

Objective non-invasive assessment of patch tests with the laser Doppler perfusion scanning technique

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The laser Doppler perfusion scanning technique is an objective, non-invasive assessment method that may be used to assess patch tests. The purpose of this paper is to focus on its clinical use in individuals with light skin, tested with non-pigmented test materials. A laser Doppler perfusion imager[®] PIM 1.0 (LDPI) is used to study different set-ups of the instrument that may affect readings. These set-ups are studied through simulated light-absorbing patch tests. The results have served as a base for clinical experiments comparing visual and LDPI assessments of normal skin, irritant patch-test reactions, and more than 25,000 allergic patch-test assessments of reactions of different intensities and control patches. This paper reviews the clinical experiments to suggest a set-up of the LDPI for patch-test readings. Such a set-up makes it possible to compare intra- and inter-individual test results and to obtain meaningful assessment values among users. Some subject-related factors that may affect reading results are studied, and the effect of non-subject related factors on readings are considered. Use of the technique is illustrated by images of perfusion and a perfusion profile of a patch-test reaction followed over time.

Key words: skin perfusion; contact dermatitis; patch-testing technique; diesel oil; sodium lauryl sulfate; nickel; budesonide; isothiozolinones; neomycin; tape; bioengineering techniques © Munksgaard, 1999.

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Despite the technological progress of the 20th century, patch tests are still traditionally assessed visually, just as they were when Jadassohn introduced them approximately a century ago. One disadvantage of the visual assessment technique is the inter-individual variance among readers' assessments. An objective, non-invasive assessment technique is desirable. This is made possible with laser Doppler perfusion scanning technology (1). The laser Doppler perfusion imager[®] PIM 1.0 (LDPI) used in this study supports this technique. It is a data acquisition and analysis system that generates images of tissue perfusion. The scanner, located in the laser head, guides a low-power helium-neon laser beam from one skin measurement site to another. At each site, light, including a part of back-scattered laser light, is detected by a photo detector in the laser head. Further processing by a computer system generates arbitrary values (0.00–10.00 V) for each measurement site in proportion to perfusion. After each reading, a color-coded picture made of scanned measurement sites is displayed on a monitor where each color indicates a different perfusion interval.

The principle used by this scanning technology is the same as that used for the laser Doppler flowmetry (2). The main advantage of the LDPI for patch-test readings is its scanning property. It makes possible quick assessment of superficial blood perfusion and eliminates the unavoidable skin touch of the conventional flowmeter that may jeopardize reading results. The technique has been used to scan superficial blood perfusion in patch-test reactions (3–11), as well as in other applications (12–23). Technical information about the LDPI has been published elsewhere and is beyond the scope of this paper (24).

Use of laser Doppler perfusion scanning technology is increasing. Obviously, an optimal set-up of the LDPI is mandatory to get a reliable assessment method that makes it possible to compare intra- and inter-individual test results, and to ensure that assessment values among users are meaningful. The purpose of this paper is to focus on the

clinical use of this technology on patch-test readings of individuals with light skin, tested with non-pigmented test-materials.

To this end, we: (a) study some factors that may affect LDPI readings by using simulated patch tests. The goal is to suggest set-ups that may be appropriate for clinical experiments with patch tests; (b) review clinical experiments based on studies in point a) and with the goal of suggesting a set-up for the LDPI for patch-test readings; (c) examine whether some subject-related factors may affect readings.

We show an example of the use of the technique for patch-test readings by retesting a subject who is allergic to budesonide. The patch test is followed over time with images of perfusion that are used to make a perfusion profile of the test.

Subjects, Materials and Methods

Instrumentation

LDPI readings and assessments were performed with a laser Doppler perfusion imager® PIM 1.0 (LDPI) and LDI 2.5 software (Lisca Development AB, Linköping, Sweden); a bar fastened to the laser head; a moveable tripod with a grip for the laser head bar allowing it to be moved; a Dell Dimension XPS P75 computer connected to the LDPI (Dell computer AB, Upplands Väsby, Sweden); a color monitor; and a color plotter.

To secure an exact reading distance between the laser head aperture and the center of the LDPI reading area, a 3 mW 670 nm Applied Laser pen® LP 30 (Applied laser systems, Grants Pass, Oregon, USA) was fastened to the laser head bar. The LDPI and the laser pen beam spots were made to cross at a desired reading distance from the laser, and at a desired angle between the LDPI laser beam spot and the center of the LDPI reading area. After this, necessary movements of the LDPI were allowed as long as they did not interfere with the angle between the laser beams' spots. After such movements, exact reading distance was secured again by making the laser beam spots cross. The laser pen was turned off during readings.

Studies on factors that may affect LDPI readings

Simulated patch-test areas were read using different set-ups of the LDPI. Background thresholds were 5.81 V and 6.10 to 9.10 V with 0.50 V intervals. Relationships among the following factors were studied.

Size and form of simulated patch tests. To simulate light-absorbing patch-test areas of varying sizes, 10 movable, white, evenly sprayed sheets,

each with a squared opening in the range of 36–361 mm² (median 169 mm²), and another 10 sheets, each with a circular opening ranging from 38–380 mm² (median 154 mm²), were used. These were placed, one at a time, on top of a 5×5 cm opening in a box covered inside with a black light-absorbing fabric. Sheets were placed randomly but centrally within the LDPI reading area and lying over the opening of the box, thus creating variable sizes and forms of simulated light-absorbing patch-test areas.

Location of patch-test areas within the reading area. The sheets with the squared openings were placed, one at a time, over the opening of the box and the sheet opening was located in the center and in each of the corners of the LDPI reading area, forming an exact square of background measurement sites on the monitor at usable set-ups of the instrument for these experiments. The opening of each sheet was placed in the corners of the LDPI reading area as close to the periphery as possible. To secure identical localization in each corner, the squares were placed so that the LDPI read exactly one row of measurement sites around the sides of each square facing the corners. These measurement sites were easily distinguished from sheet openings, because the computer system interpreted the openings as background since no back-scattered light is detected there. These tests were performed with only a 90° angle between the LDPI laser beam and the center of the LDPI reading area, allowing comparison among different locations of patch-test areas within LDPI reading areas.

Reading distance between the laser head aperture and the center of the LDPI reading area. A precision ruler and the laser pen discussed above were used to secure an exact distance between the laser head aperture and the center of the LDPI reading areas. To make the laser beams visible at the level of the sprayed sheets overlying the opening of the box, a sheet of paper was put on top of the opening of the sheets allowing proper adjustment of the beam spots prior to readings. Distances ranging from 9.0 to 22.0 cm at 1.0 cm intervals were used, and 1 mm intervals for selected readings. An increase in signal-to-noise ratio of readings was achieved by special focusing of the laser beam at various distances.

Angle between the LDPI laser beam and the center of the LDPI reading area. A digital level was gently placed on top of the box with the opening and on the box-shaped LDPI laser head in 2 perpendicular directions prior to readings. This was done to secure exact angles between the LDPI laser beam and the center of the LDPI reading area where simulated patch tests were located. Angles

ranged from 90° to 130° at 10° intervals in two perpendicular directions.

Resolution and number of measurement sites. Readings were performed using both high and low resolution, and the following numbers of measurement sites: 16×16, 32×32 and 64×64.

A mean perfusion value of 10 readings was calculated for each possible set-up. Some set-ups were omitted as clearly useless. An amplification factor of 1 was used for all readings.

Review of clinical experiments with patch tests

These experiments are based on perfusional and visual assessments of normal skin, irritant reactions caused by diesel oil, sodium lauryl sulfate and dodecane (3), and approximately 25,500 assessments of allergic reactions or control patches. The allergens used are nickel sulfate hexahydrate (7), Kathon® CG (8), budesonide and neomycin sulfate. These studies have or will be published in detail, but the purpose of this paper is to review these experiments in order to suggest a set-up of the LDPI for patch-test readings.

Effect of subject-related factors on readings

(i) *Pigmentation.* A man with vitiligo and skin type III was contacted approximately 5 months after participating in a study with the LDPI in which he had responded to neomycin sulfate patch tests. At the time of current study, he had not had any active eczema for at least 7 months, and had no other known disease than the contact allergy. He was not on any medication known to affect skin pigmentation or perfusion, and had not been treated with ultraviolet radiation for at least a few years. The study took place during a non-sunny season. Various set-ups of the instrument were used to study influence of pigmentation on readings by comparing reading results of affected and non-affected skin. A clinical junctional nevus was studied in a way similar to the vitiligo patient above to see if pigmentation of such a common skin change might affect LDPI readings.

(ii) *Movement of subjects during readings.* The potential effect of a minor movement of a subject during readings with the LDPI was tested on a healthy subject of skin type II. Various set-ups of the instrument were used.

(iii) A young woman of skin type I had participated in an LDPI study using neomycin sulfate patch tests in the dose range of 0.23–0.0085 mg/cm² with 2 control patches containing the polyvidon vehicle only (Pharmacia A/S, Hillerød, Denmark). Strips had been applied from 3 to 96 h. She developed erythema on skin exposed to the

test tape and on the patch test areas directly after the detachment of long time application strips. The erythema declined after strip detachments during the reading session. In the current study, she was retested for 72 h with patches identical to the ones used before, and using a 5×11 cm strip, also identical to the one used before, made of non-woven cellulose acetate tape coated with acrylic adhesive (Cederroth BV, Tilburg, The Netherlands). Patches were located in a staggered pattern in 2 parallel columns 1 cm apart, with a distance of 2 cm between the patches of each column. Following preparation and just as in the earlier test, the strip was kept in a light-proof, hermetically sealed envelope at 4–8°C for 4 days prior to the application. After a gentle detachment of the strip, the complete strip area was photographed 5 min later and again 25 min after the detachment under approximately the same conditions. Patch-test areas were assessed with the LDPI and visually using a distance of 17 cm between the laser head aperture and the center of the patch tests, a background threshold of 6.1 V, high resolution, an amplification factor of 1, and an angle as close to 90° as possible between the LDPI laser beam and the center of the patch tests. 10×10 measurement sites were selected for each reading. She had not had active eczema for at least 2 months prior to the study, was healthy and without drugs, and had not received any treatment with ultraviolet radiation for at least some years.

Images of perfusion and a perfusion profile of a patch test

A young man of skin type III known to be allergic to budesonide was tested with 6 µg/cm² of this corticosteroid in polyvidon printed on a 9×9 mm polyester patch (Pharmacia A/S, Hillerød, Denmark), and with a control patch printed with polyvidon only. The patches were attached to a tape strip as used above for the neomycin sulfate testing. They were located 1 cm from each other horizontally and 0.5 cm vertically. Following preparation, the strip was kept in a light-proof, hermetically sealed envelope at 4–8°C for 4 days prior to application. The patches were applied for 3 h and the patch-test areas followed with LDPI readings and visually at certain intervals ranging from 30 min after the detachment up to 264 h following the application. Visual assessments were made according to a scale accepted by the North American Contact Dermatitis Research Group (25). The same set-up was used as for neomycin sulfate patch tests above.

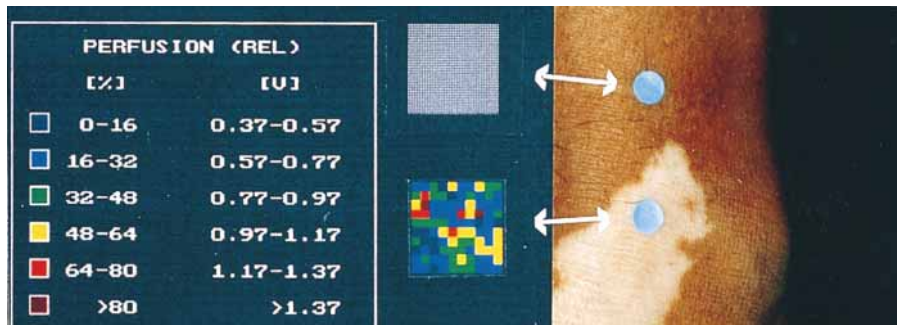


Fig. 1. LDPI readings of healthy pigmented skin of skin type III (above) and of skin with vitiligo (below). Arrows point to blue dots showing the center of readings. The skin covered by the dots is similar to immediate surrounding skin. The reading distance between the laser head aperture and the center of the reading locations is 19 cm using high resolution, a background threshold of 6.1 V, and angle as close to 90° as possible between the LDPI laser beam and the center of readings.

Results

Studies on simulated patch tests and a suggested set-up for clinical use

The light-absorbing simulated patch tests and the surrounding light-scattering sprayed sheets made enough contrast at certain set-ups to allow distinction between the 2, where the light-absorbing patch tests were interpreted as background by the computer system. All set-up factors investigated affected readings. It is beyond the scope of this paper to clarify the effect of each of these factors, the relationship between them, or the vast combinations of factors that could be tested with clinical experiments. The importance of these factors should, however, be observed. For example, only 1 mm change in the reading distance at certain set-ups may affect LDPI reading results and the LDPI is sensitive to changes in the angle between the LDPI laser beam and the center of the simulated patch-test areas. Different locations of patches

within the LDPI reading area may affect readings.

A limited number of set-up combinations were used in the clinical experiments. Thus, a suggested set-up can not be taken as the "optimal" one. All assessments are based on 9×9 mm squared patch-tests (as used by the TRUE Test™ system) located centrally within LDPI reading areas, an angle as close to 90° as possible between the laser beam and the center of patch tests, and a background threshold of 6.1 V. The measurement sites varied from 64×64 to 10×10 sites. Experiments with different reading distances and resolutions showed that a 13 cm reading distance and low resolution worked well on a study population allergic to nickel sulfate (7). However, at that distance, patch-test areas were found relatively small to work with on the monitor, and it could be difficult to assess the spatial heterogeneity of perfusion of patch tests that may be desired by some users. After subtracting perfusion values of identically tested control patches, the perfusion scale ranged from 0.00–7.82

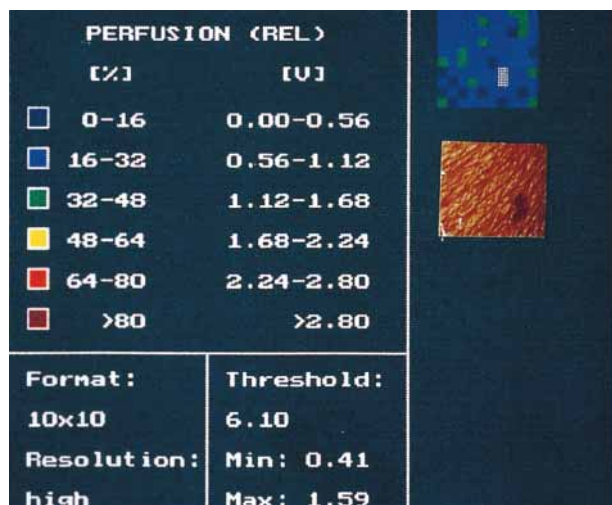


Fig. 2. An LDPI reading of a junctional nevus. The gray measurement sites of the LDPI image indicate background interpretation by the computer system. The same set-up is used as for the readings in Fig. 1 except for a 17 cm reading distance.



Fig. 3. Isolated stripes due to a minor movement of a subject during an LDPI reading.



Fig. 4. Erythema of skin approximately 5 min after the detachment of a patch-test strip. Patch-test areas are located in vertical columns between the highest and lowest blue paper dots on each side at the level of the side dots. (Please excuse one missing dot.)

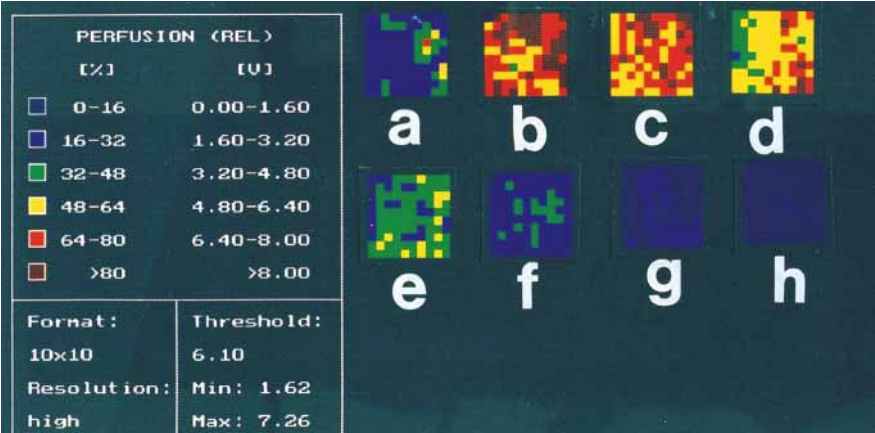
V. Further use tested high resolution using a 15-cm distance for irritant reactions (3), and a 13-cm distance for a study group allergic to Kathon® CG (8). These tests caused no size problem and seemed to allow good assessment of spatial heterogeneity of perfusion within patch-test areas. The assessment scale, after subtraction of the perfusion value of control patches, ranged from 0.00–8.17 V for



Fig. 5. Same application area as in Fig. 4, 25 min after the detachment.

the Kathon® patches. To increase the distance between the laser head and the subjects, more practical for clinical use, a 17 cm reading distance was used on a study group with budesonide. Assessment of these patch tests showed that it was possible to perform readings at that distance without an interruption by the background threshold. Assessed after subtraction of controls, perfusion of these tests ranged from 0.00–7.30 V. These tests needed no amplification of assessment values (fac-

Fig. 6. LDPI color-coded images of a budesonide patch-test reaction followed over time (a–h). For reading times, please see text to Fig. 7. Minimum and maximum values in the figure apply for the 1st reading (a).



tor 1) compared with the other allergic study groups above that needed an amplification factor of 2 to be used for a wide range use of the assessment scale. The same set-up as for the budesonide, used on another study population tested with neomycin sulfate, has worked well. Another study using balsam of Peru is in progress using the same set-up.

For the set-up for the budesonide patch tests above, investigations with 9×9 mm simulated patch tests showed the following results: A central location of the test within the LDPI reading area covers 11×11 measurement sites, regardless of whether 64×64 or 32×32 -site scans were selected. When locations in each of the corners and the center of the reading area were compared, the variation in the number of measurement sites covered by the test area varied up to 14% depending on location, when a scan area of 64×64 sites was selected (a 27 cm² reading area). This lack of agreement could be 10% with a 32×32 -site scan size. The importance of the reading distance is reflected by a 4–5% change in the number of measurement sites with a 0.5 cm deviation from the 17.0 cm distance. If the 90° angle between the LDPI laser beam and the center of the LDPI reading area was changed 10° in any direction, a 3% change in the number of measurement sites resulted, while a 30° change resulted in a 9–11% change.

Effect of subject-related factors on readings

Pigmentation affected LDPI reading results at certain set-ups. This can be seen in Fig. 1, where LDPI perfusion images of pigmented and depigmented skin (vitiligo) may be compared. The

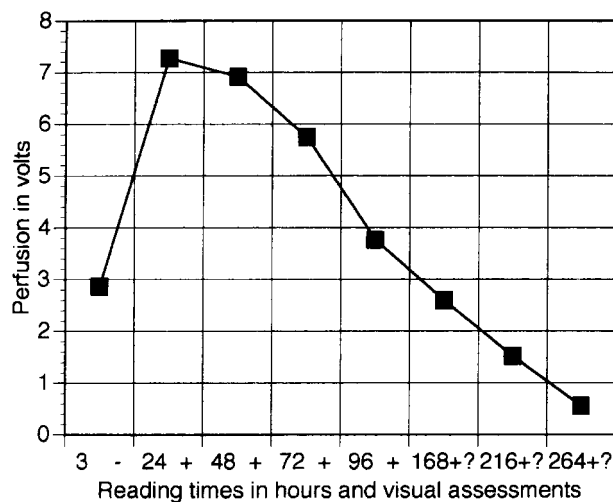


Fig. 7. Mean perfusion value of each image in Fig. 6 with concurrent visual assessments shown on the x-axis for each reading time.

LDPI image of the healthy pigmented skin shows gray-colored squares, indicating background by the computer system, while the image of the depigmented skin shows assessable perfusion at the set-up used. Fig. 2 shows the reading of the junctional nevus and adjacent skin. The nevus area is interpreted as background by the computer system at the set-up used. Thus, at certain set-ups, pigmentation may affect LDPI reading results. Which set-ups are appropriate and which are not for readings of pigmented skin is complex owing to the number of and relationships among the factors involved.

Patient movements, for example coughing or talking, could make isolated stripes on the monitor. Fig. 3 illustrates the effect of a movement. The error caused depends on the grade of movement. Careful observation during the course of LDPI readings may reveal that unacceptable movements and deep breaths will require repeated readings. There was no evidence to indicate that light breathing causes a major error.

Erythema, noticed after the detachment of the 72-h neomycin sulfate application, is shown in Figs. 4, 5. The former is taken approximately 5 min after the detachment, while the latter is taken approximately 20 min later. Perfusion values of control patches were 1.88 V and 1.71 V for the former reading, and 0.61 and 0.73 V for the latter, respectively.

Perfusion of a reaction followed over time

Fig. 6 shows LDPI color-coded images of the budesonide patch test followed over time (a-h), revealing development of a reaction that reaches its maximal intensity early before declining. The mean perfusion value of each image is shown in volts in Fig. 7 with the concurrent visual assessment for each reading time. For comparison, the peak perfusion value of the visually negative control patch was 1.06 V.

Discussion

Our experience supports the claim that the laser Doppler perfusion scanning technology is valuable for non-invasive objective assessment of patch-test reactions. It is important that users use the same set-up for patch-test readings with the LDPI if reading results are to be comparable. We would like to suggest the set-up used on the study groups above tested with budesonide and neomycin sulfate for non-pigmented patch-test materials tested on individuals with light skin. The technique may be used to investigate heterogeneity of superficial perfusion of patch tests by assessing images like the ones shown in Fig. 6, or a part of such images,

by demarcating an area of interest. Such use allows a variety of investigations, including studies on potential accumulation of test substances within patch-test areas. Superficial perfusion of patch-test surroundings may be studied. By following perfusion over time, perfusion profiles can be created, as illustrated in Fig. 7. Such profiles may be used to assess irritant or allergic quality of test materials, the effect of various factors on test responses such as concentration or dose of test substances, vehicle, occlusion, application time of testing materials, reading time of reactions, and variable test locations on the body. Depending on the purpose, such investigations may be based on a single or multiple subjects.

Pigmentation

Figs. 1, 2 show that LDPI readings are affected by pigmentation. The effect is caused by a limited amount of backscattered light from the skin to the photo detector in the laser head due to the pigmentation. This effect is dependent on the grade of pigmentation causing different light scattering in the skin, background threshold selected by the user, and the distance between the laser head and the patch tests. Such images of perfusion may not show perfusional heterogeneity of patch-test areas on the monitor, and may require change of the color scale for this. From this point of view, external factors that may affect pigmentation, such as sunbathing or treatment with ultraviolet light, should be avoided some time prior to and during patch testing. Small variations in pigmentation between subjects may influence assessments only minimally.

Pigmented skin conditions such as the junctional nevus shown in Fig. 2 may affect assessment of mean perfusion of patch tests because they may reduce the number of measurement sites assessed. They may even give false perfusion values at some measurement sites (personal communication, Professor Gert Nilsson). Our preliminary investigations suggest that pigmented patch-test materials may also affect the reading results. Thus, the grade of opacity, distribution, and area size occupied by such testing materials in relation to the underlying perfusion may influence the real perfusion assessment.

Subject movements during readings

Movement must be limited to light breathing to avoid disturbances that may cause false perfusion assessment of patch tests (Fig. 3). Because of this, the arms are probably more advantageous for testing than the back. Patients should not talk during readings.

Skin temperature

Preliminary investigations that we have done on the effect of skin temperature on perfusion assessed with the LDPI have shown for many set-ups that the instrument may be sensitive to minor skin temperature changes (unpublished results). This warrants further investigation.

Subject response to the testing device

Irritant test tape or its removal may be associated with erythema at patch-test areas (Fig. 4). Supporting this is the concurrent decline of erythema of patch tests and of surrounding skin over time (Figs. 4, 5), and our experience that such erythema is not induced in the same individual at short application times that do not cause tape irritation of skin surrounding patch tests. Such erythema, obvious after tape removal, may interfere with assessment of perfusion caused by the patch-test substances themselves. Because of this, an acclimatization period should be encouraged between the detachment of tape and before readings. Other test-related factors include irritation at patch-test borders caused by the testing device and subject-related response, such as scratching, to test materials. We have recently investigated the effect of different types of tape on the intensity of irritant patch-test reactions using the LDPI, and found the tapes to be of importance (9). We have studied possible effect on reading results caused by skin depressions (26) left by chamber edges after removal of Finn Chambers®, and have not found these *per se* to affect reading results with the set-up suggested here. A study assessing perfusion with a laser Doppler flowmetry has shown differences in irritant potential among different test procedures. The most favorable patches tested were those of TRUE Test™ and Van der Bend square chambers (27–29).

Relocation of patch-test areas for readings

We have used a skin marker (Chemotechnique AB, Malmö, Sweden) to mark the location of each patch-test area and patch-test columns directly after application of strips just outside strip borders. On top of these markings, we have put small labels, thus doubly securing markings of test areas (Figs. 4, 5). These markings and a thin flexible film with openings at the site of patch-tests should help to locate them again. For LDPI patch-test readings on the back, we have experienced difficulties in locating patch-test areas again when the position in which the subjects are lying causes the skin to stretch. Similar problems have arisen on subjects

with extensive body fat and thick skin folds. We recommend that subjects lie prone during attachment of application strips, and in a position intended for readings to minimize this problem.

Body location of patch tests

Different locations of patch tests on the body may affect visual assessment of patch tests. The LDPI may be a good instrument for detailed studies on such differences. Using patch tests or tuberculin tests, studies have shown that the back may give more positive reactions than other locations (30, 31). Furthermore, location of patch tests on the upper or lower part of the back (30) or on the left or right side of the upper back may give different test results (32).

The patch test area

Patch tests should ideally be located on a skin area that allows a parallel location of the LDPI laser head above it. The skin should be free from visible pathological skin conditions. Such testing areas may have different perfusion or pigmentation compared with other skin locations. Heterogeneity of normal skin perfusion may possibly jeopardize perfusion assessments especially of very weak reactions at certain set-ups. However, at the set-up suggested here we have experienced such heterogeneity to be of little or no importance.

Light in the test room

We have found even weak working light in the test room to interfere with LDPI readings. The minimum quantity of light that may affect LDPI reading results for different set-ups warrants further investigation. We suggest room light be reduced as much as possible during measurements, light emitted from the monitor be kept at a minimum and turned in a direction so as not to affect readings.

Measurement sites

As the LDPI reads and assesses squared areas only (Fig. 6), an unavoidable problem with circular patch-test areas is that surrounding skin becomes a part of perfusion assessments. For squares, using a larger number of measurement sites is safer than the number corresponding exactly to the patch-test areas because exact focusing of the LDPI laser beam in the center of the patch-test areas may be difficult in practice. A disadvantage with larger reading areas is that each patch-test area must be demarcated within the LDPI reading area prior to perfusion assessment, and readings take a longer

time to perform. A problem of reading the exact size of the patch-test areas is associated with test areas requiring an uneven number of measurement sites for complete capture, as the LDPI allows even numbers only. We discourage a change of the reading distance between the laser head aperture and the skin to overcome that problem if the set-up suggested here is used otherwise unchanged. This problem may be solved by changing patch-test sizes. We have used 9×9 mm squares as used in TRUE Tests™. For some test readings, we have used the set-up suggested in this paper, cautiously focused the LDPI laser beam in the center of patch-test areas, and read 10×10 instead of 11×11 measurement sites needed for complete capture of that test-area size. We have, thus, excluded from readings a border of 1×1 to prevent surrounding skin from becoming a part of the reading caused by manual inaccuracy that occurs in the focusing of the beam in the center of patch-test areas. This even excludes potential edge irritation from becoming a part of the reading. This approach may, however, interfere with exact perfusion assessment, especially if there is a relatively large difference between reactivity in test centers and in the periphery of test areas.

Focusing the laser beam and patch-test location within the LDPI reading area

Resolution and signal-to-noise ratios of readings may be increased if the laser beam is modified and focused at the exact reading distance between the laser head aperture and the center of the patch-test areas. However, after such focusing, increased accuracy is required in the adjustment of the reading distance. The laser beam should be focused in the center of the patch tests and these located in the center of the LDPI reading area as different locations may affect comparison of reading results.

Laser pen

For clinical use, we found an adjustable laser pen indispensable for securing exact reading distances. Such pens may require the use of proper eye protection. An alternative is a precision ruler of a desired length placed between the laser head and the skin. However, for reasons of accuracy, using a ruler makes touch unavoidable and thus risks true perfusion assessment of testing materials.

Palpation of test areas

Palpation is used in practice by some dermatologists for interpretation of patch-test reactions. Such palpation may be sufficient to increase per-

fusion levels of patch tests. It may be avoided by making the clinical assessment after the LDPI reading. It can be reduced by using the visual assessment scale accepted by the North American Contact Dermatitis Research Group requiring visible discrete papules for a positive (“+”) reaction (14) compared to some European systems that require erythema and infiltration only (33). Reduced test sensitivity may be expected compared to 3 European systems, as a study has shown 9% of reactions to pass undetected with the American system. However, estimation of false positives was impossible in that study as patch-testing with irritants was not included, as pointed out by the authors (33).

Control patch-test areas

To account for the effect of non-test related factors including variance in skin pigmentation and superficial skin perfusion among different test-area locations on LDPI reading results, one alternative is to perform LDPI readings of each test area to serve as a control prior to applications of patches. Such a procedure requires accuracy that may be difficult to perform in practice. Another disadvantage with that approach is that various test-related factors that may affect test results are not accounted for. Such factors include vehicle, occlusion, and tape used for application of patches. We have included vehicle-containing control patches on patch-test substance application strips and read these at the same reading session as patches containing test substance. The subtraction of the mean perfusion value of such control patches from test substance containing patches helps account for such test-related factors. However, that approach does not account for the non-test related factors accounted for by the other approach. If the set-up suggested here is used on light skin, we believe, for non-pigmented patch-test materials, that test related factors are of greater importance once applications are avoided on visible pathological skin conditions.

Relationship between LDPI assessments and visible erythematous intensity of patch tests

When they are a few days old, visually intense erythematous patch-test reactions often show low perfusion levels compared to early visually less intense reactions. This is probably caused by a dilatation of blood vessels associated with a limited movement of blood cells. An investigation using the LDPI has shown that, “relative central slowing” of blood flow can occur elsewhere than in the center of an erythematous reaction and may be due to a

localized edema (15). This is interesting as it suggests that the LDPI may not be an ideal instrument to assess all erythematous and edematous patch tests or skin disease with slow perfusion. The loss of relationship between LDPI perfusion level and erythematous intensity of skin has been observed with investigations on port wine stains, showing no difference between perfusion levels within the stains and in surrounding skin in 9 of 13 patients investigated (34).

Repeatability of patch-test results

Wårdell et al. have calculated mean perfusion from 16 consecutive LDPI readings using dithranol and a separately developed software unit with the LDPI program as a base. For their set-up and test materials, they showed that a potential error in isolated reading results is in the order of 10% (6). Seifalian et al. have also found approximately the same error by assessing 6 separate measurements at the same position on normal skin of a single volunteer exposed to heat (35). Preliminary investigations that we have done using Kathon® CG and the set-up suggested here have shown an even lower error, or below 5% (unpublished experiments).

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