

Fragrance mix I patch test reactions in 5006 consecutive dermatitis patients tested simultaneously with TRUE Test[®] and Trolab[®] test material

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Background: The prevalence of contact allergy to fragrance mix (FM) I varies from study to study, depending on factors such as test population, patch test material, and technique.

Objectives: To compare the outcome of routine patch testing with FM I TRUE Test and FM I Trolab.

Materials and Methods: A total of 5006 consecutive eczema patients were patch tested with both patch test materials according to the International Contact Dermatitis Research Group.

Results: A total of 9.9% patients tested had a positive reaction to one of the FM I mixes; 4.4% to FM I TRUE Test, 9.3% to FM I Trolab, and 3.7% to both ($P < 0.0001$). Patients with a stronger reaction to FM I TRUE Test almost all reacted to FM I Trolab, whereas the reverse situation showed a lower association. Clinical relevance of a positive patch test reaction to FM I TRUE Test was found in 73.0%, and clinical relevance of a positive patch test reaction to FM I Trolab was found in 64.3%; 68.4% of the patients with a positive reaction to FM I TRUE Test and 54.3% with a positive reaction to FM I Trolab were positive to one or more of the eight constituents of the mix.

Limitations: The study is retrospective, and supplementary testing with FM components in patients with a positive reaction to the mixes was performed in a selected group of patients. Determination of clinical relevance may be biased.

Conclusions: From this study, we cannot conclude which of the two FM I test preparations is the best for diagnostic purposes. Inclusion of both FM I tests in the baseline series to obtain a graded degree of FM I allergy for the individual patient is one option. Prospective controlled patch test studies with FM I patch test material are recommended.

Key words: clinical relevance; contact dermatitis; fragrance mix components; fragrance mix I; patch test.
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Conflicts of interest: Klaus E. Andersen is an advisor, Charlotte G. Mortz is an investigator for MEKOS A/S.

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The estimated prevalence of contact allergy to fragrance mix (FM) I [containing *Evernia prunastri* (oak moss) absolute, isoeugenol, cinnamal, cinnamyl alcohol, hydroxycitronellal, eugenol, geraniol, and α -amyl cinnamal] in patient populations varies from 7.2% to 11.5%, depending on factors such as patch test material, test technique, and test populations (1–3), when petrolatum-based test material in Finn Chambers[®] or Van der Bend chambers[®] is used. In epidemiological studies,

TRUE Test is often used, and the prevalence of contact allergy to FM I was estimated to be 1.8% among eighth-grade Danish school children in 1996 (4) and 1.6% among unselected adults in Denmark in 2006 (5).

From 1998 to 2009, we routinely patch tested with both FM I in pet. (Trolab) in Finn Chambers on Scanpor[®] tape and FM I in TRUE Test. This retrospective investigation compared the difference between the two patch test systems in relation to

number of positive reactions, strength of reaction, relevance of a positive patch test reaction to FM I, and positive patch test reactions to the constituents of FM I.

Materials and Methods

A total of 5068 consecutive eczema patients were patch tested with the baseline series, and 98.8% (5006; 3147 female, 1859 male) were tested with both FM I 450 µg/cm² (TRUE Test; MEKOS Laboratories A/S, Hillerød, Denmark) and FM I 8% in pet. with 5% sorbitan sesquioleate (SSO) added as emulsifier (Trolab; Almirall Hermal GmbH, Reinbek, Germany) (6). Finn Chambers on Scanpor (SmartPractice®, Phoenix, Arizona, USA) were used for the pet.-based patch test preparation. If a patient had a positive reaction to one of the FM I tests at the first reading, usually at D3, the individual constituents of FM I Trolab, each at a concentration of 1% in pet., were tested whenever possible, and read once at the second reading. FM I ingredients (Trolab) include 1% SSO. Therefore, SSO 20% in pet. (Trolab) was included in some tests. The patches were applied on the back for 2 days, and read according to the International Contact Dermatitis Research Group scoring scale on D3 and D5–D7. We used an extra reaction score, ? follicular, which we regarded as a doubtful reaction. All patients with a +, ++ or +++ reaction to one or both FM I test materials were regarded as positive and included in further analysis. The clinical relevance was recorded as present or past by the dermatologist seeing the patient at the last reading, depending on the overall judgement based on clinical picture, patient history, and supplementary information from tests with cosmetic products when available.

If patients were patch tested more than once in the 11-year test period, the last recordings were used for the analysis. Statistical analysis was performed using STATA 11 (StataCorp LP, College Station, TX, USA). For comparison of associations between the patch test results (positive and negative) in the two patch test systems, the McNemar χ^2 -test of paired proportions was used.

Results

Patient characteristics

The MOAHLFA index (7) for the patient group was: M, 37.1%; O, 6.8%; A, 20.1%; H, 37.9%; L, 8.2%; F, 11.7%; and age, 60.5%.

Prevalence of positive reactions to the two different FM I tests

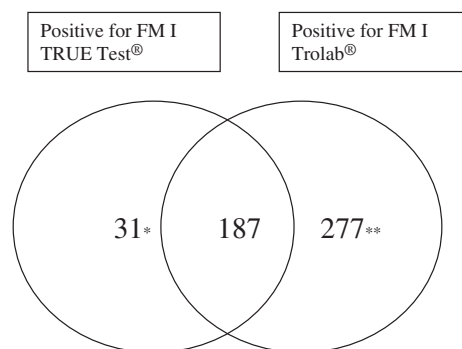
A total of 9.9% (495) had a positive reaction to one of the FM I tests; 4.4% (218) had a positive reaction

to FM I TRUE Test [147 (4.7%) female, 71 (3.8%) male]; 9.3% (464) had a positive reaction to FM I Trolab [335 (10.7%) female, 129 (6.9%) male]; and 3.7% (187) had a positive reaction to both (Fig. 1). There was a highly significant difference between the two tests in frequency of positive reactions (McNemar χ^2 -test 196, $P < 0.0001$) for both women and men.

Association between strengths of reaction to the two FM I test materials

The relationship between the strength of the reaction to FM I TRUE Test and the number of positive reactions to FM I Trolab, and vice versa, is given in Tables 1 and 2. Patients with a stronger reaction to FM I TRUE Test (+++/++) almost all reacted to FM I Trolab (98% and 100%, respectively). Conversely, only 58% with a ++ reaction and 86% with a +++ reaction to FM I Trolab had a concomitant reaction to FM I TRUE Test. The strength of reactions, doubtful reactions and follicular and irritant reactions in the two patch tests are given in Fig. 2. Doubtful reactions occurred in 24.7% (1238) of tests with FM I Trolab and in 9.2% (462) of tests with FM I TRUE Test.

Of the 462 patients with a doubtful reaction with FM I TRUE Test, 46.3% (214) also had a doubtful reaction and 22.3% (103) a positive reaction with FM I Trolab; 1.7% (8) had a follicular reaction, and 29.7% (137) were negative with FM I Trolab.



FM I TRUE Test®	FM I Trolab®		
	Negative	Positive	Total
Negative	4511	277	4788
Positive	31	187	218
Total	4542	464	5006

Fig. 1. Patch test results in 5006 patients tested with fragrance mix (FM) I both in TRUE Test® and in pet. (Trolab®).

*Of the 31 (29+ and 2++) patients positive only in FM I TRUE Test, 19 had a ? reaction to FM I from Trolab®, and 12 had no reaction.

**Of the 277 (193+, 78++, and 6+++) patients positive only in FM I Trolab, 103 had a ? reaction to FM I TRUE Test, 12 had a follicular reaction, and 162 had no reaction.

Table 1. Relationship between the strength of reaction to fragrance mix (FM) I TRUE Test® and the number of reactions to FM I Trolab®

Strength of TRUE Test® FM I test reaction	Number of patients	Number (%) of patients with concomitant reaction to FM I Trolab®
+	119	90 (75.6)
++	86	84 (97.7)
+++	13	13 (100.0)
All	218	187 (85.8)

Table 2. Relationship between the strength of reaction to fragrance mix (FM) I in pet. (Trolab®) and the number of reactions to FM I TRUE Test®

Strength of Trolab® FM I test reaction	Number of patients	Number of patients (%) with concomitant reaction to FM I TRUE Test®
+	238	45 (18.9)
++	184	106 (57.6)
+++	42	36 (85.7)
All	464	187 (40.3)

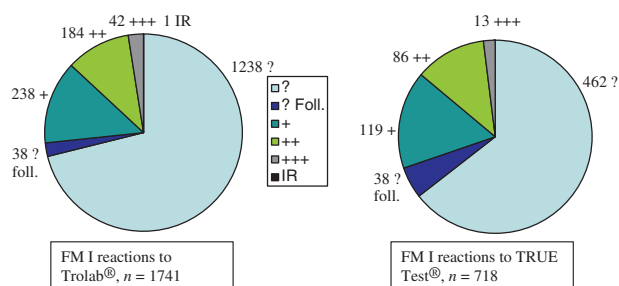


Fig. 2. Number of doubtful, irritant and graded positive reactions to fragrance mix (FM) I from TRUE Test and Trolab.

Table 3. The strength of reaction in the 187 patients with a positive patch test reaction to both fragrance mix (FM) I TRUE Test® and FM I Trolab®

	FM I Trolab®			
FM I TRUE Test®	+	++	+++	Total
+	43	43	4	90
++	2	63	19	84
+++	0	0	13	13
Total	45	106	36	187

Most of the tests with doubtful reactions to FM I Trolab [80.0% (990/1238)] were negative with FM I TRUE Test; 17.3% (214) had a doubtful reaction, 1.2% (15) a follicular reaction and 1.5% (19) a positive reaction to FM I TRUE Test.

The strengths of reactions for the 187 test courses with positive reactions to both FM I tests are given in Table 3.

Results of repeated tests in the same patients

A total of 236 patients were tested more than once in the 11-year period (219 were tested twice and 17 three times). One hundred and ninety-eight were negative for both FM I tests in all test sessions, and 38 patients (36 tested twice, 2 tested three times) had at least one positive reaction to one of the FM I tests.

Eight of 15 of those with one or more positive reactions to TRUE Test were positive in all test sessions, 2 reacted only at the first test, 4 reacted only at the second test, and one had 2 negative test results, and the third test result being positive. Thus, for FM I TRUE Test, reproducible test results occurred in 229/236 patients (97.0%).

Thirteen of 33 of those with one or more positive reactions to Trolab FM I were positive in all tests, 9 reacted only at the first test, and 10 only at the second test; in 1 patient, the first test result was positive and the two following were negative. Thus, for FM I Trolab, reproducible test results occurred in 216/236 patients (91.5%).

Contact allergy to FM I and associated clinical relevance

The clinical relevance of a positive patch test reaction to FM I TRUE Test was found in 73.0% (130/178), and the clinical relevance of a positive patch test reaction to FM I Trolab was found in 64.3% (222/345). The relationship between strength of reaction in the two tests and associated clinical relevance is given in Table 4. One hundred and three of 462 patients with a doubtful reaction to FM I TRUE Test had a positive reaction to FM I Trolab, and 48 of these were judged to be of clinical relevance.

Contact allergy to FM I and association with positive patch test reactions to constituents of FM I

One hundred and seventy-one of 218 patients with a positive reaction to FM I TRUE Test were tested with the eight constituents of FM I, and 68.4% (117/171) were positive to one or more of the components in the mix (Table 5); 48%

Table 4. The relationship between strengths of reaction in the two tests and associated clinical relevance

Strength of FM I patch test	% (number) of patients with clinical relevance of FM I TRUE Test®	% (number) of patients with clinical relevance of FM I Trolab®
+	64.1 (59/92)	52.7 (87/165)
++	82.2 (60/73)	70.6 (101/143)
+++	84.6 (11/13)	91.9 (34/37)

Table 5. The relationship between strengths of reaction in the two tests and positive reactions to the constituents of fragrance mix (FM) I

Strength of FM I patch test reaction	% (number) of patients with a positive reaction to FM I TRUE Test® and with reaction to constituents of the mix	% (number) of patients with a positive reaction to FM I Trolab® and with reaction to constituents of the mix
+	54.3 (50/92)	29.2 (50/171)
++	83.3 (60/72)	74.8 (110/147)
+++	100 (7/7)	96.7 (29/30)

(36/75) of patients with a doubtful reaction to FM I TRUE Test were positive to one or more of the ingredients.

Three hundred and forty-eight of 464 patients with a positive reaction to FM I Trolab were tested with the mix constituents, and 54.3% (189/348) reacted to one or more of the components.

A positive patch test reaction to FM I TRUE Test gave a positive reaction to *E. prunastri* (oak moss) absolute in 36.8% of patients, to isoeugenol in 23.4%, to cinnamal in 17.5%, to cinnamyl alcohol in 16.4%, to hydroxycitronellal in 16.4%, to eugenol in 8.2%, to geraniol in 4.1%, and to α -amyl cinnamal in 2.9%.

A positive patch test reaction to FM I Trolab gave a positive reaction to oak moss absolute in 23.6% of patients, to cinnamal in 16.4%, to hydroxycitronellal in 16.1%, to isoeugenol in 14.9%, to cinnamyl alcohol in 12.6%, to eugenol in 8.3%, to geraniol in 4.9%, and to α -amyl cinnamal in 2.9%.

The emulsifier SSO 20% in pet. (Trolab) was included in only a part of the test period, and was tested in 94 of the patients; 3 of those positive with FM I Trolab reacted to SSO 20% in pet. Of the 189 patients with a positive reaction to FM I Trolab and one or more of the constituents, 4 reacted to eight ingredients, and 1 to all eight fragrances and SSO.

Discussion

Patients with stronger reactions to FM I TRUE Test (++/+++) almost all reacted to FM I Trolab (98% and 100%, respectively). The reverse situation also showed a dose–response pattern, but only 58% with a ++ reaction and 86% with a +++ reaction had a concomitant reaction to FM I TRUE Test. FM I Trolab gave many doubtful reactions (25% of the tested patients); 80% of these had no reaction to FM I TRUE Test. Furthermore, of the 238 with a + reaction to FM I Trolab, only 45 (18.9%) had had a positive reaction to FM I TRUE Test, suggesting that some of the reactions

could be non-specific/non-allergic reactions. FM I TRUE Test gave doubtful reactions in 9% of the test population, and of these, 22% had a positive reaction to FM I Trolab, suggesting that some of the doubtful reactions to FM I TRUE Test were weak positive allergic reactions. The interpretation may be that weak positive reactions to FM I Trolab should be evaluated carefully for clinical relevance, because some of these reactions are probably non-specific. On the other hand, when testing with FM I TRUE Test, some cases of fragrance sensitivity may be missed.

SSO 5% is added to FM I Trolab to obtain a satisfactory dispersion of the eight constituents at 1% in the pet. vehicle. The individual ingredients of Trolab include 1% SSO. Reactivities to FM I and its constituents are changed in a specific pattern by the addition of SSO. Both irritant and allergic reactions seem to be increased (6). Only 94 of the patients were patch tested with SSO, as SSO was included in only part of the test period.

One of the challenges in diagnostic patch testing is differentiation in scoring between a doubtful (+?) reaction and an irritant reaction. In our hands, FM I very rarely gives irritant reactions, whereas patchy erythema and few follicles may occur and be registered as irritant reactions by others. To quote Fisher: “There is no morphological way of distinguishing a weak irritant patch test from a weak allergic test” (8–10). Repeated tests and the use of serial dilution tests help in interpretation.

A higher percentage of clinically relevant reactions was found by testing with FM I TRUE Test than by testing with FM I Trolab, and FM I TRUE Test was a better predictor than FM I Trolab for positive reactions to one of the ingredients in the mix. This difference was most pronounced for weak positive reactions (+ reactions); stronger reactions (++ reactions) gave the same result with both tests. Other studies have also shown that the clinical relevance of reactions to FM I increases with the strength of the reactions (6, 11). The determination of clinical relevance is challenging, and depends on several factors, such as patient history, patch tests performed, reading, and the skill and interest of the dermatologist (12). Information about possible fragrance intolerance was not systematically obtained prior to patch testing, and readings of the two FM patch tests were not ‘blinded’ for the dermatologist.

It has also been reported that there is a discrepancy between the number of positive reaction to FM I and reactions to the individual constituents of the mix. Johansen et al. (13) found that only 54.4% of their patients sensitized to FM I from Trolab reacted to the constituents of the mix, which is in accordance with our data. The discrepancy between reactions to FM I and to the ingredients in the mix has been

explained by: (i) false-positive reactions (irritant) to the mix; (ii) false-negative reactions to the constituents; and (iii) two or more ingredients in the mix forming a new allergen (compound allergy) (14). It has been proposed that the test concentrations of the constituents (13) and also of the mix (15) are too low, and may give rise to false-negative reactions. The FM I ingredients are tested in 1% in pet., which is the same concentration as in the FM mix, and it may be too low. The optimal test concentration for FM I constituents is not documented in published dose–response studies (16).

Most reactions were caused by *E. prunastri*, isoeugenol, and cinnamal, which, again, is in accordance with other studies (13, 14). Concomitant reactions to more constituents of the FM I mix was seen especially for chemically related ingredients such as cinnamal/cinnamyl alcohol.

The reproducibility of patch tests is important for their use as diagnostic tools in allergic contact dermatitis. Retesting of 236 patients during the 11-year period showed that TRUE Test FM I gave more reproducible results than Trolab FM I. However, the time period was long, and some patients could have acquired sensitization during the test period. There was no indication of a risk of patch test sensitization, as has been suggested by others (17).

Studies performed with investigator-loaded Finn Chambers generally give more heterogeneous results than those performed with manufacturer-loaded test systems such as TRUE Test or Epiguick® (18–21). However, the reproducibility is strongly allergen-dependent (21). Gollhausen *et al.* (18) reported a 37.9% non-reproducibility rate for Finn Chambers, as compared with 17.9% for TRUE Test. Concordance between the two methods varied from 57% to 78% (18, 22–24).

One study, including 207 eczema patients, compared TRUE Test and the IQ chamber® system for 21 allergens; 19 patients were positive for FM I in IQ Chamber, 12 in TRUE Test, and 9 in both tests; another study compared Finn Chamber tests with TRUE Test in 167 patients, and found 14 reactions to FM I; 7 were positive in Finn Chamber tests, 1 in TRUE Test, and 6 in both tests (25, 26). Furthermore, 167 persons in Heidelberg were patch tested with FM I from TRUE Test and in pet. A poster abstract did not report any noteworthy difference in reactivity to the two test materials (27).

The analysis of this large database does not show which of the two FM I test preparations is the best for diagnostic purposes. We recommend testing with both test systems to show fragrance contact allergy. Diagnostic patch testing is a biological assay with inherent variability and pitfalls. Inclusion of both FM I tests in the baseline series to obtain

a graded degree of FM I allergy for the individual patient is one option. With investigator-loaded Finn Chambers, the major problem comprises false-positive reactions, whereas with TRUE Test, the major problem comprises false-negative reactions. Doubtful FM I TRUE Test reactions may be clinically relevant in many cases. Weak positive reactions to FM I Trolab should be evaluated carefully for clinical relevance. Some of the 1+ reactions may be non-specific, and the clinical relevance increases with the strength of reaction. A recent study from Germany suggests that only ++ and +++ reactions should be considered as allergic (28). On the other hand, when testing only with FM I TRUE Test, some cases of clinically relevant contact sensitization may be missed. Prospective controlled studies with FM I patch test materials are needed. To quote Frances Storrs: “When we dermatologists advise our patients to avoid all fragranced products on the basis of a very weak positive (maybe irritant) fragrance mix patch test reaction, we deprive them of one of life’s pleasures” (12).

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