

A hand immersion test under laboratory-controlled usage conditions: the need for sensitive and controlled assessment methods*

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Exaggerated test conditions were frequently used to investigate the cutaneous tolerance of detergent products in the past. As the sensitivity of newly designed biometric methods is steadily improving, the trend towards more realistic test conditions should be encouraged. A hand immersion test under laboratory-controlled usage conditions is presently described, fulfilling such principles. Panelists soaked their hands in 2 different hand dishwashing liquids, 2× daily for 10 min each (with successive in-solution/out-of-solution cycles) for 4 consecutive days. Products were at usual dilution for dishwashing liquids and were randomized between the dominant and non-dominant hands of panelists. Visual scoring of erythema and dryness developing on the whole hands (scoring scales including interdigital areas and joints) during the week did not allow discrimination between the 2 products. However the dominant hands were significantly more susceptible to alterations than the non-dominant hands, regardless of product attribution. In contrast, skin electrical measurements (Corneometer® CM800 and Skicon® 200) on the dorsum of the hands (muscle mass between thumb and index) and squamometry analysis of tape stripping (harvested from the same site) yielded significant differences between the 2 products. In conclusion, a hand immersion test under realistic conditions has been described, which discriminates between products when sensitive assessment methods are used to explore skin sites partially protected from daily-life skin aggressions.

Key words: hand immersion test; irritation; mildness; stratum corneum stripping; conductance; biometry; detergent; hand dominance. © Munksgaard, 1999.

Accepted for publication 17 October 1999

In the investigation of the cutaneous tolerance of detergent products, exaggerated test conditions are frequently used in order to amplify the differences between products. In the Frosch-Kligman soap chamber test (1) or its variants, products are applied to the skin for a long period of time (2–4), sometimes undiluted (5, 6) and usually under occlusion (7). To move towards interaction between the product and the skin closer to that in the daily life of the consumer, exaggerated usage tests were developed. These tests were adapted to the type of products that had to be tested. Exaggerated arm wash (8), flex wash (9) or hand wash (10) tests seemed the most appropriate to study the interaction of body cleansing products with the skin. For foam baths or dishwashing liquids, exaggerated

hand or arm immersion tests (11) were often more relevant. In all of these tests, however, products were applied repetitively, and for more time than in normal use. In the past, when sensitive methods to detect faint skin alterations were not available, such exaggerated procedures were necessary. Products were compared based on the clinical effects of the products, such as inflammatory reaction or xerosis. Progressively, bioengineering measurement techniques have been included (2, 8) to improve the discriminatory power of the tests, but the exaggerated application conditions were maintained.

This study describes a hand immersion test under laboratory-controlled usage conditions, in which 2 hand dishwashing liquids are compared by a type of contact with the hands and the resultant cutaneous reaction, which is more relevant to consumer experience. The importance of well-designed randomization of products is emphasized, as well

* Part of this paper was presented as a poster at the June 1998 ISBS Meeting in Boston, USA.

as a recommendation for sensitive assessment methods and appropriate measurement sites.

Materials and Methods

Products

2 hand dishwashing liquids were used in the test; they were both at a surfactant concentration of 42–45%.

- product A=majority of anionic surfactants-based dishwashing liquid
- product B=majority of nonionic surfactants-based dishwashing liquid

Tests

All tests were performed on healthy, fully informed and consented volunteers, according to the principles of good clinical practice.

Frosch-Kligman soap chamber test (1). 300 μ l of 2.25% (v/v) solutions of products A and B were used in 25-mm diameter Hill Top Chambers® (Hill Top, Cincinnati, OH, US). On day (D) 1, chambers were applied to the volar forearms of the volunteers ($n=25$). 24 h later, chambers were removed, sites rinsed under running tap water and patted dry without rubbing. 30 min later, products were re-applied under the same conditions and on the same sites for 6 h, when they were removed and sites rinsed and dried as above. On D 3, 4 and 5, skin reaction was evaluated on the different sites for erythema and dryness, and products re-applied for 6 h each day. On D 8, subjects reported to the testing laboratory for a final evaluation of dryness.

Products A and B were included in a series of 8 products which were randomized within subjects. 6 out of the 8 products were not related to this work. Visual scoring of cutaneous reactions was done by a trained evaluator, according to the following scales: erythema: 0=no evidence of erythema; 1=slight redness, spotty and diffuse; 2=moderate, uniform redness; 3=strong, uniform redness; 4=fiery red. Dryness: 0=no evidence of scaling; 1=fine scaling, whitish in appearance; 2=moderate scaling; 3=large flakes mixed with small scales; 4=large flakes over the whole surface.

Hand immersion test

Panelists ($n=30$) soaked their hands in solutions of products A and B 2 $\times$ a day, Monday through Thursday, for 10 min per soak. There was a period of 5 h between the 2 daily soak sessions. When soaking, subjects followed successive cycles with the hands in the solutions for 20 s and out of the solutions for 30 s (12 full cycles over the 10 min soak session). At the end of each session, hands

were rinsed under running tap water and patted dry without rubbing.

For the test period and for 3 days prior to the test, panelists were asked not to apply lotions or any topical product to their hands. The 2 products A and B were diluted at 0.2% (v/v) in a volume of 2 l and used at 40°C. Temperature at the end of a soaking session was between 35 and 37°C. The assignment of a product to a particular hand was constant for the duration of the test; randomization of products between hands was based on hand dominance.

Evaluations. Visual scoring of erythema and dryness was done by a trained evaluator, using an illuminated magnifying ($\times 2$) lens, before each soaking session and on the morning of the 5th day. The following scoring scales, taking into account the complete surface of the hands, were used: erythema: 0=no evidence of erythema; 1=barely visible pink color, non-extended, mainly over joints; 2=barely visible pink color over extended area, or slight pink color over joints; 3=obvious pink color, non-extended, mainly over joints; 4=obvious pink color over extended area; 5=moderate pink color, difference clearly visible between the hand and the non-soaked wrist; 6=marked pink color; 7=deep pink color; 8=deep pink color and slight fissuring; 9=deep pink color, fissuring and edema. Dryness: 0=skin smooth, soft, not dry at all; 1=slight flaking, limited area; 2=moderate flaking, limited area; 3=marked flaking on limited area or moderate flaking over extended area; 4=marked flaking over extended area; 5=like 4, with slight cracking; 6=like 4 with moderate cracking; 7=like 4 with slight fissuring; 8=like 4 with moderate fissuring; 9=like 4 with severe fissuring.

Instrumental measurements of skin surface hydration/dehydration were performed before the morning session of each day and on the morning of the 5th day, after the panelists had waited for 20 min, at rest, in a room at $20 \pm 2^\circ\text{C}$ and 50% relative humidity. Measurements were performed on the muscle mass on the back of hands, between the thumb and the index finger; the hands were laid flat on the table with the thumb separated from the hand and the other fingers held together. 2 instruments were used for this purpose, the Corneometer® CM800 (Courage & Khazaka, Köln, Germany) and the Skicon® 200 (I.B.S., Shizuoka-ken, Japan) equipped with an 8 contact prongs probe (Measurement Technologies, Cincinnati, OH).

Stratum corneum tape strippings were collected by means of D-squame® adhesive discs (Cuderm, Dallas, TX, USA) from both hands, on the morning of D 1 (baseline, lateral side of the back of hands) and on the morning of D 5 after all other

evaluations (same site as instrumental measurements). They were processed to be analyzed by squamometry (12); briefly, they were stained for 60 s with PMS (Polychrome Multiple Stain, Dermatologic Lab Supply, Bluffs, USA), rinsed and the intensity of staining was quantified by Chroma C*

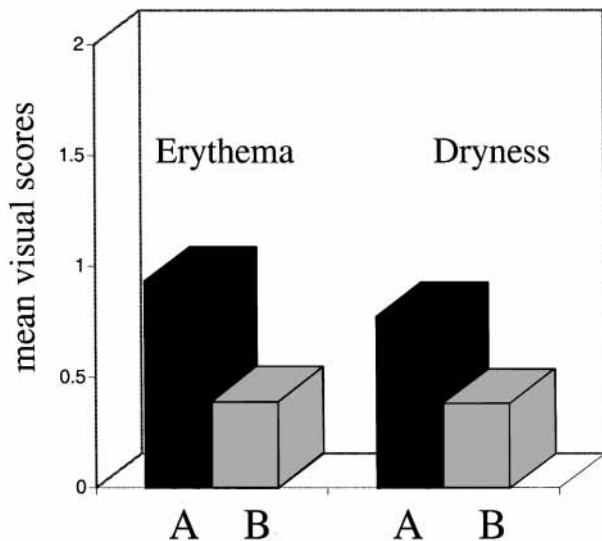


Fig. 1. Frosch-Kligman soap chamber test, comparison of products A and B. Products were applied at 2.25% (v/v) in a Frosch-Kligman soap chamber test. The mean score of erythema at D 5 and of dryness at D 8 is given for product A and product B.

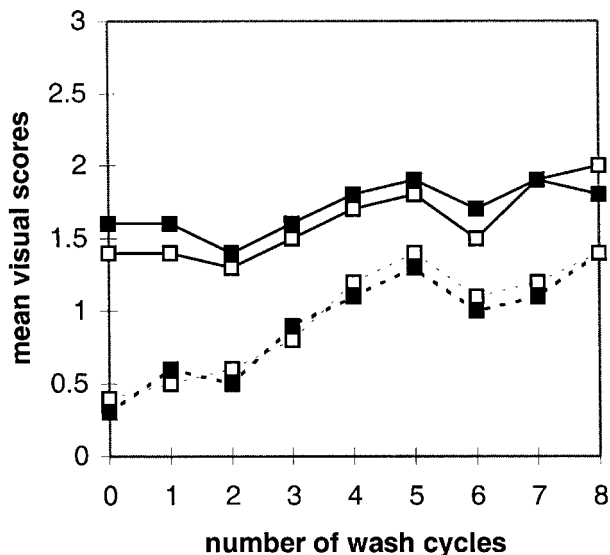


Fig. 2. Hand immersion test – comparison of products A and B. Erythema (continuous lines) and dryness (dotted lines) were evaluated by a trained assessor on 0 to 9 scoring scales taking into account the whole hands. Mean scores at each assessment session are given for product A (occluded squares) and B (open squares). No significant difference was observed between products A and B.

measurement using the Minolta Chroma Meter® CR200 in L*c*h* mode.

Statistical analysis: A paired *t*-test comparison between products A and B was performed at the most relevant days (D 5 for erythema, D 8 for dryness) in the Frosch-Kligman soap chamber test. In the hand immersion test, a paired *t*-test was performed for each wash cycle in order to compare (i) the effect of products A and B, and (ii) the skin condition of the dominant hand to that of the non-dominant hand of each subject, regardless of the product solution in which the hands were soaked. For instrumental measurements (Corneometer®, Skicon®, Chroma Meter®), statistics were performed on the differential values versus the baseline, at each time point.

Results

The 2 test dishwashing liquids were clearly differentiated in a Frosch-Kligman soap chamber test (FK-SCT), with product B being significantly milder to skin than product A (Fig. 1). However, both products induced limited cutaneous response under these test conditions.

In the hand immersion test (HIT), visually scored dryness slightly increased during the week

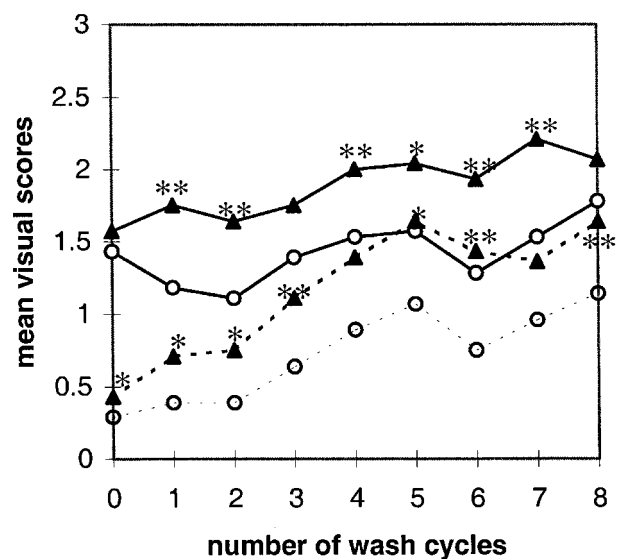


Fig. 3. Hand immersion test – comparison of dominant and non-dominant hands. Erythema (continuous lines) and dryness (dotted lines) were evaluated by a trained assessor on 0 to 9 scoring scales taking into account the whole hands. Mean scores at each assessment session are given for the dominant hands of panelists (occluded triangle) and their non-dominant hands (open circle), regardless of the product solutions in which the hands were soaked. Statistically significant differences between the 2 hands are indicated by * (>95% of confidence level in a paired comparison *t*-test) and ** (>99% of confidence level in a paired comparison *t*-test).

Table 1. Skin properties measured at the baseline

	Comparison of hands dedicated to prod. A versus hands dedicated to prod. B			Comparison of preferred hands versus non-preferred hands whatever product assignment		
	product A	product B	<i>t</i> -test: A versus B	dominant hand	non-dominant hand	<i>t</i> -test dom. versus non-dom.
Corneometer® measurements	42±2	42±2	NS	43±2	41±2	NS
Skicon® measurements	153±11	154±11	NS	146±10	161±11	sign. <i>p</i> <0.05
Chroma C* of D-Squames® discs	22.1±1.1	23.8±1.3	NS	23.7±1.3	22.1±1.1	NS

Characterization by bioengineering techniques of the baseline skin condition, on a site partly protected from daily aggressions, of hands assigned to product A and to product B (left side of the table), and of dominant versus non-dominant hands (right side). Chroma C* is a measure of the intensity of PMS staining of stratum corneum stripping. NS=non-significant at 95% CL.

Table 2. Skin properties measurements after the 8 soaking sessions

	Comparison of hands soaked in prod. A versus hands soaked in product B			Comparison of dominant hands versus non-dominant hands whatever the product in which they soaked		
	product A	product B	<i>t</i> -test: A versus B	dominant hand	non-dominant hand	<i>t</i> -test dom. versus non-dom.
Corneometer® measurements	42±2	46±2	sign. <i>p</i> =0.001	44±2	43±2	NS
Skicon® measurements	118±11	154±29	sign. <i>p</i> <0.001	134±12	138±14	NS
Chroma C* of D-Squames® discs	25.6±0.9	21.2±0.7	sign. <i>p</i> <0.001	23.8±1.0	22.9±0.8	NS

Characterization by bioengineering techniques of the skin condition after the soakings, on a site partly protected from daily aggressions, of hands assigned to product A and to product B (left side of the table), and of dominant versus non-dominant hands (right side). Chroma C* is a measure of the intensity of PMS staining of stratum corneum stripping. NS=non-significant at 95% CL.

for both products (Fig. 2), but overall remained relatively low (maximum mean score of 1.3 on a 0 to 9 scale). No significant difference between the 2 products was observed. Erythema barely increased over the week, without any difference between the products (Fig. 2).

All individual data were re-analyzed regardless of the soaking solution, but rather comparing the evolution of the skin condition on the dominant and on the non-dominant hands of panelists. In this case, significant differences between the 2 hands were observed (Fig. 3). The mean dryness level was already different at baseline, with the higher level of dryness obtained for the dominant hands. With soakings, the difference between the 2 hands progressively increased, remaining statistically significant for most assessment sessions. Furthermore, the degree of statistical significance for the difference between the hands increased. The mean erythema level was similar for the dominant and non-dominant hands at the baseline; however, after the 1st soaking session, a significant difference between the 2 hands appeared, with a higher mean level of erythema observed for the dominant hands of the panelists.

Sensitive bioengineering methods were used on a skin site of the hands partly protected from external daily aggressions (by opposition to finger joints, palms, etc.); they included capacitance measurements at the surface of skin with the

Corneometer®, conductance measurements with the Skicon®, and squamometry analysis of stratum corneum tape strippings. At the initiation of the test, there was no measured difference between the hands dedicated to product A and those dedicated to product B (Table I). However, a significant difference existed for the Skicon® measurements when the dominant hands of the panelists were compared to their non-dominant hands; the dominant hands had a stratum corneum slightly less well hydrated.

After the 8 soaking sessions, the situation was different (Table 2). Products A and B were clearly differentiated from each other, with the stratum corneum being less well hydrated (significant with the Corneometer® and the Skicon®) and more altered (significant by squamometry) with product A than with product B. At the same evaluation time, the dominant and the non-dominant hands of the panel showed similar mean Corneometer®, Skicon® and squamometry values.

Discussion

This study describes a hand immersion test under laboratory-controlled usage conditions, in which the type of interaction of dishwashing liquids with the hands as experienced by consumers at home is respected. Under the non-exaggerated conditions of the test, 2 dishwashing liquids were clearly dif-

ferentiated from each other by using some of the most sensitive biometric techniques currently available, such as electrical skin surface properties measurements and squamometry (13). The need to perform the measurements on a skin site partly protected from daily aggressions is also demonstrated. Indeed, clinical assessment (dryness, erythema) of the skin condition of the whole hand could not differentiate the 2 products from each other, and showed progressive impairment of the skin over finger joints, and on the fingers palm and interdigital area over the test week. Such impairments mainly developed on the dominant hand of the panelists, exposed to more physical and chemical trauma than the other hand. Rapidly, a significant difference even appeared between the dominant and non-dominant hands for the clinical evaluations, regardless of the product solution in which the hands had been soaking. This is likely to be explained by increased skin sensitivity of the hands of the panelists participating in the test due to 2 major reasons: (i) they stopped using hand lotion during the test week and for the 3 days prior to the beginning of the test and (ii) they were submitted to 2 additional daily soaking sessions during the test period as compared to their usual dishwashing activity. Under such conditions, the hands become more sensitive and daily aggressions, more frequent on the dominant hand, cause slight skin irritation especially on the sites most exposed to external trauma. This could explain why whole hand evaluation did not discriminate between products, while showing obvious differences between dominant and non-dominant hands. Measurements performed on a site better protected from these aggressions would be more appropriate to discriminate between product treatments, as shown in this study by using 3 biometric evaluation methods, squamometry, skin surface conductance and capacitance, already known to be able to detect stratum corneum alterations at the subclinical level (10, 13, 14). Squamometry was first developed to evaluate skin xerosis (12, 15) and later shown to correlate with the irritant potential of surfactants or surfactant-based products applied to skin without causing xerosis (16, 17). The Corneometer® and the Skicon® are both based on the measurement of electrical properties of the surface of the skin; the Corneometer® measures skin capacitance, and the Skicon® high frequency skin conductance. The Corneometer® has been described as being more sensitive than the Skicon® to demonstrate stratum corneum dehydration (18–20), and the Skicon® to be more sensitive to follow improvement of stratum corneum hydration (18–20). In this study, the Skicon® appeared to be more sensitive than the Corneometer® in measuring dif-

ferences. At the end of the test, both instruments were able to identify a significant difference in the skin surface hydration level due to product treatment; product A leaving the stratum corneum slightly less well hydrated than product B. Such a ranking of products corresponds to the same ranking as that predicted from the Frosch-Kligman soap chamber test. However, only the Skicon® showed an obvious decrease in skin surface hydration with product A, as compared to the baseline value. It was also the only instrument to show a significant difference between the dominant and non-dominant hands at the baseline assessment. From these observations, it can be concluded that the Skicon® is not only the most sensitive instrument with which to measure hydration improvement, but also to measure slight decreases in skin hydration from a 'normal' value. For more dehydrating products or test conditions, we do not contest the higher discrimination potential of the Corneometer®, as described by others (18–20).

Finally, differences in squamometry and skin electrical values induced by the 2 dishwashing liquids, were previously observed between sensitive hands and non-sensitive hands populations (21). This suggests that using on a regular basis less mild dishwashing liquids could be a risk factor to increase hand skin sensitivity, and hence the propensity to make hands more responsive to daily aggressions.

In conclusion, the present hand immersion test under nonexaggerated conditions is a novel way to discriminate between consumer products, under several key conditions: (i) randomization of products based on panelists' hand dominance; (ii) use of very sensitive assessment methods, such as skin surface electrical properties measurement or squamometry; (iii) evaluation of the effect of the product on the back of the hands, on a zone partly protected from daily aggressions.

When such rigorous conditions are all added to the usual rules to be followed for a proper test (22), nonexaggerated test conditions can be used to compare the cutaneous tolerance of products without causing irrelevant skin irritation.

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