least 20 s after steady-state conditions had been achieved. The increase in flux due to inflammation was expressed as the difference between the mean reading at each site and the mean background reading determined at three adjacent control sites (15).

5) *Scanning laser Doppler velocimetry*: Triplicate scans, each taking 6 min to complete, were made using a scanning laser Doppler instrument 72 h after application of allergen. The instrument used differs from conventional laser Doppler velocimeters in that all contact with the skin surface is avoided, the laser light being reflected to and from the skin by a motor-driven mirror. The area of interest is scanned in a rectilinear manner and the data processed by a computer to form a 250 × 250 pixels image of blood flux, measured on a scale with 256 subdivisions and displayed on a monitor screen for subsequent analysis. At the closest scanning distance, each pixel represents an area on the skin surface of 1 mm². A full technical description of the instrument (11) and details of the method of data analysis (16) has been published. In brief, an average maximum blood flux was calculated at each site to which allergen had been applied and this value was compared with the average background blood flux. The area of inflammatory reaction at each site was calculated from the number of pixels showing a blood flux value greater than two standard deviations above the mean background flux (16).

RESULTS

Dose responses

Seven of the 10 patients had no detectable response as assessed by visual grading at the lowest concentration of allergen. In 1 patient it was not possible to measure change in skin fold thickness, and in 3 patients area of reaction using the scanning LDV could not be calculated as the areas of reactions defined by the scanning LDV, but not by visual assessment, overlapped.

The readings obtained from the different methods were plotted against the logarithm of the dose of allergen. Skin fold thickness, erythema, blood flux and area of reaction were plotted as the change in value relative to normal skin. Instrumental readings were performed in triplicate and were plotted as the means. Dose-response curves for 1 subject using the different measurement methods are shown in Fig. 1.

The following parameters were determined from the dose-response curves in each patient.

1. Detection threshold.
 - i) Clinical: dilution producing response of + or more as defined by the ICD group.
 - ii) Skin fold thickness: dilution producing a change in skin fold thickness equal or greater than 0.2 mm.
 - iii) Instrumental methods: dilution producing a reading greater than zero plus twice the median standard deviation (SD).
2. Plateau.
 - i) Clinical: Two or more consecutive dilutions producing a maximum grade in any individual patient or any dilution producing grade 3.
 - ii) Skin fold thickness: Two or more maximal readings differing by less than 0.2 mm.
 - iii) Instrumental methods: 2 or more maximal readings differing by less than twice the median SD.
3. Range of the linear portion of the dose-response curve.

Table I. Threshold dilution expressed as percentage of allergen concentration used in standard battery for the different measurements

Subject	Visual grade	Skin fold thickness	Erythema index	Conventional LDV (flux)	Scanning LDV (flux)	Scanning LDV (area)
1	0.02	0.02	0.2	0.02	0.02	0.02
2	0.2	0.2	0.2	0.2	0.002	0.002
3	0.002	0.2	0.02	0.2	0.002	0.02
4	3.125	12.5	3.125	6.25	3.125	6.25
5	0.02	0.2	0.00002	0.02	0.02	0.002
6	1.56	1.56	1.56	1.56	1.56	*
7	1.56	3.125	1.56	1.56	1.56	1.56
8	0.001	0.0001	0.0001	0.0001	0.0001	*
9	0.001	*	0.0001	0.1	0.01	0.1
10	1.56	1.56	1.56	1.56	1.56	*

* Results not available for technical reasons.

This was defined as the number of dilutions between the threshold and plateau values. In the cases in which no plateau was observed, the plateau value was taken as the maximum concentration used plus one.

The detection threshold of the objective methods (Table I) did not differ significantly from that assessed visually ($p > 0.05$, Wilcoxon matched pairs signed rank test). There was also no significant difference between the different objective methods ($p > 0.05$). A plateau, with no detectable increase in response with increasing allergen concentration, was observed in 9 of 10 patients using clinical grading, 2 of 9 patients using change in skin fold thickness, 4 of 10 patients using reflectance spectrophotometry, 9 of 10 patients using conventional LDV, all of 10 patients using blood flux (scanning LDV) and 2 of 7 patients using increase in area of reaction (scanning LDV).

The range of the linear portion of the dose-response curve was significantly greater ($p < 0.05$, Wilcoxon matched pairs signed rank test) when measured as change in skin fold thickness, erythema or increase in area of reaction (scanning LDV), rather than blood flux (conventional or scanning LDV). No significant difference was found between responses measured as change in skin fold thickness, erythema or increase in area of reaction (scanning LDV) (Table II).

In order to compare measurement error between the different instrumental methods, the ratio of the mean standard deviation to the maximal value was calculated. This was sig-

nificantly greater with measurements made using the conventional LDV compared with other instruments ($p < 0.05$, analysis of variance).

Relationship between clinical grading and objective methods

Fig. 2 shows the change in skin fold thickness, erythema index, blood flux (conventional and scanning LDV), and area of reaction (scanning LDV) in relation to the clinically graded groups. As there were only two ?+ reactions, these have been included in the negative reaction group. This clearly shows that each clinical grade encompasses a wide range of responses when assessed by objective methods.

DISCUSSION

We have used the responses to a range of concentrations of common contact dermatitis-producing allergens to compare conventional measurement methods with a novel scanning laser Doppler velocimeter that allows measurement of both intensity and area of inflammation (11). The dilutions used produced a wide range of clinical grades in most of the patients and in 7 of the 10 patients at least one of the dilutions produced a sub-threshold response enabling us to address the question of sensitivity. The observation that the area of increased blood flux defined by the scanning LDV is greater than that assessed visually for strong positive reactions is similar to the findings of Baillie et al. who used thermography to

Table II. Number of dilutions forming the linear part of the dose-response curve for the different measurement methods

(Where no plateau was obtained, number of dilutions equals number to maximum value plus one.)

Subject	Visual grade	Skin fold thickness	Erythema index	Conventional LDV (flux)	Scanning LDV (flux)	Scanning LDV (area)
1	1	4	1	1	1	4
2	3	3	3	1	2	5
3	4	4	5	2	1	3
4	2	2	2	1	2	3
5	1	1	3	0	1	3
6	0	7	7	4	0	*
7	1	4	7	7	1	7
8	1	7	7	0	5	*
9	3	*	7	1	2	3
10	0	7	5	0	5	*

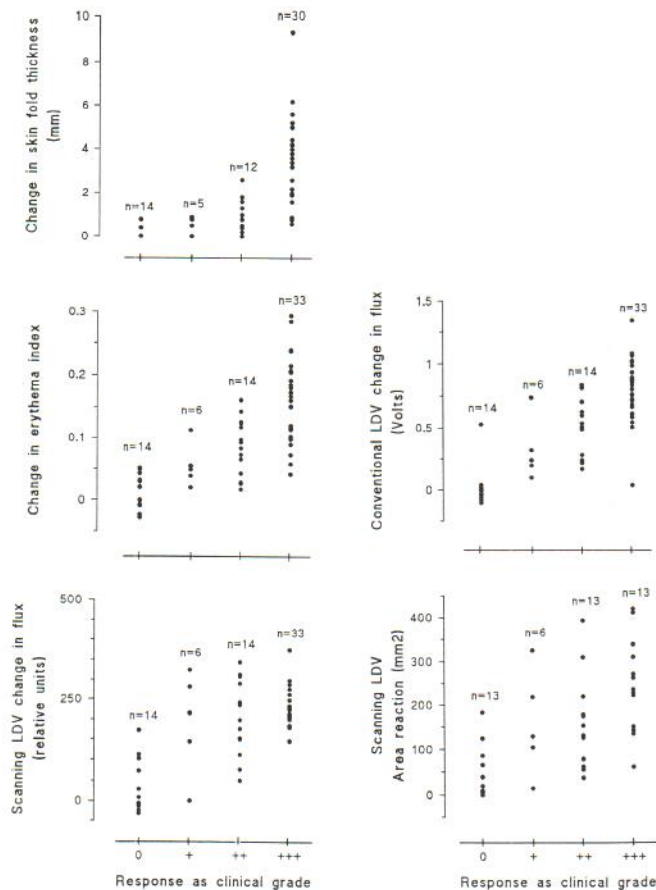


Fig. 2. Response as change in skin fold thickness, erythema index, blood flux (conventional or scanning LDV) and area of reaction (scanning LDV) in relation to the clinical grade.

quantify patch test reactions (8). Patch testing with these allergens confirmed the expected log dose-response relationships shown previously by us and others (9, 17); however, substantial differences were observed in the shape of the dose-response curve within individuals depending on the measurement method used. Clinical reading of patch test responses is based on the combined assessment of both redness and swelling. Most instrumental methods measure a specific feature of the inflammatory response. The differences in the shape of the dose-response curves reflect the change in the relative importance of vascular changes and oedema/cellular infiltration as the intensity of the allergic reaction increases.

The usefulness of any method used to quantify patch test responses will depend on a number of factors, and the requirements of a dermatologist in the routine contact dermatitis clinic will clearly be different from those for research purposes. Visual grading is subjective and produces non-continuous data which is unsuitable for dose-response analysis. In order to understand the functional components of allergic contact hypersensitivity reactions and study factors that modify the response, objective measurement methods are required. The usefulness of any method will depend on a number of factors and will include sensitivity, measurement error, range of the linear portion of the dose-response curve and ease of use. Direct comparison of the sensitivity and range of the

linear portion of the dose-response curve of methods that measure different components of ACH reactions is difficult. We chose to define detection threshold and plateau using criteria which took into account the measurement variation inherent in each technique.

No significant difference in detection threshold was found between the different objective methods. The measurement error of the instrumental methods was expressed as the ratio of the mean standard deviation to the plateau or maximal value obtained, as the number of increments between no response and maximal response was not uniform for the different methods. This value was significantly greater when responses were measured using the conventional LDV. The measurement error using skin fold thickness could not be determined, as tissue compression during measurement will influence multiple readings.

Comparison between individuals and study of factors influencing ACH reactions are best done using the linear part of the dose-response curve. The number of doses forming the linear part of the dose-response curve was significantly greater when measured as change in skin fold thickness, erythema or increase in area of reaction (scanning LDV), rather than blood flux (conventional or scanning LDV), permitting comparisons to be made over a wider range of allergen concentrations. Although a plateau response was seen in 9 of the 10 patients when assessments were made clinically or by measurement of blood flux, a plateau was seen less often with other measurement methods and the estimation of the plateau value that we made for comparative purposes will have underestimated its true value.

In terms of ease of use, the measurement of erythema with the reflectance instrument and blood flux with the conventional LDV is time-consuming, as only a small area can be measured at one time and multiple readings at different sites require the light guide to be repositioned between each measurement. The technique of skin fold thickness measurement using Harpenden calipers, although simple to perform, cannot be used in sites in which skin folds cannot be raised, does not permit determination of measurement error and is more likely to be affected by observer bias. The scanning LDV is simple to use and permits rapid measurement of cutaneous blood flux and area of reaction over large areas of skin without contact with the skin surface.

Instrumental methods are unlikely to replace subjective clinical grading in the routine assessment of patch test responses in the contact dermatitis clinic. The main indication would be as a means of differentiating negative from doubtful patch test reactions. In this study, using a series of 10-fold dilutions of antigen, none of the objective methods was found to be superior to visual grading in terms of detection threshold. This is at variance with the conclusions of Staberg et al. (2), who studied a range of responses to single concentrations of multiple allergens and concluded that it was possible to separate positive, doubtful and negative patch test reactions using a conventional LDV. Other studies using a variety of objective methods have tended to support our findings (4, 7, 17).

There is no single best method for measuring allergic con-

tact hypersensitivity reactions. Present instrumental methods are no more sensitive than the eye in detecting a threshold response and therefore offer no advantage in the assessment of equivocal positive patch tests in the contact dermatitis clinic. The conventional LDV was least suited for measurement of ACH reactions as measurements are time-consuming, show detectable changes over a more limited range of allergen concentrations, and have a larger measurement error than the other methods. Useful data over a wide range of allergen concentrations can best be obtained by measurement of skin fold thickness, erythema or area of reaction using the scanning laser Doppler velocimeter. The scanning laser Doppler velocimeter has the added advantages of being able to measure area of reaction without contact with the skin surface and to measure reactions at all skin sites.

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REFERENCES

1. Wilkinson DS, Fregert S, Magnusson B, et al. Terminology of contact dermatitis. *Acta Derm Venereol (Stockh)* 1970; 50: 287-292.
2. Staberg B, Klemp P, Serup J. Patch test responses evaluated by cutaneous blood flow measurements. *Arch Dermatol* 1984; 120: 741-743.
3. Serup J, Staberg B, Klemp P. Quantification of cutaneous oedema in patch test reactions by measurement of skin thickness with high frequency pulsed ultrasound. *Contact Dermatitis* 1984; 10: 88-93.
4. Van der Valk PGM, Kruis-De Vries MH, Nater JP, et al. Eczematous (irritant and allergic) reactions of the skin and barrier function as determined by water vapour loss. *Clin Exp Dermatol* 1985; 10: 185-193.
5. Mendelow AY, Forsyth A, Feather JW, et al. Skin reflectance measurements of patch test responses. *Contact Dermatitis* 1986; 15: 73-78.
6. Serup J, Staberg B. Differentiation of allergic and irritant reactions by transepidermal water loss. *Contact Dermatitis* 1987; 16: 129-132.
7. Prens EP, Van Joost T, Stekete J. Quantification of patch test reactions by transcutaneous PO₂ measurement. *Contact Dermatitis* 1987; 16: 142-146.
8. Baillie AJ, Biagioni PA, Forsyth A, Garioch J, McPherson D. Thermographic assessment of patch-test responses. *Br J Dermatol* 1990; 122: 351-360.
9. McLelland J, Shuster S. Contact dermatitis with negative patch tests. *Br J Dermatol* 1990; 122: 623-630.
10. Higgins EM, McLelland J, Friedmann PS, Matthews JNS, Shuster S. Oral cyclosporin inhibits the expression of contact hypersensitivity in man. *J Dermatol Science* 1991; 2: 79-83.
11. Essex TJH, Byrne PO. A laser Doppler scanner for imaging blood flow in skin. *J Biomed Eng* 1991; 13: 189-194.
12. Moss C, Friedmann PS, Shuster S, Simpson JM. Susceptibility and amplification of sensitivity in contact dermatitis. *Clin Exp Immunol* 1985; 61: 232-241.
13. Diffey BL, Oliver RJ, Farr PM. A portable instrument for quantifying erythema induced by ultraviolet radiation. *Br J Dermatol* 1984; 111: 663-672.
14. Farr PM, Diffey BL. Quantitative studies on cutaneous erythema induced by ultraviolet radiation. *Br J Dermatol* 1984; 111: 673-682.
15. Farr PM, Diffey BL. The vascular response of human skin to ultraviolet radiation. *Photochem Photobiol* 1986; 44: 501-507.
16. Quinn AG, McLelland J, Essex T, Farr PM. Measurement of cutaneous inflammatory reactions using a scanning laser Doppler velocimeter. *Br J Dermatol* 1991; 125: 30-37.
17. Eun HC, Marks R. Dose-response relationships for topically applied antigens. *Br J Dermatol* 1990; 122: 491-499.