

Assessment of irritant skin reactions using electrical impedance – a comparison between 2 laboratories

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To assess interlaboratory variability in the measurement of skin electrical impedance (IMP), we evaluated irritant reactions to sodium lauryl sulfate (SLS) (2%) and nonanoic acid (NAA) (40%) in 2 laboratories. We studied the patch test responses in 40 healthy male and female volunteers between 20 and 30 years of age (20 in each laboratory) with an instrument for measuring IMP. 2 other bioengineering methods and visual scoring were also used to facilitate further illumination of any findings. A strict protocol including all details of the measurement procedure was carefully implemented in both laboratories. The skin reactions were evaluated at 23 h and at 3, 7 and 14 days after exposure. Our findings show that both irritants caused distinct dynamic responses detectable with the bioengineering techniques. Interestingly, the IMP baseline values varied between the 2 laboratories. Moreover, at early stages in the development of irritation (day 1), the irritants induced changes in IMP indices in the opposite direction, which accords with the concept of various IMP patterns of contact dermatitis caused by different irritants. Although the absolute values of IMP differed in both laboratories, the dynamic response patterns were the same.

Key words: bioengineering methods; electrical impedance; irritancy; multicentre study; nonanoic acid; sodium lauryl sulfate. © Blackwell Munksgaard, 2003.

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Contact dermatitis is a skin condition induced by contact with exogenous agents, e.g. solvents and detergents. For many years, visual assessment was the only way to evaluate patch tests. During the last few decades, bioengineering techniques, providing objective and quantitative data, have been used in the field of experimental contact dermatitis. In brief, all bioengineering devices are based on a particular physical principle and, therefore, assess only a fragment of biological phenomena, which, in reality, are the results of complex events.

In the present study, we used visual assessment and 3 non-invasive instruments, including IMP, to classify and quantify experimentally induced skin reactions. The irritants chosen, i.e. sodium lauryl sulfate and NAA, have been extensively used as model irritants in tests of skin irritation. The aim was to compare the data obtained by 2 laboratories with special reference to the IMP instrument, in the context of monitoring irritation phenomena and the process of recovery.

Materials and Methods

Test subjects

The subjects comprised 40 healthy non-smoking subjects. Of these, 4 males and 16 females, mean age 25 (20–28) years, were from the Department of Dermatology at Huddinge University Hospital (here called HUDDINGE), and 5 males and 15 females, mean age 25 (20–30) years, were from the Department of Environmental and Occupational Dermatology at Norrbacka, Stockholm County Council, Stockholm (here called NORRBACKA). The measurements were performed from September to November 2001. The Regional Ethics Committee of Karolinska Institutet approved the study protocol and informed consent was obtained from all participants.

Test substances

NAA (SIGMA) was used at a concentration of 40% in 99.7% L-propanol (v/v), and SLS

(SIGMA) was used at a concentration of 2% in distilled water (w/v). These concentrations were based on previous tests, in which both irritants produced similar ranges of response, as judged by visual scores. We used the following controls: water, L-propanol, empty chamber and no chamber.

Assessment

Visual assessment. The test reactions were read according to the simple scoring system: 0 = negative, 1/5 = doubtful, 1 = weak (weak erythema and slight oedema), 2 = moderate (moderate degree of: erythema, oedema, scaling or roughness), 3 = strong (marked degree of erythema, oedema, scaling or roughness) (1).

Transepidermal water loss. Transepidermal water loss (TEWL) (2) was measured at Huddinge with the Evaporimeter[®] EPI (Servomed AB, Kinna, Sweden). The measurements at Norrbacka were performed with Derma LAB[®] CE0543 (Cortex Technology, Hadsund, Denmark).

Laser Doppler flowmetry. Laser Doppler flowmetry (LDF) (3) was performed with Periflux 4001 (Perimed AB, Stockholm, Sweden) at Huddinge and with Periflux 5010 (Perimed AB) at Norrbacka, both of which had the same type of probe (probe n 413).

Electrical impedance. IMP was determined in both laboratories with 2 IMP spectrometers (4), model SciBase I (SciBase AB, Huddinge, Sweden). The instruments were validated using a calibrated test plug and identical electrode systems (precision gold electrodes on ceramic platform), implying that any differences could not be ascribed to lack of calibration. The instrument records impedance spectra, both magnitude and phase, at 31 logarithmically distributed frequencies in the range of 1 kHz–1 MHz in an area below the probe. The IMP device facilitates measurements to 5 depths. In this study, only depth 5 was used. At the time of the study, no formal guidelines for the instrument had been published. In short, we used a set of 4 indices, representing changes with frequency in the 4 main aspects of IMP in the complex number space (5):

$$\text{Magnitude index, MIX} = \text{abs}(Z_{20\text{kHz}}) / \text{abs}(Z_{500\text{kHz}});$$

$$\text{Phase index, PIX} = \arg(Z_{20\text{kHz}}) - \arg(Z_{500\text{kHz}});$$

$$\text{Real part index, RIX} = \text{Re}(Z_{20\text{kHz}}) / \text{abs}(Z_{500\text{kHz}});$$

$$\text{Imaginary part index, IMIX} = \text{Im}(Z_{20\text{kHz}}) / \text{abs}(Z_{500\text{kHz}}).$$

Test procedure

6 sites, on the midvolar aspect of the non-dominant forearm of each participant, were randomly assigned for a test substance or control. The substances were applied in 12 mm Finn Chambers[®] (Boule Nordic AB, Stockholm, Sweden) for 22 h. Each chamber contained 50 µL of the test solution on a paper disk. Ink marks around the sites ensured correct placement of the chambers and instrument probes. Readings were taken before application (day 0), 23 h after application of the patches (day 1) and on days 3, 7 and 14 of the study. 1 h before the second visit, the volunteers removed their own patches. All the measurements were made after at least 20 min rest on a bed with the study forearm immobilized by an inflatable pillow. Readings were made in the following order: visual scoring, TEWL, LDF and IMP. Before the IMP measurement, the skin is moistened for 60 s with physiological saline to reduce the normally high impedance of the stratum corneum. None of the volunteers was allowed to use skin care products for at least 1 day before the study or to drink coffee 2 h before the measurements.

Laboratory calibration

To reduce the interlaboratory variation due to handling of the instruments, a pilot study was performed on 2 subjects from both laboratories. The measurements of TEWL and with LDF were made in accordance with published guidelines (2, 3). Based on the pilot study, a strict study protocol was devised by the authors. Each IMP measurement is shown on the computer screen as 2 graphs representing the 31 frequencies, 1 graph for the phase angle and 1 for the magnitude. Depending on the shapes of the curves, each measurement is judged as acceptable or not. If a measurement is considered unacceptable because of its form of the graphs, additional inundation time was employed. The authors worked together to ensure that they used the same definitions for acceptable and unacceptable IMP curves. The readings were made at: Huddinge by NK (all), Norrbacka by SJ (IMP and visual) and by CD (TEWL, LDF and visual).

Statistical analysis

We analysed the data using multifactor-repeated measurement ANOVA with repeated measures of 2 factors: (1) Huddinge and Norrbacka laboratories and (2) time, i.e. days 0, 1, 2, 7 and 14. The *P*-values were corrected by the Bonferroni

procedure (6) and $P \leq 0.05$ was chosen as the level of significance.

Results

Environmental factors. In Huddinge Laboratory, the average air temperature and relative humidity were 24.8°Celsius (range 23.6–25.8°Celsius) and 35% (24.6–48.8%), respectively. Those in Norrbacka laboratory were 21.8°Celsius (18.6–26.1°Celsius) and 36.4% (15.6–79.8%), respectively, throughout the study.

Visual scoring. These results are summarized in Table 1. On day 14, 4 subjects at Huddinge and 9 in Norrbacka showed positive reactions to NAA, all of whom had received a score of 1, apart from 2 in Norrbacka who were scored as 2. On day 14, visual scoring of the SLS-induced reactions was negative in Huddinge for all subjects, but in Norrbacka, 9 volunteers showed a positive reaction scored as 1 and 1 person had a reaction scored as 2.

Transepidermal water loss. The dynamics of the changes in TEWL were similar in the Huddinge and Norrbacka subjects for both SLS-induced (Fig. 1a) and NAA-induced (Fig. 1b) reactions.

Laser Doppler. Both laboratories showed a significant increase in LDF values with highest values on day 1 in NAA-induced reactions and on day 3 in SLS-induced reactions (data not shown).

TEWL and LDF measurements were performed to obtain objective data to support the visual scoring.

Electrical impedance

SLS-induced reactions. The dynamics of the patterns were essentially similar in both laboratories, for SLS-induced reactions (Figs 2a,b). Interestingly, on day 0, we found a significant difference between the MIX indices in the laboratories, i.e. the values

were higher in the Norrbacka subjects. On days 1, 3, 7 and 14, the mean MIX index in the SLS-induced reactions was significantly lower in both laboratories than on day 0 (Fig. 2a). The PIX index was similar in both laboratories on day 0 (Fig. 2b).

NAA-induced reactions. The dynamics of the patterns were essentially similar in both laboratories, for NAA-induced reactions (Figs 3a,b). The baseline value, day 0, for the MIX index was significantly higher in Norrbacka subjects (Fig. 3a). The mean values of the PIX index did not differ in the 2 laboratory groups on day 0. In both laboratories, PIX decreased on days 1 and 3 and normalized on day 7 (Fig. 3b).

Discussion

Visual scoring is the commonest and most practical method used for clinical assessment of patch tests. Because it is subjective, long training (7), a simplified scoring system (8), a sufficient number of subjects (9) and careful design are required. In our study, visual evaluation of the clinical findings despite some quantitative differences tended to be similar. To objectify these experimental findings, we used 3 non-invasive techniques (TEWL, LDF and IMP).

TEWL changes were in accordance with previous findings by others (5). Even after 14 days, complete barrier recovery after acute irritation with 2% SLS had still not occurred. In a study by Wilhelm et al. (10), using a chronic irritation model, it took 20 days after the last surfactant treatment with 7.5% SLS in polypropylene chamber patches for the TEWL values to normalize. Because the skin's response to irritants includes an increase in blood flow, measurement of cutaneous blood flow has successfully been used to quantify the results of the patch test, which was also the case in this study (data not shown).

Table 1. Results of visual scoring and application of 2% sodium lauryl sulfate (SLS) and 40% nonanoic acid (NAA) in Huddinge ($n=20$) and Norrbacka ($n=20$) laboratories on days 0 (before application of the substance), 1, 3, 7 and 14

Days scores	SLS – number of subjects					NAA – number of subjects				
	0	1	3	7	14	0	1	3	7	14
Huddinge laboratory										
0	20	10	6	13	20	20	1	1	3	16
1	0	8	14	6	0	0	11	10	11	4
2	0	2	0	1	0	0	8	9	6	0
3	0	0	0	0	0	0	0	0	0	0
Norrbacka laboratory										
0	20	0	0	4	10	20	0	0	3	9
1	0	12	18	12	9	0	7	4	8	9
2	0	7	2	4	1	0	13	15	9	2
3	0	1	0	0	0	0	0	1	0	0

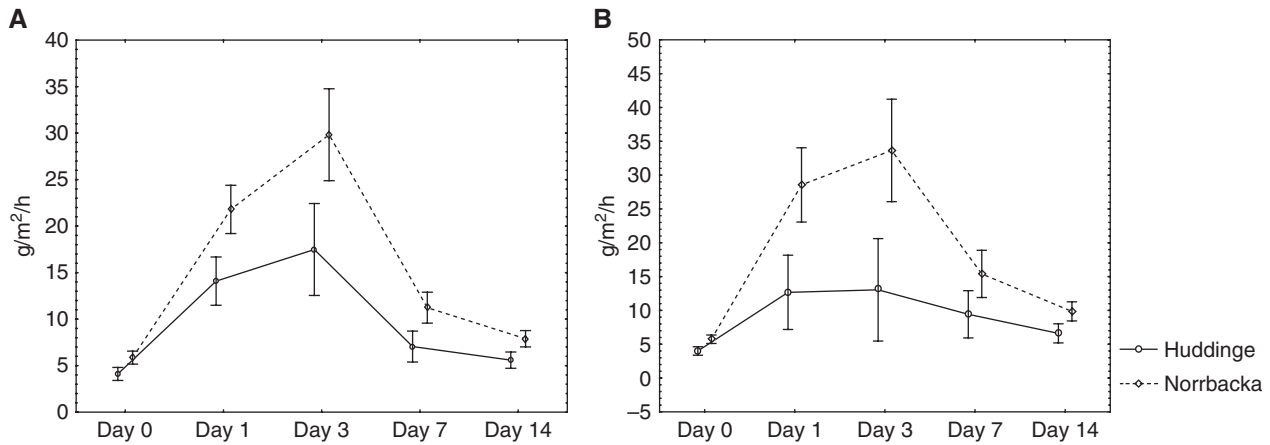


Fig. 1. Means and 95% confidence intervals for transepidermal water loss measurements of skin reactions to 2% sodium lauryl sulfate (a) and 40% nonanoic acid (b) in 2 laboratories: Huddinge ($n=20$) and Norrbacka ($n=20$), on days 0 (before application of the substance), 1, 3, 7 and 14.

The analysis of data using the IMP instrument for impedance measurements was performed with indices (4). Previous studies on skin irritation have shown that bioimpedance modelling by index extraction is a simple and sensitive method (11). In a study using SLS, benzalkoniumchloride and NAA, it was shown that each one produced histologically different effects, 24 h after removal of the patches. The patterns of the indices were distinctive, which suggested that one can characterize these reactions by IMP parameters (5). These changes in patterns were confirmed by our study as regards the difference between SLS- and NAA-induced skin reactions in the early stages of the development of a response to an irritant. We found time-dependent differences in the response to SLS and NAA, detectable on day 1, but not later on, which is in accordance with previously published data, time-dependent differences between SLS- and NAA-induced reactions in the Langerhans' cell population (12), the composition

of the inflammatory cell infiltrate (13) and the elementary content (14) have been reported.

It has been proposed that the impedance of the skin, amongst other factors, is affected by (1): the state of hydration of the SC (15), (2) changes in the lipid content of the normal human SC (4) and (3) the number of SC layers (16). The same factors affect skin barrier function, and such similarities suggests that measurements of IMP should be suitable for objective assessment of the condition of the skin barrier.

Our study shows that although there were some differences between the values in the 2 laboratories, which prevented us from pooling the results, the findings regarding dynamic measurement changes were similar. The significant difference in IMP baseline is interesting and deserves some consideration. This difference may be due to:

(1) Operator variability in preparation of the skin for the IMP measurement. All steps in the procedure – e.g. the time for wetting the skin,

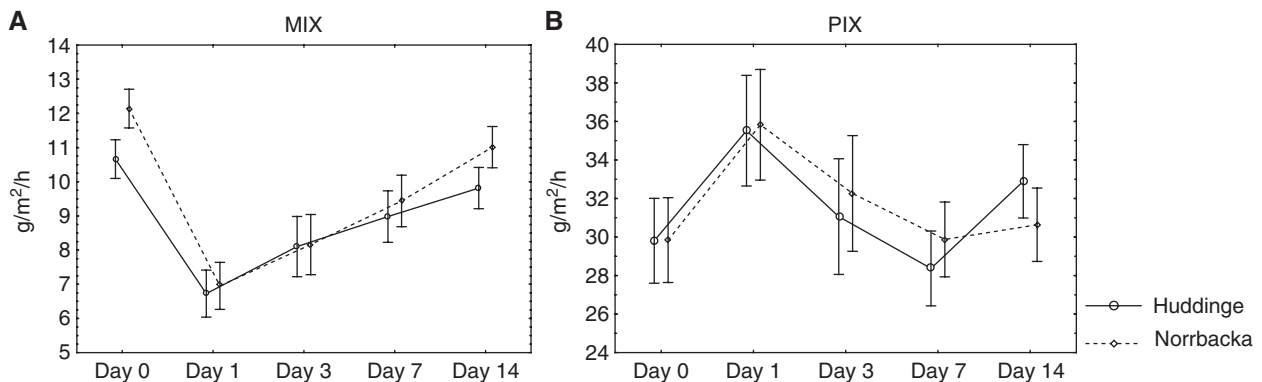


Fig. 2. Means and 95% confidence intervals for measurements of skin reactions to 2% sodium lauryl sulfate in Huddinge ($n=20$) and Norrbacka ($n=20$), as reflected by 2 impedance indices: magnitude index (MIX) (a) and phase index (PIX) (b), on days 0 (before application of the substance), 1, 3, 7 and 14.

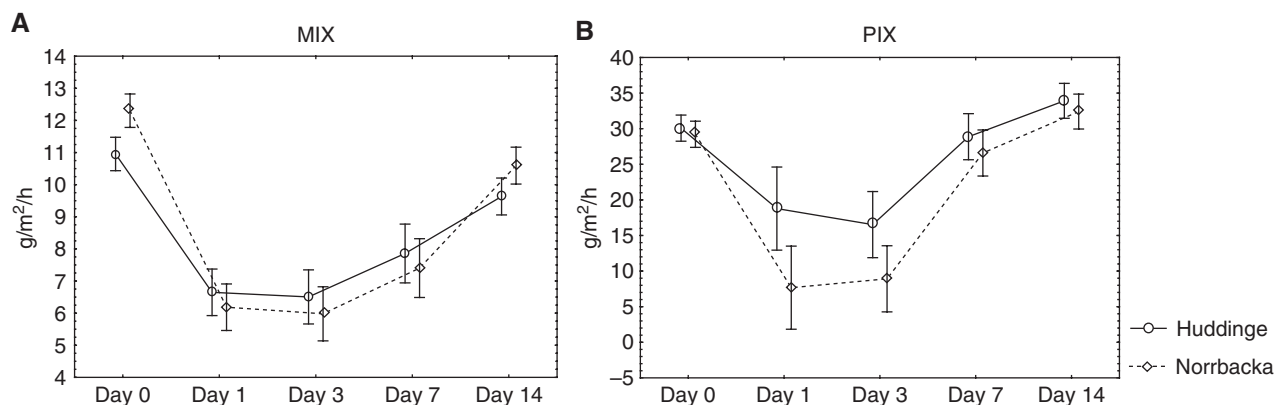


Fig. 3. Means and 95% confidence intervals for measurements of skin reactions to 40% nonanoic acid in Huddinge ($n = 20$) and Norrbacka ($n = 20$), as reflected by 2 impedance indices: magnitude index (MIX) (a) and phase index (PIX) (b), on days 0 (before application of the substance), 1, 3, 7 and 14.

pressure of the probe, judging of the IMP curves and repetitive measurements – may be of importance. Future standardization of the procedure is needed to increase the accuracy and reproducibility of the data for interlaboratory studies and comparisons.

(2) Differences in reactivity between subjects. In our groups, the subjects had been carefully selected and matched for age and sex before the study. However, some differences may have been present regarding their sensitivity to irritants as evaluated with TEWL and IMP. Responses to irritants differ with various irritants, doses and concentrations, frequency of exposure, season, sex, age, skin type/colour, anatomical site, atopic status and phase of menstrual cycle (1). More and larger studies are needed on the effects of these factors on the impedance measurements.

(3) Differences in instruments. TEWL devices, although calibrated in the same unit ($\text{g/m}^2\text{h}$), give different results due to probe geometry and type of sensing elements. Therefore, calibration in a laboratory cannot overcome such physical differences when the devices are used in clinical work (17). For the IMP instrument, calibration was adequate in this study.

In conclusion, distinct changes in 4 impedance indices may be used to classify irritant contact reactions, although further clarification of the factors that affect skin impedance are needed. More advanced data analysis, using more of the information embedded in the impedance spectra, may increase the precision and comparability of studies on irritants with IMP.

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