

Standardization of the TRUE Test imidazolidinyl urea and diazolidinyl urea patches

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The preservatives imidazolidinyl urea (IMID, Germall 115) and diazolidinyl urea (DU, Germall II) are commonly used in cosmetic products and are well-known sensitizers. The aim of the present study was to establish the optimal patch test concentration in hydrophilic dried-in vehicle (TRUE Test) for IMID and DU. 181 patients were included in the study. Of these, 150 were patients referred for patch testing, 12 were patients with known allergy to IMID and 19 were patients with known allergy to DU. 76 consecutive patients and the 12 IMID-allergic patients were patch tested with a dilution series IMID (0 to 600 $\mu\text{g}/\text{cm}^2$), formaldehyde (180 $\mu\text{g}/\text{cm}^2$) and DU (200 $\mu\text{g}/\text{cm}^2$). 74 consecutive patients and the 19 DU-allergic patients were patch tested with a dilution series of DU (0 to 600 $\mu\text{g}/\text{cm}^2$), formaldehyde (180 $\mu\text{g}/\text{cm}^2$) and IMID (200 $\mu\text{g}/\text{cm}^2$). A positive dose-response relationship was found. The number of doubtful reactions decreased with increasing test concentrations. No late reactions were observed. A patch test concentration in hydrophilic dried-in vehicle (TRUE Test) of 600 $\mu\text{g}/\text{cm}^2$ was found to be adequate and safe for both IMID and DU.

Key words: imidazolidinyl urea; Germall 115; diazolidinyl urea; Germall II; TRUE Test; formaldehyde; dose-response; concomitant reaction; allergic contact dermatitis; contact allergy; patch testing technique. © Munksgaard, 2001.

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Imidazolidinyl urea (IMID) and diazolidinyl urea (DU) are preservatives frequently used in cosmetic products. Both IMID and DU are well-known sensitizers, and frequent cases of allergic contact dermatitis reactions to both chemicals have been reported (1–8). IMID (trade name: Germall 115) was responsible for 3% of the reactions in a patch test study of patients with cosmetic reactions (1). Contact dermatitis reactions to IMID have been reported occasionally since its introduction in 1970 (2–4). Another member of this chemical group, DU (trade name: Germall II), was introduced on a large scale in 1982. Since then several cases of sensitization have been observed (5–7). Data from the North American Contact Dermatitis Group (NACDG) show a response rate of 2.5% to 3.7% (8). A Swedish multicentre patch test study shows a response rate of 1.8% to DU (2% aq.) and 1.3% to IMID (2% aq.) (9) in patients referred for eczema.

Both IMID and DU release small amounts of formaldehyde by hydrolysis in aqueous conditions. When a positive reaction to a formaldehyde-releasing compound occurs concurrently with a posi-

tive reaction to formaldehyde, the question arises as to whether the allergic reaction is caused by the compound itself or by the formaldehyde released. The literature does not provide a definite answer to this question, but most data indicate that contact allergy toward the compounds themselves actually exists. Concomitant reactions between DU and IMID have also been reported (10–12). *In vitro* skin permeation studies through pig skin indicate that IMID and DU do possess their own sensitizing ability, as only very limited amounts of formaldehyde permeate the skin (13).

Because of the ability of both IMID and DU to release formaldehyde upon hydrolysis, it was very important to choose a patch test vehicle in which hydrolysis of these compounds is unable to occur. One of the concepts of TRUE Test is that all the allergens are incorporated in dry vehicles, and therefore the optimal vehicles for these compounds were selected between substances such as polyvidone and different celluloses, which are all capable of making gels that can be dried into thin layers. Complete stability of both IMID and DU was seen when the vehicle polyvidone was used. This means

that no free formaldehyde is present when the patch is applied.

The aim of the present study was to establish the optimal test patch test concentration in a hydrophilic dried-in vehicle for IMID and DU.

Materials and Methods

181 patients, 65 men and 116 women, were included in the study. Patients with widespread dermatitis were excluded. Patients participated after written informed consent, and the local ethical committees have approved the study.

Patients were recruited from dermatological patch test clinics in Gentofte and Odense University Hospitals, Denmark, Malmö Sjukhus, Sweden, and Dortmund Hautklinik, Germany. Of the 181 patients included, 150 were consecutive patients referred for patch testing, 12 were patients with a pre-

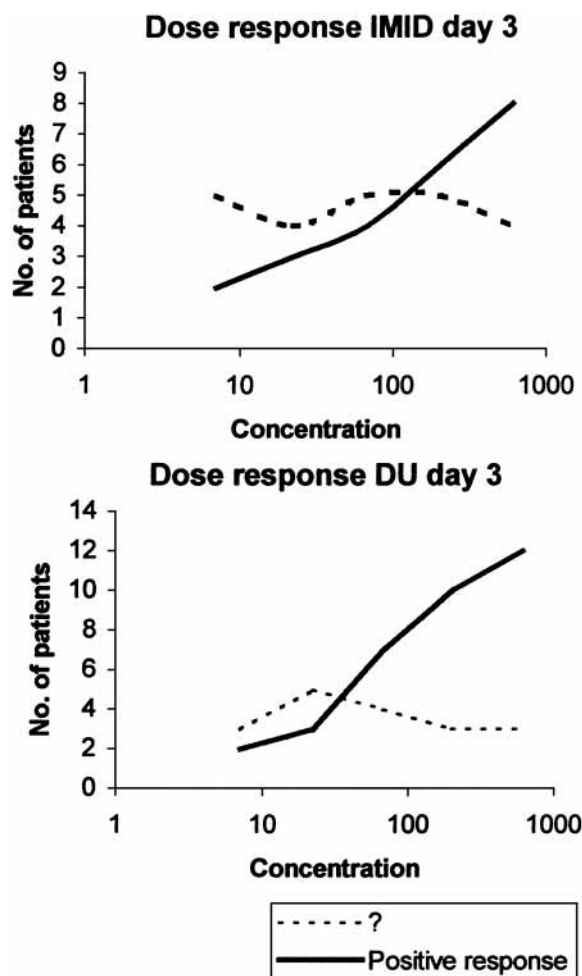


Fig. 1. The dose-response relationship for imidazolidinyl urea and diazolidinyl urea at D3. The number of patients with doubtful reactions decreased with increased test concentration. The x-axis is logarithmic.

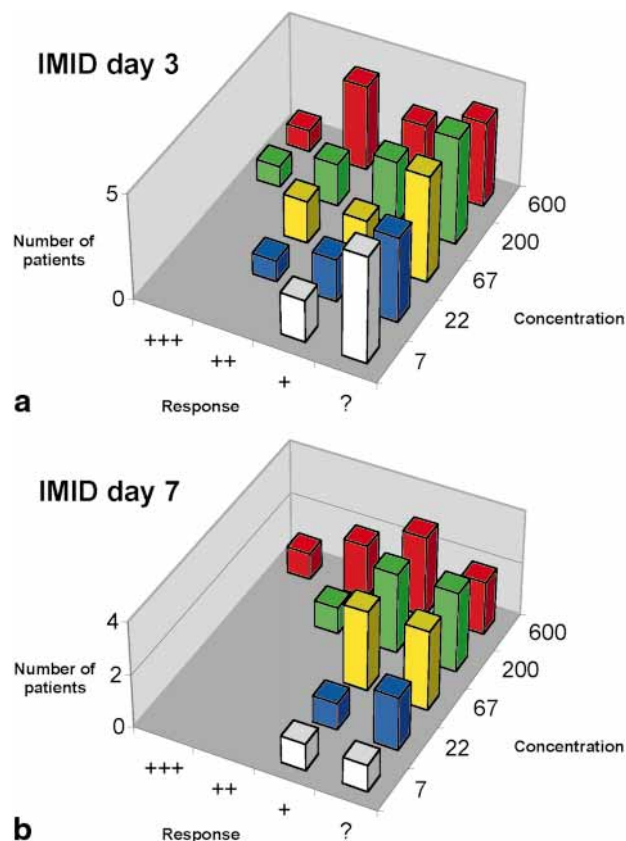


Fig. 2. (a, b) The degree of response for imidazolidinyl urea at D3 and D7. The concentrations are given in $\mu\text{g}/\text{cm}^2$: 600 in red, 200 in green, 67 in yellow, 22 in blue and 7 $\mu\text{g}/\text{cm}^2$ in white bars.

vious positive patch test to IMID and 19 were patients with a previous positive patch test to DU. Consecutive patients referred for testing were randomized to patch testing with a dilution series of either IMID or DU in a blinded study design. 76 consecutive patients and the 12 IMID-allergic patients were patch tested with 6 concentrations of IMID (TRUE Test: 0, 7, 22, 67, 200, and 600 $\mu\text{g}/\text{cm}^2$), a Finn Chamber with 2% IMID (in aq. or in pet. depending on the tradition of the patch test clinic), 1 formaldehyde patch (TRUE Test: 180 $\mu\text{g}/\text{cm}^2$) and 1 DU patch (TRUE Test: 200 $\mu\text{g}/\text{cm}^2$). 74 consecutive patients and the 19 DU-allergic patients were patch tested with: 6 concentrations of DU (TRUE Test: 0, 7, 22, 67, 200, and 600 $\mu\text{g}/\text{cm}^2$), a Finn Chamber with 2% DU (in aq. or in pet. depending on the tradition of the patch test clinic), 1 formaldehyde patch (TRUE Test: 180 $\mu\text{g}/\text{cm}^2$), and 1 IMID patch (TRUE Test: 200 $\mu\text{g}/\text{cm}^2$). Test tapes were produced with patches mounted in 3 different combinations. For both allergens polyvidone was used as a vehicle. Patch tests were removed after 2 days, and reactions were evaluated after 3 or 4 days and after 7 days. A follow-up, in most cases a telephone interview, was performed after 3 weeks.

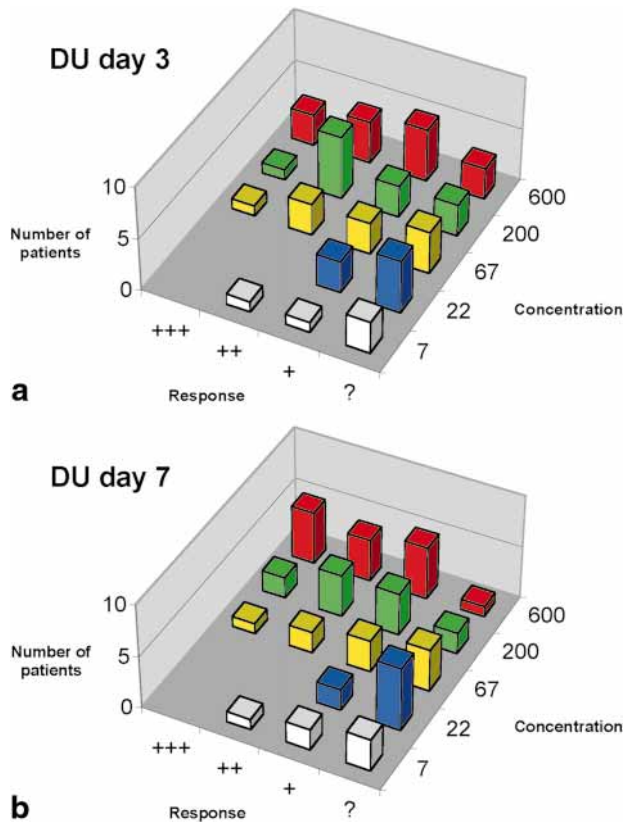


Fig. 3. (a, b) The degree of response for diazolidinyl urea at D3 and D7. The concentrations are given in $\mu\text{g}/\text{cm}^2$: 600 in red, 200 in green, 67 in yellow, 22 in blue and 7 $\mu\text{g}/\text{cm}^2$ in white bars.

Difference in response to the varying test concentrations was evaluated by the Friedman 2-way analysis by ranks. P -values <0.05 were considered statistically significant. When statistical significant differences between patches were found multiple comparison between patches was performed.

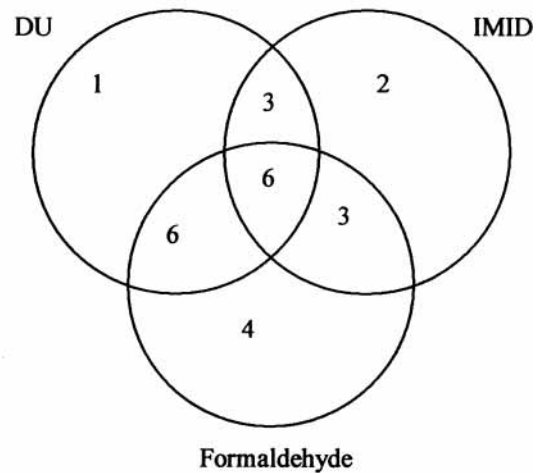
Results

The results of patch testing at day (D) 3 and 7 for IMID and DU are given in Figs. 2 and 3, respectively. A linear dose-response relationship was found for both IMID and DU (Fig. 1).

Testing with IMID

Of the 12 IMID patients, 2 did not respond to any of the patches. Of the 76 consecutive patients patch tested with IMID, 2 patients reacted positively to IMID (dilution series as well as Finn Chamber), and were included in the IMID group in the analysis of the data. 1 patient reacted to IMID (1+) in Finn Chamber at D7, but not to any test concentration in the dilution series.

Day 3



Day 7

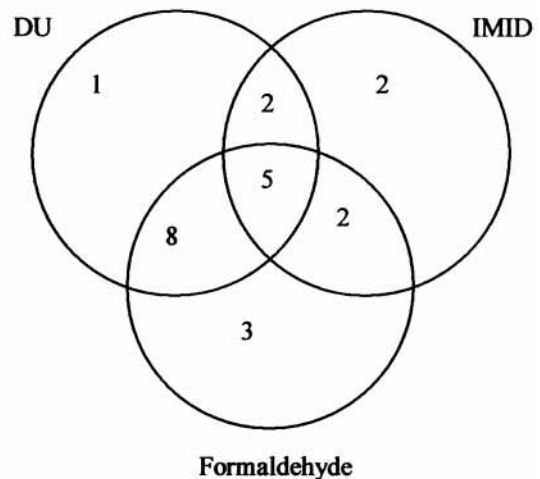


Fig. 4. Concomitant reaction. The distribution of positive responses.

Testing with DU

Of the 19 DU patients 3 did not respond to any of the patches. Of the 74 consecutive patients patch tested with DU, 1 reacted positively to DU (both TRUE Test dilution series and Finn Chamber), and was included in the DU group in the analysis of the data. None of the consecutive patients had doubtful reactions to DU in any concentration at D3. At D7 1 patient had a doubtful reaction to DU 600 $\mu\text{g}/\text{cm}^2$. 1 patient reacted to DU (1+) in Finn Chamber at D3 and D7, but not to any test concentration in the dilution series.

For IMID, a statistical significant difference in response was found between 600 $\mu\text{g}/\text{cm}^2$ and 7 $\mu\text{g}/\text{cm}^2$ as well as 67 $\mu\text{g}/\text{cm}^2$ ($p < 0.05$) at D3. At D7

statistically significant differences in the response was found for IMID between 600 $\mu\text{g}/\text{cm}^2$ and 7 $\mu\text{g}/\text{cm}^2$ as well as 22 $\mu\text{g}/\text{cm}^2$ ($p < 0.05$). No statistical significant differences were found between response rate to 200 $\mu\text{g}/\text{cm}^2$ patches and 600 $\mu\text{g}/\text{cm}^2$.

Statistically significant differences in the response to different concentration was found for DU at D3 between 600 $\mu\text{g}/\text{cm}^2$ and 7 $\mu\text{g}/\text{cm}^2$ as well as 22 $\mu\text{g}/\text{cm}^2$ ($p < 0.05$), and for DU at D7 the patch with 600 $\mu\text{g}/\text{cm}^2$ demonstrated significantly higher response than the 3 lowest concentrations. Number of doubtful reactions decreased with increasing test concentration.

During testing with IMID 2 patients experienced flare-up of their previous eczema. At follow-up 3 weeks after patch testing, 10 patients (2 IMID-allergic and 8 DU-allergic) had persisting local reactions. No late reactions were observed.

The distribution of positive responses to patch test with IMID, DU and formaldehyde is given in Fig. 4. Of a total of 23 patients with previous positive patch tests to either IMID or DU, only 5 did not react to patch testing with formaldehyde.

Discussion

Comparison of test concentrations of IMID and DU varying from 0–600 $\mu\text{g}/\text{cm}^2$ shows a statistically significant dose-response relationship. The number of doubtful reactions was reduced when test dose was increased (Fig. 1). Although no severe adverse reactions occurred, a flare-up of previous eczema was seen in a few patients. Late reactions were not seen. Together these data indicate that 600 $\mu\text{g}/\text{cm}^2$ was the most advantageous of the included test concentrations for both IMID and DU. Doubtful reactions may be interpreted as either weak positive or irritant reactions. The fact that the number of doubtful reactions decreased when test dose was increased, indicates that the threshold for irritancy was not reached, and that doubtful reactions in most cases should be read as a weak positive response (Figs. 2, 3). Theoretically, the test dose could then be further increased. However, the sensitivity of the test was found to be sufficient, since an allergic response was reproduced in 83% and 84% of the IMID and the DU allergic patients, respectively. The sensitivity was not found to be significantly different from that of the Finn Chamber test. The flare-up of eczema noted in 2 subjects also indicates that the test dose should not be further increased. It can be concluded that a patch test concentration of 600 $\mu\text{g}/\text{cm}^2$ of IMID or DU in hydrophilic dried-in vehicle (TRUE-Test) is adequate and safe.

It has been debated whether allergic reaction to IMID or DU is based on contact allergy to the

compounds themselves or based on contact allergy to formaldehyde. A positive response to IMID or DU may then theoretically be due to a positive reaction to the released formaldehyde. It can be calculated that the maximum dose of formaldehyde capable of being released in the IMID and DU patches at the highest concentrations is 23 and 43 $\mu\text{g}/\text{cm}^2$ respectively, compared to 180 $\mu\text{g}/\text{cm}^2$ in the formaldehyde patch in the TRUE Test standard series. Studies of dilution series of formaldehyde demonstrate that a few patients react positively to formaldehyde concentrations as low as 23 to 43 $\mu\text{g}/\text{cm}^2$ (14–16). In the present study, 5 out of 23 patients reacted at D7 to DU or IMID alone, while 18 out of 23 reacted to 1 of the biocides and to formaldehyde, or to formaldehyde alone (Fig. 4). In a study of 2400 consecutive patients, it was reported that of the 13 patients positive to DU, 7 had no reaction to formaldehyde (7). In a study of 639 patients including 58 DU positive patients, 12% reacted to DU alone, 7% to DU+IMID, 7% to DU+IMID+formaldehyde; and 74% to DU+formaldehyde (10). A recent study demonstrated that 39% of the IMID-positive patients had a concomitant formaldehyde reaction (17). Our results show that although some positive reactions may be caused by release of formaldehyde, specific positive allergic reactions to the biocides are also likely to occur.

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