

Comparison of 2 tests for clinical assessment of formaldehyde exposure

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The objective of the study was to obtain further experience on the applicability of 2 tests for clinical assessment of formaldehyde exposure. About 1300 products brought in by consecutive formaldehyde-sensitive eczema patients during a 2-year period were tested with both the chromotropic acid test and the acetylacetone test. The chromotropic acid test was modified by including a 2nd reading after 2 days. The outcome was identical for about 90% of the products, and 74% of the products that were positive on day 2 were also positive on day 1. More than 1/2 of the tests that scored as traces or caused problems on day 1 became positive on day 2. For products analyzed with both the chromotropic acid test (day 2 readings) and the acetylacetone test, about 80% gave identical results. Positive reactions were frequently found for hair shampoo, creams and lotions, soap and skin cleansers, make-up and dishwashing liquids. The samples causing discolouration or problems in the tests were mostly concentrated in specific product categories. Very few products caused problems in both test methods. Thus, the most convenient test can be used as the 1st choice and, for product categories causing discolouration or problems with a specific test, the other test method can be used.

Key words: allergic contact dermatitis; formaldehyde; clinical exposure assessment; cosmetics; household products; chromotropic acid test; acetylacetone test. © Munksgaard, 1996.

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Formaldehyde in products for industrial, household or personal use is of importance for induction and elicitation of allergic contact dermatitis from formaldehyde. Careful instruction and information on occurrence of formaldehyde and exposure to formaldehyde in products can improve the prognosis for these patients (1, 2). Information on occurrence of contact allergens can be obtained from several sources: e.g., product labelling or material safety data sheets, chemical analysis, contact with suppliers or manufacturers, data bases or information centres on toxic materials (3). Each of these methods have their advantages and disadvantages, depending on the allergens in focus.

Formaldehyde tests for evaluation of products are an important tool in the clinical investigation of formaldehyde exposure, as formaldehyde can occur in products from different sources: e.g., added by the manufacturers, hidden content in raw materials (4), residues from synthesis (5), decomposition of components (6), and by contamination from packages coated with formaldehyde resin (7, 8). 2 tests for formaldehyde – the chromo-

tropic acid test (9) and the acetylacetone test (10) are available for use in clinical laboratories.

Earlier, we have reported problems concerning the reliability of the chromotropic acid test (2, 11). Problems concerning false-positive reactions (12, 13) and false-negative reactions or discolouration (6, 14), with certain types of products or ingredients, have been described by others. Gryllaki-Berger et al. (15) made a comparative study using the chromotropic acid test (direct and indirect method), the acetylacetone test and HPLC (high-performance liquid chromatography). They found that the acetylacetone test was a more efficient method for formaldehyde detection in a clinical laboratory.

The chromotropic acid test (indirect method) with a single reading on day 1 has routinely been used in our laboratory. In order to investigate a modification of the chromotropic acid test, and to obtain further experience on the applicability in a clinical laboratory, both the chromotropic acid test and the acetylacetone test have been carried out at the Department of Dermatology, Gentofte Hospi-

tal, on products brought in by formaldehyde-sensitive eczema patients during a 2-year period.

The purpose of the present paper is to describe our experience of duplicate testing a large number of products with both the chromotropic acid test and the acetylacetone test.

Materials and Methods

Test methods for detection of formaldehyde

The chromotropic acid test. Formaldehyde reacts with chromotropic acid to form a violet derivative. The sample (0.5–1.0 g) is placed in a glass test tube with a ground-glass stopper. Solid samples are wetted with water. A smaller test tube containing the reagent (2 ml) (chromotropic acid from BDH, sulfuric acid 96% suprapur from Merck) is placed in the sample test tube, which is sealed and kept in the dark (9, 16). The results were read after 1 day and 2 days. For samples containing formaldehyde the reagent developed a violet colour. Weak reactions were scored as traces. Tests giving yellowish, brownish or sometimes black colours in the reagent were scored as discolouration. Samples giving a colour not compatible with discolouration and clearly not positive were scored as "problems".

The day 2 reading, which is a modification according to previous descriptions of the method, was included in order to solve some of the problems with samples showing traces at the day 1 reading.

Standard solutions of 5 and 10 $\mu\text{g/ml}$ (ppm) formaldehyde were positive on both day 1 and day 2. A solution of 2.5 $\mu\text{g/ml}$ (ppm) showed traces on day 1 and became positive on day 2, and a solution of 1 $\mu\text{g/ml}$ (ppm) was negative on day 1 and showed traces on day 2.

The acetylacetone test. Formaldehyde reacts with acetylacetone in the presence of ammonia to form a yellow derivative. The sample (0.5 g) is placed in a glass test tube with a ground-glass stopper, mixed with the reagent (2.5 ml) and heated at 60°C for 10 min. Solid samples are used as they are. Fats and ointments are mixed with an emulsifier (Triton X 100) (10, 16). Tests containing

formaldehyde developed a yellow colour. Coloured products caused problems in reading the test, as they made it difficult or impossible to see the yellow colour, i.e., the method cannot be applied to this type of product.

Standard solutions of 2.5, 5 and 10 $\mu\text{g/ml}$ (ppm) formaldehyde were positive in the acetylacetone test. A solution of 1 $\mu\text{g/ml}$ (ppm) showed a weak positive reaction.

Results

Consecutive formaldehyde-sensitive eczema patients were offered an analysis of products with which they were in contact. A total of 1324 products from 97 patients were analyzed from January 1993 to December 1994.

The majority (63%) of the products tested were cosmetics and toiletries, such as creams, lotions, hair shampoo, soap, skin cleansers, deodorants, etc., followed by household products (18%), such as cleaning agents, dishwashing liquids, textile washing, textile finishes, occupationally used products and paper samples, etc.

Modification of the chromotropic acid test

For 1177 products, the readings of the chromotropic acid test were performed on both day 1 and day 2 (147 products not tested day 2) (Table 1). Evaluation of this modification of the chromotropic acid test showed that the 2 readings were identical for about 90% of the products tested (1060/1177): 157 products were positive in both readings, 801 were negative, 12 showed traces and 90 discolouration or problems in the reading. For products giving positive results on day 2, approx. 74% were positive on day 1 (157/211). All samples positive on day 1 were also positive on day 2. For 40 products showing traces on day 1, 28 became positive on day 2 (12 still traces on day 2), and among 27 products giving problems in the reading on day 1, 18 became positive on day 2.

A total of 211 products (18% of 1177 tested) were positive in the chromotropic acid test on day

Table 1. Readings on day 1 and day 2 for 1177 products analyzed with the chromotropic acid test, January 1993–December 1994

Readings day 1	Reading day 2					Total
	negative	positive	traces	discolouration	problems	
negative	801	8	32	14	13	868
positive	0	157	0	0	0	157
traces	0	28	12	0	0	40
discolouration	1	0	0	84	0	85
problems	3	18	0	0	6	27
total	805	211	44	98	19	1177

Table 2. Test results for 1134 products analyzed with both the chromotropic acid test (readings day 2) and the acetylacetone test, January 1993–December 1994

Chromotropic acid test – day 2	Acetylacetone test					Total
	negative	positive	traces	discolouration	problems	
negative	728	10	1	0	28	767
positive	11	180	1	0	18	210
traces	22	7	4	0	11	44
discolouration	87	3	0	1	4	95
problems	8	4	0	0	6	18
total	856	204	6	1	67	1134

2. Positive reactions were frequently found for hair shampoo (36/103), creams and lotions (77/294), dishwashing liquids (11/44), soap and skin cleansers (33/137), and make-up (10/44).

Comparison of the chromotropic acid test and the acetylacetone test

A comparison for 1134 products analyzed with both the chromotropic acid test (day 2 readings) and the acetylacetone test showed that for 81% of the products (919/1134) the 2 tests gave identical results: 180 products were positive in both tests, 728 products were negative, 4 showed traces, and 7 discolouration or problems in the readings (Table 2).

For 113 products causing discolouration or other problems in the chromotropic acid test, 95 were negative in the acetylacetone test, 10 caused problems and 1 discolouration in the acetylacetone test too, and only 7 were positive. For 67 products causing problems in the reading of the acetylacetone test (the colour of the sample made it impossible to see the yellow colour), 28 were negative in the chromotropic acid test, 18 were positive, 11 showed traces and 10 caused discolouration or problems in the chromotropic acid test too. Among 215 products giving different results in the 2 tests, 94 caused discolouration in the chromotropic acid test and 61 caused problems in the acetylacetone test.

Product types with frequent positive reactions in both test methods were the same as mentioned above: hair shampoo (35/103), creams and lotions (71/286), soap and skin cleansers (32/137), make-up (7/33) and dishwashing liquids (7/42).

Perfumes and eau de cologne (11/16), after shave preparations (5/9), textile finishes (19/35) and general cleaners (16/56), frequently caused discolouration in the chromotropic acid test. Coloured samples such as dishwashing liquids (6/42), hair shampoo (11/103), general cleaners (6/56) and some solid samples caused problems in reading the acetylacetone test. Very few products caused problems in both test methods.

Discussion

The equipment necessary for performing the chromotropic acid test and the acetylacetone test is a little different. For the chromotropic acid test (9, 16), the reagent can be stored for about 2 weeks and the laboratory work is quick; a disadvantage is that the samples have to stay in the dark for 24 h/48 h in a place free from formaldehyde (new laboratory equipment and furniture can cause problems), and the readings are not available until the next day or even the day after that. For the acetylacetone test as described by Fregert et al. (10), fresh reagent has to be made weekly; the procedure in the laboratory is slightly more time-consuming, as it includes heating; but in favour of this test is the fact that the readings are available the same day. In summary, the chromotropic acid test is more easily performed, but the acetylacetone test provide the answers on the 1st day of testing.

Most samples (85%) were analyzed without problems in both the chromotropic acid test and the acetylacetone test. The samples causing discolouration or problems in the tests were mostly concentrated in specific product categories. In the chromotropic acid test, products containing alcohol often caused discoloration, i.e., perfumes and eau de cologne. Textile finishes and occasionally cleaning agents caused discoloration too. In the acetylacetone test, products such as coloured creams and yellow products made the readings impossible. The acetylacetone test can be used as an alternative for samples causing discoloration in the chromotropic acid test, although a few samples caused problems in both test methods and could not be analyzed in an ordinary clinical laboratory. Thus, as these 2 tests are both valuable tools in a clinical laboratory, it is important to be aware that the prognosis for contact eczema in formaldehyde-sensitive eczema patients depends on the ability to elucidate their exposure to formaldehyde. A comprehensive knowledge of the individual formaldehyde exposure can improve the prognosis for these patients considerably (1, 2).

The present study did not include analyses with

HPLC or another method giving the "true answer" on the formaldehyde content in the products. Therefore false-positive or false-negative results in the chromotropic acid test and the acetylacetone test cannot be excluded.

Solutions of 5 µg/ml (ppm) formaldehyde or more were positive in the chromotropic acid test on day 1, a solution containing 2.5 µg/ml (ppm) showed traces on day 1 and became positive on day 2, and a solution of 1 µg/ml (ppm) was negative on day 1 and showed traces on day 2. According to Blohm (9), the violet colour develops within 15 min for a solution of 500 µg formaldehyde in 5 ml water (100 ppm), within 1 h for 10 ppm and after 24 h for less than 1 ppm. For the acetylacetone test, solutions of 2.5 µg/ml (ppm) or more were positive and a solution of 1 µg/ml (ppm) showed a weak positive reaction. These results imply that the detection limit of both the chromotropic acid test with a day 2 reading and the acetylacetone test is 1 ppm.

Gryllaki-Berger et al. (15) reported that the reaction of the chromotropic acid test took up to 14 days to stabilize. The formaldehyde concentrations for these products will, according to the present study and Blohm (9), be less than 1 ppm, and it can be questioned whether the formaldehyde concentration in samples that take 14 days to stabilize a positive reaction in the chromotropic acid test is clinically relevant. According to a study with 120 h of continuous patch testing in formaldehyde-sensitive subjects, formaldehyde levels below 30 ppm should be tolerated by sensitized subjects (17).

The main findings of including a 2nd reading on day 2 in the chromotropic acid test were that all readings that were positive on day 1 were also positive on day 2, and that most samples read as traces (28/40) and samples causing problems (18/27) in the readings on day 1 became positive on day 2. Based on these results, we recommend a modification of the chromotropic acid test to include a 2nd reading on day 2 for products not giving positive test results on day 1. Thus, a day 2 reading in the chromotropic acid test can be regarded as a valuable and simple improvement of the test, which eliminated more than 1/2 of the tests scored as traces or causing problems on day 1.

Our clinical experience of using both the chromotropic acid test and the acetylacetone test for a 2-year period, and testing about 1300 products brought in by about 100 formaldehyde-sensitive patients, is that the results of the 2 tests were the same for more than 80% of the products. Depending on whether quick laboratory work is given priority (the chromotropic acid test) or the test results are needed quickly (the acetylacetone test), the most convenient test can be selected as the 1st choice for products suitable for both test methods.

For product types causing problems with a specific test, the other test method can be used.

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