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Clinical Standardization of the TRUE Test™ Formaldehyde Patch

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Formaldehyde is an ubiquitous allergen in industrial and domestical products. High concentrations of formaldehyde are rare in our environment. Small amounts, however, can be found almost everywhere; in cosmetics, household products, building material, paint, medicaments, fabric and paper [1]. Formaldehyde exposure is difficult to estimate because the chemical – besides being manufactured, imported and used as such – is incorporated into a large variety of products and reactants in chemical processes. Exposure often occurs in a hidden fashion since at least 80 trade names and synonyms are used in the marketing of formaldehyde-releasing compounds [2, 3].

Since more than 30 years formaldehyde has been present in standard test series worldwide, and 1–4% of patients tested on suspicion of contact dermatitis react positively to this allergen [4, 5]. The relevance, however, of a positive formaldehyde patch reaction in relation to low exposure of formaldehyde is disputed [1, 6].

Patch test material must be standardized in order to obtain reliable and reproducible test results. The TRUE Test™ is a ready to use system with a high degree of standardization [7, 9].

Formaldehyde is a highly reactive, water-soluble gas, with a tendency to polymerize. The incorporation of formaldehyde in a dry patch was a challenge. Many different ideas were tried to fix formaldehyde to a patch [10]. The solution was to use a proallergen of formaldehyde. Extensive studies concerning the break-down of different N-hydroxymethyl derivatives which cleave in water solutions and then release formaldehyde were carried out [11].

Based on these studies, N-hydroxymethylsuccinimide (HMS) was selected as a pro-allergen candidate for the formaldehyde patch and proved to fulfil the requirements. HMS applied to the surface of the skin gets in contact with humidity and is almost instantly transformed to formaldehyde and succinimide. This has been confirmed in an in vitro study which showed that the same amounts of formaldehyde penetrated through human skin from a formaldehyde solution and an equivalent amount of HMS in a TRUE Test patch.

The TRUE Test formaldehyde patch, when stored in pouches with desiccant paper, is completely stable for at least 24 months at 8 °C.

It is important that the carrier succinimide does not give rise to any unwanted side reactions. The dermal toxicity of succinimide has been tested with 20% topical applications and 5% intradermal injections in guinea pigs. This caused no irritant reactions. Guinea pig maximization tests with succinimide showed no allergenic potential [11]. Succinimide has been used in the treatment of nephrolithiasis and epilepsy in oral doses up to 10 g/day. This reduced the concentration of vitamin B_6 in serum but no other side effects were reported [12, 13].

The present study describes the clinical studies involved in the development of the formaldehyde patch of TRUE Test.

Materials and Methods

Test Material

Patches were formulated from the proallergen HMS in the vehicle polyvidon (PVP) by printing it on flexible polyester carrier foil sheets. After drying, the printed sheets were cut in patches, mounted on hypoallergenic tape and covered with protective siliconized polyethylene foil. The test strips were packed with desiccant paper in an air-tight laminated envelope keeping the patch absolutely free from water.

HMS in PVP was formulated as TRUE Test patches with the amounts formaldehyde equivalent to 0.01, 0.02, 0.03, 0.04, 0.08, 0.10, 0.12, 0.15, 0.19, 0.26, 0.33, 0.57 and 1.12 mg/cm^2; analytical values obtained with colorimetric method after hydrolysis. A TRUE Test patch with 0.81 mg/cm^2 HMS contains 0.19 mg/cm^2 of formaldehyde and will expose the skin to the same amount formaldehyde as a Finn Chamber test with 15 µl 1% formaldehyde solution. The area of the TRUE Test patch is 0.81 cm^2.

For the purpose of comparison, control patch tests were performed with formaldehyde 1% in water and dilutions thereof (Chemotechnique Diagnostics AB, Malmö, Sweden). 15 µl of the formaldehyde preparations were applied in Finn Chambers (Epi-Test, Helsinki, Finland).

The patch test series were applied on the upper back. Left/right application varied at random. With few exceptions, the test strips remained on the back for 48 h and were evaluated after 72 or 96 h. The tests were evaluated according to the ranking scale recommended by ICDRG [14].

Patients

Healthy volunteers without known sensitivity to formaldehyde, consecutive patients with contact dermatitis, and patients with previous patch tests to formaldehyde were included after informed consent. The studies were approved by ethical committees.

Experimental Design

Group 1: First Irritancy Study. Nine healthy volunteers, 3 women and 6 men with no known skin disease and not sensitive to formaldehyde were patch tested with TRUE Test HMS patches with 0.12, 0.57 and 1.12 mg/cm^2 formaldehyde.

Group 2: First Dose-Response Study in Formaldehyde-Sensitive Patients. A comparative study on 25 patients with previous positive patch test reactions to formaldehyde were rechallenged with (a) TRUE Test HMS patches with 0.02, 0.03, 0.04, 0.08, 0.12 and 0.15 mg/cm^2 formaldehyde, and (b) formaldehyde aqueous tests 0.015, 0.032, 0.063, 0.13, 0.25, 0.5 and 1.0%.

Group 3: First Dose-Response Study on Consecutive Eczema Patients. A comparative study on 120 consecutive patients with contact dermatitis were tested with (a) TRUE Test HMS patches with 0.01, 0.02, 0.04, 0.08, 0.12, 0.15 mg/cm^2 formaldehyde, and (b) formaldehyde aqueous test 1.0%.

Group 4: Second Dose-Response Study in Formaldehyde-Sensitive Patients. A comparative study on 24 patients with previous positive patch test reactions to formaldehyde were tested with (a) TRUE Test HMS patches with 0.15, 0.20, 0.26 and 0.33 mg/cm^2 formaldehyde, and (b) formaldehyde aqueous test 0.1, 0.3 and 1.0%.

Group 5: Second Dose-Response Study on Consecutive Eczema Patients. A comparative study on 255 consecutive patients, 96 males and 159 females, with contact dermatitis were tested with (a) TRUE Test HMS patches with 0.11, 0.19, 0.26 and 0.33 mg/cm^2 formaldehyde; (b) formaldehyde aqueous tests 1.0%; (c) formaldehyde standard test as used at the different clinics either in aqueous or petrolatum vehicle and placebo patches, and (d) vehicles PVP, and succinimide equivalent to the amount included in the active HMS-TRUE Test patches.

Statistical Methods

Descriptive methods were used.

Table 1. Minimal dose/concentration for positive reactions in patients tested with TRUE Test formaldehyde patches and with Finn Chambers dosed with 15 µl formaldehyde in water

Minimal Finn Chamber formaldehyde concentration, %	Minimal TRUE Test formaldehyde dose, mg/cm^2							
	neg	0.15	0.12	0.08	0.04	0.03	0.02	0.01
neg	1[a]	1		1				
1.0	1	1			1			
0.5			1	1				
0.25	3			1		4		
0.13	1			2		1		
0.063						1		2
0.032								1
0.015				1				

[a] Questionable to both methods.

Results

Group 1. Five of 9 patients demonstrated irritant reactions to the 1.12 mg/cm^2 TRUE Test formaldehyde patch, and 2 of them also to 0.57 mg/cm^2 patch. No irritation was observed with the 0.12 mg/cm^2 patch.

Group 2. The dilution series were positive with both methods in 17 of 25 patients, to TRUE Test formaldehyde patches only in 2 patients and to formaldehyde aqueous in Finn Chambers only in 5 patients (table 1). Questionable reactions were registered for both methods in 1 patient. Comparisons of the two methods indicate that a TRUE Test formaldehyde patch with 0.15 mg/cm^2 formaldehyde induces reactions equivalent to formaldehyde aqueous tests in a concentration between 0.5 and 1.0%.

Group 3. Three of 120 patients had positive and relevant allergic reactions to both the TRUE Test formaldehyde patch and the formaldehyde aqueous test. No irritant reactions occurred.

Group 4. Thirteen of the 24 patients previously positive in formaldehyde tests demonstrated positive reactions to both TRUE Test formaldehyde patches and the formaldehyde aqueous tests. One patient had reactions to the TRUE Test formaldehyde path only. Comparison of the two methods showed that TRUE Test formaldehyde patches with 0.20 and with 0.26 mg/cm^2 formaldehyde gives an equivalent test response as a formaldehyde aqueous 1% test.

Table 2. Reactions to TRUE Test HMS patches and formaldehyde 1% control Finn Chamber patches in 255 consecutive patients

Patient No.	Reactions to TRUE Test HMS patches formaldehyde, mg/cm^2				Control formaldehyde 1% aq.
	0.33	0.26	0.19	0.11	
Positive reactions					
106	++	IR/?	+	–	+
123	++	+	–	–	IR/?
144	+	+	+	IR/?	+
228	++	++	–	+	–
250	++	++	++	++	–
347	+	–	–	+	++
519	IR/?	IR/?	IR/?	IR/?	+
529	IR/?	IR/?	IR/?	–	+
550	+	+	+	+	+
Irritant reactions					
112	IR/?	IR/?	IR/?	–	IR/?
146	IR/?	IR/?	IR/?	–	IR/?
212	IR/?	–	–	–	–
224	IR/?	–	–	–	–
244	IR/?	–	–	–	–
344	–	–	–	–	IR/?
439	IR/?	IR/?	–	–	–
510	IR/?	IR/?	–	–	–
516	IR/?	IR/?	IR/?	–	–
526	IR/?	IR/?	IR/?	–	IR/?

Group 5. All four active TRUE Test formaldehyde patches in the dilution series showed positive test results. Nine patients had positivity to formaldehyde; 4 to both methods, 2 to formaldehyde aqueous tests with IR/? reactions on TRUE Test formaldehyde patches, and 3 to TRUE Test formaldehyde patches with IR/? reaction for 1 and negative for 2 on the formaldehyde aqueous tests. Details about positive and irritant/questionable reactions are given in table 2.

Four of the patients with positive formaldehyde reactions and 1 with IR/? reactions to both test methods had a relevant history of allergy to formaldehyde.

Statistical Results. The acceptable frequency of irritation in group 5 was set to at most 10%, and based on assumed point estimate of 5% for the recommended dose with n = 255. The upper 95% confidence limit for this frequency was about 7.5% and indicates that the HMS dose equivalent to 0.18 mg/cm^2 formaldehyde is acceptable.

Discussion

Formaldehyde differs in its sensitizing profile from other allergens [15]. A background of combined type I and type IV sensitivity has been proposed [16]. Its protein binding to the skin may influence the test pattern. Formaldehyde is also a difficult allergen to test with, as the optimal test concentration/dose is close to the threshold of irritation [17]. Therefore, careful optimization of the allergen dose in relation to the irritant dose is necessary. Our goal of patch test optimization is to include at least 90% of sensitive patients. The standard method for patch tests with formaldehyde is to use 1 or 2% formaldehyde solution stabilized with methanol either in Finn Chambers or on an AL-test. A 2% formaldehyde solution is somewhat irritant with Finn Chamber technique [17]. Although these methods are not optimized, they should still be acceptable standards for comparison. Reports on incorporation of formaldehyde patch test material petrolatum or other semisolid vehicles are not convincing [17].

The discovery that the use of the stable pro-hapten HMS is an excellent indicator of formaldehyde allergy is a technical breakthrough. Studies in vitro and in vivo on animals prove that the concept of using the pro-allergen HMS is an accurate method for testing formaldehyde sensitivity [11].

The result from the first groups indicate that TRUE Test formaldehyde patches with both 0.57 and 1.12 mg/cm^2 formaldehyde is irritant and thus a too high dose. Test results from the second group gave indications that the most highly dosed TRUE Test formaldehyde patch with 0.15 mg formaldehyde/cm^2 had a marginally too low dose, whereas the clinical study on patients with contact dermatitis (group 3) indicated adequate performance with a TRUE Test formaldehyde patch of 0.15 mg/cm^2 formaldehyde.

The tests of group four confirm excellent performance of TRUE Test formaldehyde patches with 0.20–0.26 mg/cm^2 formaldehyde in patients sensitized to this compound. Those 11 of 24 now negative but previously positive to formaldehyde have probably fallen in sensitivity below the detection level. Own experience (T.F.) has indicated that about 60% is a normal reproducibility rate of formaldehyde reactions on retesting patients with previous positive reactions during the last few years.

The test results from group 5 gave the reasonable number of 9 patients with positive formaldehyde reactions of 255 tested (3.5%) for either or both methods.

In conclusion, the clinical studies verify that a HMS-TRUE Test is an excellent method to determine formaldehyde allergy in humans, and that a dose of 0.18–0.20 mg formaldehyde/cm^2 is optimal. Therefore, a TRUE Test formaldehyde patch of 0.18 mg formaldehyde/cm^2 has been chosen for the standard TRUE Test series.

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