

Objective assessment of nickel sulfate patch test reactions with laser Doppler perfusion imaging

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The laser Doppler perfusion scanning technique was used to assess the superficial blood flow of nickel sulfate hexahydrate patch test reactions. There was good agreement between laser Doppler and visual assessments when the highest assessment values of reactions were studied. Earlier detection of reactions was possible with the laser technique. There was great inter-individual variance in perfusion between identically tested patients. 4 patients were visually negative when a TRUE TestTM patch test dose of 0.20 mg/cm² was applied for 48 h and the test area read 4× up to 168 h. These 4 showed a dose-related increase in perfusion and visually positive reactions using longer application times. The instrument allowed a dose reduction not possible for visual assessments. Reading transparent patches in contact with the skin through transparent semi-occlusive plastic foil or through windows in the tape strip over the patches, allowed us to detect perfusion at 48 h, where a longer application would have been needed using tape and visual assessments.

Key words: allergic contact dermatitis; patch test optimization; serial dilution; reading time; exposure time; reaction-pattern analysis; skin perfusion; patch testing technique; nickel; laser Doppler perfusion imaging; bioengineering methods. © Munksgaard, 1998.

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The laser Doppler perfusion imager (LDPI), an instrument for laser Doppler perfusion scanning, was introduced in 1993 (1). It uses a helium-neon laser operating at 632.8 nm. The instrument is based on the same principle as laser Doppler flowmetry (2). Its main advantage over flowmetry lies in its ability to scan tissue without touching it. LDPI can capture superficial skin perfusion and display spatial heterogeneity of the tissue under study on a monitor for assessment. The technique has been used to measure blood perfusion in patch test reactions (3–6) and in other applications (7–10).

The aims of this study were as follows

(A) To find out by using the LDPI: (i) whether objective assessment of perfusion of nickel sulfate hexahydrate patch test reactions is possible; (ii) whether there are different inter-individual perfusion profiles in identically tested patients.

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(B) To compare LDPI and visual assessment.

(C) To find out: (i) whether dose or application time affect LDPI or visual assessment of the patch tests in the study group; (ii) the “ideal” dose, application time and reading time(s) of nickel sulfate hexahydrate patch tests in the study group for each of the reading techniques; (iii) whether it is possible to assess perfusion of patch tests by reading through transparent test patches and thin opaque semi-occlusive plastic foil or windows in a tape strip, while applications remain in place.

Patients, Materials and Methods

Study populations

9 Caucasian patients, 8 women and 1 man (patient no. 3), aged 26–66 years (median age 29 years), were randomly drawn from the outpatient files of patch tested patients at the Department of Dermatology, Karolinska Hospital, Stockholm. They had a relevant history of nickel allergy and had been tested with the Swedish standard patch test series within the previous 2 years. All had reacted to

2.5% nickel sulfate in petrolatum (Chemotechnique Diagnostics AB, Tygelsjö, Sweden), read at 24 h after a 48-h application using Finn Chambers® on Scanpor® tape (Epitest Ltd Oy, Tuusula, Finland) (11). Patients who had reacted to more than 1 other allergen in the series were excluded. 4 patients had skin type III (nos. 1, 2, 7, 9), 2 had skin type II (nos. 4, 5), 2 had skin type IV (nos. 3, 6) and 1 skin type I (no. 8). Exclusion criteria were atopic disease, medication or disorder known to affect perfusion or allergic reactions, treatment with ultraviolet radiation or patch testing or history of eczema within the previous 2 months. Patients with visible pathological skin conditions were excluded if it was not possible to place patch tests outside such areas. The intensities of the patients' prior patch test readings were unknown to the investigators. The patients were asked to inform the investigators of any late reactions appearing after the study. No such reactions were reported.

Test materials and applications

9×9 mm transparent polyester patches coated with hydroxypropyl cellulose film containing nickel sulfate hexahydrate chemically analysed to 0.20 mg/cm², 0.067 mg/cm², 0.022 mg/cm², 0.0073 mg/cm² and 0.0024 mg/cm² (Pharmacia A/S, Hillerød, Denmark) were used. 1 patch of each dose plus 1 similar hydroxypropyl-cellulose-coated control patch were applied to 5×11 cm non-woven cellulose acetate tape strips with acrylic adhesive (Cederroth BV, Tilburg, The Netherlands). Patches were applied to the strips, in random order unknown to the investigators, in 2 staggered parallel columns of 3 patches each. The vertical distance between the patches was 2 cm and the horizontal distance 1 cm.

The test strips were applied vertically in a randomized order on the patients' upper backs, 1 strip for each application time. Applications and readings varied by patients as shown in Table 1. Reading times were counted from the application of

strips. No testing was done during the summer months.

In addition, 2 similar strips were prepared, 1 with 10×12 cm Tegaderm™ (3M Svenska AB, Sollentuna, Sweden), a thin semi-occlusive adhesive plastic foil instead of the tape, and 1 with approximately 8×8 mm punched out windows in the cellulose tape strip over the patches. The size of the latter strip was 5×13 cm. The distance between the patches was the same as for the strips above, except for the patches of the strip with the punched-out windows, where the vertical distance was 3 cm between the patches. These strips were applied on patient no. 9 and read at 24 h, 48 h, 72 h, 96 h and 168 h.

All test materials were prepared within 4 days of application and were kept at 4–8°C in airtight and light-proof envelopes until use.

Test readings

After application of strips, patches were identified through their coverings, allowing marking of patch test areas vertically and horizontally outside strip borders. These markings, and a flexible reading board made of film, facilitated exact identification of patch test areas after detachment of strips.

Reactions were first read with the LDPI and then assessed visually. Most of them were followed up to 168 h, which required on average approximately 230 patch-test assessments per patient (excluding "reading through" materials). Each patient session was completed within 2.5 h. Strips planned for long applications were removed ahead of time in patients with strong test reactions. Contact with chemicals or water on the back was allowed only after the last reading. Ultraviolet radiation treatment, sunbathing or scratching were prohibited, as was medication known to affect allergic reactions or perfusion. Room temperature was kept between 22–34°C. Back skin temperature ranged between 28.9 and 31.4°C.

Table 1. Application and reading times; "+" indicates those actually performed

Application time (h)	Reading time(s) (h)	Patient no.								
		1	2	3	4	5	6	7	8	9
6	6, 24, 48, 72, 96, 168	+	+	+	+	+	–	–	–	–
18	18, 42, 66, 90, 162	+	+	+	+	+	–	–	–	–
24	24, 48, 72, 96, 168	+	+	+	+	+	+	+	+	–
48	48, 72, 96, 168	+	+	+	+	+	+	+	+	+
72	72, 96, 168	+	+	+	+	+	+	+	+	–
96	96, 168	+ ^{a)}	+	+	+	+	+	+	+ ^{a)}	+
168	168	–	–	–	–	–	–	+	–	+

^{a)} Test strip removed at 48 h.

Table 2. Highest APVs and highest visual assessments for a 48-h application and readings at 48 h, 72 h and 96 h; dose: 0.067 mg/cm²

Patient no.	APV	Visual assessment
6	5.70	+++
8	4.48	+++
1	3.34	++
5	3.20	++
2	2.14	+
9	1.02	—
3	0.88	—
7	0.57	—
4	0.02	—

Visual assessments and photo-documentation

Test reactions were visually assessed according to a standard accepted by the North American Contact Dermatitis Research Group (12). 30 min acclimatization was aimed at before LDPI assessments. During this time, photo-documentation was performed.

LDPI reading parameters

LDPI readings were performed with the patients comfortably prone. They were asked to breathe easily and not to talk or move, as this could affect the readings. The only light allowed during readings was that from the computer monitor which was set to a minimum.

Perfusion was read and analysed with an LDPI PIM 1.0 using LDI 2.5 software (Lisca Development AB, Linköping, Sweden). The instrument was set to read 32×32 measurement sites for each reaction. This allowed 4 readings in approximately 6–7 min including manual stops between readings with the instrument set for sequential measurements.

Readings were performed at 13-cm distance, where the instrument, after a special modification, was well focused, thus increasing the signal-to-noise ratio. Low resolution was used with a background threshold of 6.1 V. The perfusion value of each measurement site was indicated in arbitrary units (V). The perfusion scale ranged from 0.00–10.00 volts and the amplification factor was 2. The laser head was adjusted parallel to the reading area with a spirit level at exactly 13 cm from the laser head aperture. A 3 mW 670 nm Applied Laser pen® LP 30 (Applied Laser Systems, Grants Pass, Oregon, USA) mounted on the laser head bar had its beam cross the LDPI beam at that distance prior to readings. This allowed accuracy in the distance between laser head and skin. The laser head was adjusted as parallel to the skin as possible and the beam

was centered within the patch test areas prior to readings.

Assessment of perfusion

The 32×32 measurement sites used for LDPI readings cover a larger area than needed to catch reactions. Unpublished experiments with light-absorbing material have shown, for the set-up as above, that 7×7 measurement sites cover 9×9 mm patch test areas and 6×6 measurement sites cover each window of the strip with the punched out windows. By using these numbers of measurement sites, the mean perfusion value of each patch test area could be calculated. Such a mean perfusion value of each control patch was subtracted from its corresponding allergen patches applied equally long and read at the same patient session. The smaller assessment areas of the windows were assessed the same way. Perfusion obtained this way is here termed “adjusted perfusion” (AP) and its

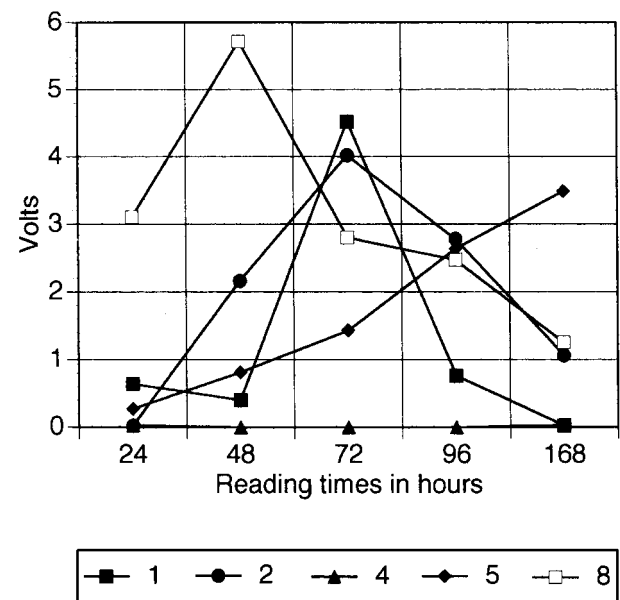


Fig. 1. Inter-individual difference in AP profiles illustrated for selected patients for a 24-h application using a dose of 0.20 mg/cm².

Table 3. Comparison between APVs (V) and visual assessments for patient no. 6 tested with a nickel sulfate hexahydrate dose of 0.20 mg/cm² applied for 24 h

Reading times (h)	APV	Visual assessment
24	3.83	—
48	6.09	++
72	0.87	++
96	0.60	+++
168	0.09	?

Table 4. Application (A) and reading (R) times (h), generating highest APVs for different patients and doses

Patient no.	1	2	3	4	5	6	7	8	9
dose (mg/cm ²)	A/R	A/R	A/R	A/R	A/R	A/R	A/R	A/R	A/R
0.20	24/72	72/72	96/168	96/168	96/168	48/72	72/72	72/72	96/96
0.067	72/72	72/72	96/168	96/168	48/168	72/72	72 ^{ab} /96 ^{ab}	72/72	48/48
0.022	72/72	72/72	96/168	b)/b)	72/96	48/72	96/168	72/72	48/48
0.0073	72/72	72/72	b)/b)	b)/b)	72/96	48/72	b)/b)	72/72	b)/b)
0.0024	72/72	b)/b)	24/24	b)/b)	96/96	96/96	b)/b)	48/48	b)/b)

a) An application and a reading at 168 h gave a higher APV. The time indicated is shown for comparison, as this application was not performed on all patients.

b) Low APV (less than 0.63 V) with a visually negative reaction. The lowest APV in the table for a positive reaction was 0.67 V.

value, “adjusted perfusion value” (APV), indicated in V.

Ethical approval was obtained from the Ethical Committee of the Karolinska Institute, Stockholm (no. 95/130).

Results

All patients showed increased AP and visually positive reactions at some time during the study.

The LDPI

The fixed number of measurement sites used in this study (7×7 or 6×6) completely captured areas of increased perfusion for most readings. It was possible to objectively assess variable grades of hypersensitivity with the LDPI. This is shown in Table 2 for an application and readings used by some test centers.

AP profiles varied between identically tested patients. This is illustrated in Fig. 1 showing APVs at different readings for selected patients.

Comparison between LDPI and visual assessment

The LDPI generally detected the early phase of reactions before the visual picture became positive. Highest assessment values of visually assessed reactions occurred later than highest APV assessments. There was good agreement between highest values assessed with the LDPI and visually for reactions of different intensities. This good agreement is shown in Table 2. Such good agreement between the reading techniques rarely applied for assessments at single time points, as illustrated in Table 3.

Other results

Generally, the shorter the application time or the lower the dose, the lower the assessment values regardless of reading technique used. However, this was not as clear for visual assessments, as that as-

essment scale had a relatively narrow range. Application time or dose did not affect the timing of the highest reaction assessment values regardless of reading technique. At very low doses or very short application times, AP profiles were less distinct or lost.

Different application times and reading times generated the highest APVs for different patients and doses, as shown in Table 4. The same applied for visual assessments. Please note for comparison reasons in the table, that the 96-h strip was removed ahead of time for patients no. 1 and 8 because of discomfort, and that only 48 h, 96 h and 168 h applications were performed on patient no. 9.

Some patients required long applications. APVs of such a patient tested at 0.20 mg/cm² are illustrated in Fig. 2 for all applications and readings,

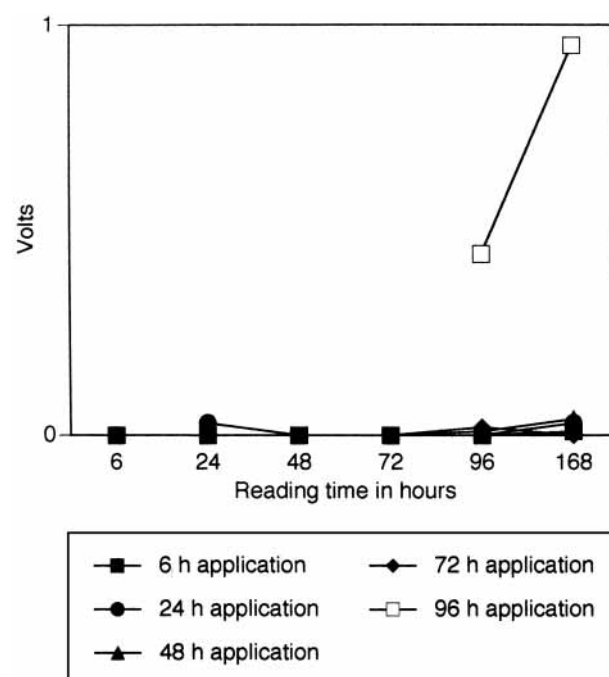


Fig. 2. AP profiles of patient 4 for all applications except for 18 h at a dose of 0.20 mg/cm².

Table 5. Ranking of patients by dose required to detect an AP or a visually positive reaction

Dose/area (mg/cm ²)	AP	Visual assessment ^{a)}
0.20		7, 3 ^{b)}
0.067		4
0.022		
0.0073		1, 2
0.0024	8, 1, 3, 6, 7, 5, 9, 2, 4	8, 5, 6

Where 2 or more patients are indicated at a certain dose, level they are shown in order of decreasing reaction intensities. 6 h, 18 h, 96 h and 168 h application times are excluded for comparison, as this was not performed on all patients; however, 24 h and 72 h applications are included although not performed on patient no. 9.

^{a)} Patient no. 9 showed visually positive reactions only at a dose of 0.20 mg/cm² needing a 96-h application.

^{b)} Patient no. 3 was positive at 0.067 mg/cm² or 0.20 mg/cm² at a 96-h application not indicated here for comparison reasons.

Table 6. APVs for patient no. 9 tested with 0.20 mg/cm² nickel sulfate hexahydrate with reading of testing materials performed through a TegadermTM foil or through punched-out windows on the test strip; APVs are indicated in volts

Application and reading time (h)	Tegaderm TM foil	Strip with windows
24	0.06	0.05
48	2.39	1.75
72	1.91	1.86
96	1.32	0.42
168	0.00	not performed

except for the 18-h application for the sake of simplicity (the highest APV for that application was 0.03V). APVs are low except for the 96-h application, which was the only one to show a positive visible reaction occurring at the 168-h reading.

Table 5 shows the lowest dose needed to detect AP or a visually positive reaction for identically tested patients except for 24-h and 72-h applications which are included, although not performed on patient no. 9. The ranking order of patients by APVs varied with dose.

Testing patients with 1/3rd of a TRUE TestTM dose of 0.20 mg/cm² (product information, Glaxo Wellcome, Inc., Research triangle park, NC 27709, USA) captured high or moderately high APVs for all patients tested with a 72-h application read at 96 h and 168 h (8 patients). For visual assessments, a full TRUE TestTM dose, a 96-h application and readings at 96 and 168 h, would be required to detect allergy of all patients thus tested (7 patients).

Testings with "reading through" materials

With LDPI, it proved possible to objectively assess AP of testing materials still in contact with the

skin. This is illustrated in Table 6. As a rule, low doses showed similar perfusion profiles, only generating lower APVs. Highest AP was detected at 48 h or 72 h, regardless of dose or "reading through" material used.

Discussion

This investigation indicates that the laser Doppler perfusion imager (LDPI) is a valuable instrument to objectively assess intensity of nickel sulfate hexahydrate patch test reactions. Technical information about the LDPI has been published elsewhere (1). The good agreement between APVs and visual assessments at their highest values regardless of reaction intensity is interesting, as is the difference in APVs between visually positive and negative patients at the reading times in Table 2. Poor agreement between APVs and visual grading at the same point for many readings (Table 3) is explained by the earlier appearance and disappearance of AP as compared to visual assessments.

To detect AP, the mean perfusion value of a control vehicle patch was subtracted from those of the allergen patches on the same strip or foil applied equally long and read at the same session. A control patch can be expected to give a good reference value, but is affected by test- and non-test-related factors, such as irritation, spatial heterogeneity of skin blood perfusion (10, 13–14) and pathological skin conditions. Such factors can affect assessment of perfusion and probably explain low APVs for some patch tests and why a few visually negative control patches showed higher perfusion values than the corresponding low perfusion allergen patches with no visible reactions. However, this occurred rarely and at low test doses or short application times only.

The "ideal" time for application of patch tests has been under debate (15). Most investigators have compared 24 h and 48 h, with different agreement rates (16–22). Test result comparisons between investigations using the same application times can be questionable because of different reading times. One investigation compared 48 h versus 72 h applications and found slightly more positive patients after 72 h application for certain allergens but not for nickel sulfate (23), which is not in agreement with present study. By replenishing a 48-h test with new material for another 48 h, relevant allergic responses have been disclosed that were not apparent after the first test (24). One investigation found equivalent reactions comparing a 5-h application of higher nickel sulfate concentrations with a 48-h application (25).

A longer application than 48 h, as traditional at many test centers, is required for some nickel-al-

lergic patients in our study group. For most patients and doses, the AP is higher after 72-h applications than after shorter ones (Table 4). 4 patients (nos. 3, 4, 7, 9), with clinical relevance for nickel sulfate allergy would have been missed using a 48-h application and the strongest dose whichever reading technique was used. For 3 of these (nos. 3, 4, 9), the highest APVs were captured using 96-h applications, and 72-h for the 3rd (no. 7) at the same dose. This is illustrated in Table 4 for all 4 and additionally in Fig. 2 for patient 4. In these cases, visual reactions at optimal applications and readings were classical allergic “++” or “+++” reactions with a large increase in APVs. Such late responders are probably less sensitive to nickel sulfate hexahydrate than other patients, requiring high doses to elicit visible allergic reactions (Table 5). These 4 patients generated much lower APVs than the others at all doses and applications (except at the weakest dose where APVs were very low). It is possible that even longer applications than used in this investigation are necessary for some patients to disclose their hypersensitivities to nickel sulfate hexahydrate. Our finding indicate that the use of long applications is an alternative to serial dilutions and intradermal tests for verifying a questionable patch test response. However, this can only be justified in selected cases.

Studies investigating different reading times sometimes ignore application times, which can make patch test results questionable. The finding of different reactivity patterns of identically tested patients in this material supports the necessity of double readings, which result tallies with many studies (26–31).

All our patients already generated AP at the lowest allergen dose, but 6 required higher doses to elicit visible reactions (Table 5). The lowest allergen dose sometimes gave different ranking of identically-tested patients by APVs. This is probably explained by the relatively higher influence various test- and non-test-related factors can have on the ranking of patients with very low APVs.

No single application or reading time is optimal for all tested patients (Table 4). Except for the lowest dose, longer application and reading times were required for optimal testing for patient nos. 3, 4 than for patient nos. 2, 6. This is probably because the former were less sensitive.

The dose of 0.067 mg/cm² (1/3rd of a TRUE TestTM dose) captured high or moderately high APVs for all patients tested with a 72-h application read at 96 h and 168 h (8 patients). This lower allergen dose can be expected to reduce the risk of sensitization, the booster effect, the possibility of cross-reactions and the “excited skin syndrome”. However, this application time, longer than the 48 h used

at some test centers, possibly increases the risk of irritant false-positive reactions, active sensitization and patient discomfort. Before such a standard testing procedure can be proposed, a larger number of patients including controls would have to be tested, with only 1 dose at a time to eliminate the enhancing effect strong reactions can possibly have on weaker ones (32–34).

For visual assessments, a dose reduction to 0.067 mg/cm² as for LDPI assessments was not possible, as at least the allergy of patient no.9 would pass undetected even if both 96-h and 168-h applications were used (patients no. 1 and 8 were not thus tested). Of the application times used in this study, 96 h would be required with readings at 96 h and 168 h to detect allergy of all patients thus tested (7 patients).

Testings with “reading through” materials

Reading patch tests through TegadermTM, a transparent foil or windows in the tape strip, introduces the possibility of following reactions without jeopardizing assessment of perfusion due to detachment of strips. A comparison between uncovered and TegadermTM-covered irritant reactions has shown no difference in perfusion values, indicating that the TegadermTM foil itself does not influence LDPI readings (6). This technique was used on patient no. 9 who had a clinically relevant allergy where a 48-h test with a tape strip and readings at 48 h, 72 h, 96 h and 168 h showed “?”+” or “–” visual readings at the strongest dose. With the TegadermTM “reading through” foil, an increased AP was already detected at 48 h (Table 6). This patient needed a 96-h application tape strip and the highest dose to elicit reactions visually. This allergy would have been missed using a 48-h application with visual readings as above, but was detected by the LDPI using only 1.2% of a TRUE TestTM dose and a TegadermTM plastic foil after a 48-h application.

Reading patch tests through punched out windows in the tape strip always generated the highest APVs of reactions later than reading corresponding patches through the TegadermTM foil, regardless of dose. As these observations are based on a few readings only, further testings are need in order to draw a conclusion.

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