Concentrations and stability of methyl methacrylate, glutaraldehyde, formaldehyde and nickel sulfate in commercial patch test allergen preparations

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Summary

Background. Epicutaneous patch tests are used to reproduce allergy and diagnose allergic contact dermatitis. Reliable allergen test preparations are required.

Objectives. The purpose of the present study was to measure the actual concentrations of nickel(II) sulfate hexahydrate (NiSO4), methyl methacrylate, formaldehyde, and glutaraldehyde, and to compare them with the labelled concentrations, in commercial patch test allergen preparations found in dermatology clinics where patch testing is routinely performed.

Materials and methods. The commercial in-date and out-of-date patch test allergen preparations concentrations of NiSO4, methyl methacrylate, formaldehyde and glutaraldehyde from one to three participating clinics were analysed with chromatographic or wet chemical techniques.

Results. NiSO4 and formaldehyde concentrations were at or above the labelled concentrations; however, formaldehyde loss occurred with storage. NiSO4 particulate was uniformly distributed throughout the petrolatum. ‘In-use’ methyl methacrylate reagent syringes all contained ≤ 56% of the 2% label concentration, with no observable relationship with expiration date. Lower methyl methacrylate concentrations were consistently measured at the syringe tip end, suggesting loss resulting from methyl methacrylate’s volatility. The concentrations of glutaraldehyde patch test allergen preparations ranged from 27% to 45% of the labelled (1% in pet.) concentration, independently of expiration date.

Conclusions. Some false-negative methyl methacrylate, formaldehyde or glutaraldehyde patch test results may be attributable to instability of the test preparations.

Key words: concentration and stability; formaldehyde; glutaraldehyde; methyl methacrylate; nickel sulfate; patch test allergens.
Stability of the allergen in the epicutaneous patch test preparation is extremely important for the demonstration of contact allergy and the proper diagnosis of allergic contact dermatitis. Currently, the TRUE Test® ready-to-use system includes a very limited number of contact allergens, and is currently approved by the US Food and Drug Administration, although use of the traditional Finn Chambers® prepared with commercially available patch test allergens in syringes has been reported to be more suitable for detecting clinically relevant moderate or weak patch test reactions (1). Finn Chambers® are individually prepared with commercially available patch test allergens, which are most often incorporated in petrolatum, and occasionally water, and supplied in syringes or, occasionally, dropper bottles. A few studies have investigated the stability of specific patch test allergen preparations. These include thiurams (2), mercapto mix (3), disiocyanates (4–6), limonene hydroperoxide (7), triglycidyl isocyanurate (8), methyl dibromo glutaronitrile (9), and acrylates /methacrylates (10). In these studies, potential patch test preparation stability problems were identified for diphenylmethane-4,4'-disiocyanate, methyl methacrylate, and triglycidyl isocyanurate. Reactions between test components found in thiuram disulides and mercapto test mixes, forming new chemical species, were also reported. In the present study, commercial nickel(II) sulfate hexahydrate (NiSO₄), methyl methacrylate, glutaraldehyde and formaldehyde patch test preparations were obtained from several dermatology clinics, and assayed for specific allergen concentrations. These specific allergen reagents were chosen because of their chemical properties, or because of clinician concerns that false-negative test results may be, at least in part, attributable to the test reagent. In addition, the consistency of allergen within the pet. throughout the reagent syringe was assessed.

Materials and Methods

Commercial patch test allergen preparations manufactured by two vendors (MAN 1 and MAN 2) were obtained from up to three different North American Contact Dermatitis Group (NACDG) member clinics. Three clinics provided one or more of the patch test preparations for glutaraldehyde and methyl methacrylate, two clinics provided NiSO₄, and one provided formaldehyde. Both expired and within-date reagents were provided to the National Institute for Occupational Safety and Health (NIOSH) laboratory. In addition, the laboratory purchased patch test allergen preparations directly from MAN 2, stored them immediately at −16°C upon arrival, and assayed them shortly thereafter. All patch test preparations were supplied by the vendors in 3-ml syringes, except for the 1% formaldehyde from MAN 2, which was supplied in a plastic dropper bottle. NiSO₄ was supplied as a particulate dispersed within pet. Methyl methacrylate was also in pet. Preparations of glutaraldehyde in pet. contained 5% sorbitan sesquioleate (Span™ 83) as an emulsifier. Formaldehyde was provided in an aqueous solution.

A battery of solvents was screened for optimal ability to dissolve the pet., and pet. was determined to have the greatest solubility in xylens. Xylens were found to be suitable solvents for the methyl methacrylate and NiSO₄ sample preparation: however, Span™ 83 interfered with organic solvent extraction of glutaraldehyde from pet. Glutaraldehyde could not be assayed by dissolving the patch test preparations in xylens and injecting them directly into the gas chromatography–mass spectrometry apparatus, as was done for methyl methacrylate, owing to instability of glutaraldehyde within the heated gas chromatography (GC) injector. High quantitative recovery of glutaraldehyde was obtained by melting the pet. and extracting the glutaraldehyde in hot water. Reagent-specific analytical details are provided below. Regression analyses for all standard plots resulted in $R^2$ values of > 0.99.

Methyl methacrylate extraction and analysis

Aliquots of ~200 mg of methyl methacrylate in pet. were extruded from the syringes. Each aliquot was weighed and dissolved in 1 ml of xylens (Fisher Scientific, Waltham, MA, USA). Methyl methacrylate (Fisher Scientific)-spiked European Pharmacopoeia grade white pet. (Sigma Chemical Co., St Louis, MO, USA) standards were prepared and run in parallel with the samples. Standards ranged from 0.50% to 4.0% wt/wt methyl methacrylate in pet. A blank standard was also run. One microlitre of each extract was injected (injector temperature, 300°C) onto a Restek Rxi-5MS, 30 M, 0.25-mm internal diameter, 0.25-μm film thickness GC column (Restek, Bellefonte, PA, USA), and eluted from the column with 1.2 ml/min helium and the following GC (Agilent 6890; Agilent Technologies, Santa Clara, CA, USA) temperature gradient. The initial oven temperature of 45°C was held for 3 min, and the temperature was then increased at 5°C/min to 100°C. Methyl methacrylate was detected with an Agilent 6890C mass spectral detector in total ion current (TIC) mode. Samples were quantified by calculating the concentration by use of the sample TIC area under the curve, from the standard plot of the methyl methacrylate standards run concurrently.
NiSO₄ extraction and analysis
NiSO₄ exists as a dispersed solid in pet. NiSO₄ (Sigma Chemical Co.)-spiked pet. standards were prepared by first dissolving the pet. in xylenes and then spiking with 1.5 m ammonium hydroxide and NiSO₄. Standards were run in parallel with the samples. Xylenes were added to each sample, blank and standard (1.3–21.4 mg NiSO₄/200 mg pet.). NiSO₄ is insoluble in xylenes, and the Ni precipitate was quantitatively recovered by centrifugation at 14 000 g for 1 min. The NiSO₄ particles were washed twice with 5 ml of xylenes. Xylenes were removed with a glass transfer pipette. Five hundred microlitres of 1.5 m NH₄OH (Sigma Chemical Co.) was added to each sample and standard, and the vials were capped and vortexed until all NiSO₄ crystals were dissolved. The samples were then transferred to a clean glass tube for subsequent absorbance measurement of the resultant complex at 580 nm on a Beckman Coulter DU 800 spectrophotometer (Beckman Coulter, Somerset, NJ, USA). The efficiency of extraction of NiSO₄ was determined to be 105% ± 6% in preliminary studies.

Formaldehyde analyses
The formaldehyde stock standard of 37% in water (Sigma Chemical Co.) was standardized by acid titration, as detailed in the NIOSH Manual of Analytical Methods Method #3500 (11). The formaldehyde patch test preparation is supplied in water, so extraction was not required. The formaldehyde concentration was measured with the method of Hauser (12). In short, 10μl of formaldehyde patch test preparation, blank or standard was added to 990μl of 0.05% 3-methyl-2-benzothiazolone hydrazine solution (Ricca Chemical Co., Arlington, TX, USA). After 1 hr of incubation at room temperature, 200μl of 1.3% wt/vol sulfamic acid (Sigma Chemical Co.) and 1% wt/vol ferric chloride (Fisher Scientific) solution in water was added, and the reaction was allowed to proceed for 12 min at room temperature. The resultant complex was measured spectrophotometrically at 608 nm on a Beckman Coulter DU 800 spectrophotometer. A linear plot of formaldehyde standards was obtained from 0.25 to 4.0 μg of total formaldehyde, and sample concentrations were determined from that plot.

Glutaraldehyde extraction and analyses
Glutaraldehyde stock standard 70% in water (Sigma Chemical Co.) was standardized by colorimetric titration, as detailed in the US Pharmacopeia (13). Standards were made by adding 0–50 μg glutaraldehyde/g pet. containing 5% sorbitan sesquioleate (Ricca Chemical Co.) that had been melted at 70 °C with stirring and then allowed to solidify at −16 °C. Standards were compounded immediately prior to extraction. Glutaraldehyde was extracted from each pet./sorbitan sesquioleate standard and sample by the addition of 1 ml of distilled, deionized water to 200 mg of glutaraldehyde patch test preparation. Standards and patch test preparations were then heated to 70 °C for 5 min to melt the pet., vortexed, and immediately centrifuged at 14 000 g for 1 min. The top pet. plug was removed, and the water extract was collected for analysis by the method of Hauser (12), as described above. Compounded standards and samples were extracted and analysed concurrently. Extraction efficiency was determined to be 103% ± 2%, which would be the same for both compounded standards and samples.

Results
Table 1 lists the results of chemical analyses of patch test preparations that were provided by NACDG participating clinics. Each listed preparation had different lot numbers, with varying amounts of reagent left within the syringe when it was supplied to the laboratory. NiSO₄ was uniformly dispersed through the patch test preparation syringes, as assessed by microscopic examination and by chemical analyses of one 2.5% NiSO₄ allergen syringe (2.6% ± 0.2% for 23 aliquots across the syringe). Both the NiSO₄ and formaldehyde patch test preparation syringes contained allergen concentrations greater than or equal to the concentrations specified on the labels. NiSO₄ particulate diameters ranged from 1.47 to 24.21 μm, with an average sof 11.3 ± 8.27 μm, as determined by microscopic image analysis. The excellent stability of these particles in pet. was also evident, as the allergen syringe that was 2.8 years older than the stated expiration date had 28% more NiSO₄ than the labelled concentration. Significantly, loss of formaldehyde from patch test preparation syringes was noted, with storage at <8 °C according to the manufacturer’s instructions, when they were assayed ~1 year later, but still within the expiration date (Table 1).

A gradient was observed across the methyl methacrylate patch test preparation syringe (Fig. 1). This is most likely attributable to the volatility of methyl methacrylate; however, it can also self-polymerize. ‘In-use’ methyl methacrylate syringes all had ≤56% of the reported label concentration, with no observable relationship with expiration date. The sample taken near the tip end of the syringe, with potentially greater air exposure, was assessed separately, and the concentration was consistently lower than the reagent concentration behind it.
Concentrations of glutaraldehyde in patch test preparations obtained from the dermatology clinics were also extremely low. Only one of the glutaraldehyde patch test preparations was within the expiration date, but this syringe, labelled as containing 1% glutaraldehyde, contained only 0.2% glutaraldehyde, which was the lowest concentration in the three glutaraldehyde syringes tested. Preliminary assessment of a commercial glutaraldehyde syringe purchased by the laboratory and used for methods development was found to contain \( \sim 70\% \) of the labelled 0.2% concentration of glutaraldehyde, which ranged from 0.12% to 0.16% across 19 aliquots from tip to plunger of the syringe. The 0.2% glutaraldehyde patch test preparation was discontinued, and is no longer commercially available. The assay date was 1.5 years prior to the expiration date of this glutaraldehyde patch test syringe. No concentration gradient was observed from tip to plunger across the syringe. In a separate study, a 1% preparation of glutaraldehyde in pet., was compounded in the laboratory and divided among three syringes per storage condition. Glutaraldehyde stability was assessed for up to 2 months, and significant storage time-dependent loss of glutaraldehyde (\( > 20\% \)) was observed at \( \geq 5 \) days at room temperature (Fig. 2). Syringes stored at \( -16^\circ C \) for 2 months had less loss than that observed for syringes stored at room temperature; however, \( > 40\% \) loss was still observed at day 60.

### Discussion

A number of chemical and physical properties of patch test allergen formulations may influence a patient’s patch test reaction, and thus the ability of the clinician to diagnose contact allergy. These properties include the nature of the vehicle, irritation potential, allergen stability, and percutaneous penetration (14). Pet., the most common vehicle, continues to be the vehicle of choice, as it is both non-allergenic and non-irritating, although scattered reports of pet. allergy with eczema at the site of allergen application can be found in the literature.

Regardless of the nature of the vehicle, the allergen must be sufficiently stable within that vehicle to deliver a consistent dose to the skin. Several studies have examined the stability of specific patch test allergens in pet., and have shown potential storage problems for some

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**Table 1.** Concentrations of allergens in patch test preparations from dermatology clinics

<table>
<thead>
<tr>
<th>Patch test preparations obtained from dermatology clinics</th>
<th>Years to expiration</th>
<th>Measured concentration ± SD (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Labelled concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5% Nickel(II) sulfate hexahydrate, MAN 1</td>
<td>0.7</td>
<td>3.1 ± 0.7</td>
<td>124</td>
</tr>
<tr>
<td>2.5% Nickel(II) sulfate hexahydrate, MAN 1</td>
<td>0.5</td>
<td>2.6 ± 0.11</td>
<td>104</td>
</tr>
<tr>
<td>2.5% Nickel(II) sulfate hexahydrate, MAN 2</td>
<td>−2.8</td>
<td>3.2 ± 0.3</td>
<td>128</td>
</tr>
<tr>
<td>2% Methyl methacrylate, MAN 1</td>
<td>0.7</td>
<td>0.91 ± 0.18</td>
<td>45.5</td>
</tr>
<tr>
<td>2% Methyl methacrylate, MAN 2</td>
<td>−2.4</td>
<td>1.40 ± 0.03</td>
<td>70</td>
</tr>
<tr>
<td>2% Methyl methacrylate, MAN 1</td>
<td>0.7</td>
<td>0.89 ± 0.01</td>
<td>44.5</td>
</tr>
<tr>
<td>2% Methyl methacrylate, MAN 1</td>
<td>−0.4</td>
<td>1.13 ± 0.05</td>
<td>56.5</td>
</tr>
<tr>
<td>2% Methyl methacrylate, MAN 2</td>
<td>−2.4</td>
<td>0.30 ± 0.02</td>
<td>15.0</td>
</tr>
<tr>
<td>1% Glutaraldehyde, MAN 1</td>
<td>−3.7</td>
<td>0.27 ± 0.05</td>
<td>26.8</td>
</tr>
<tr>
<td>1% Glutaraldehyde, MAN 2</td>
<td>0.83</td>
<td>0.20 ± 0.03</td>
<td>20.0</td>
</tr>
<tr>
<td>1% Glutaraldehyde, MAN 2</td>
<td>−1.6</td>
<td>0.45 ± 0.11</td>
<td>45.4</td>
</tr>
<tr>
<td>1% Formaldehyde, MAN 1 (re-assay after storage at (&lt; 8^\circ C))</td>
<td>1.1</td>
<td>1.1 ± 0.12</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>(0.1)</td>
<td>(0.32 ± 0.005)</td>
<td>(33.1)</td>
</tr>
<tr>
<td>1% Formaldehyde, MAN 1 (re-assay after storage at (&lt; 8^\circ C))</td>
<td>2.0</td>
<td>1.1 ± 0.03</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(0.45 ± 0.04)</td>
<td>(58.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Tip refers to the aliquot immediately at the dispensing tip of the reagent syringe.

<sup>b</sup>Two individual aliquots from each methyl methacrylate, nickel(II) sulfate hexahydrate and formaldehyde reagent syringe were analysed independently. Glutaraldehyde was assayed from aliquots of \( \sim 200 \) mg of the entire content from each patch test syringe, resulting in 4–21 aliquots, depending on the amount of pet. contained in the syringe on receipt at the laboratory.
In the original, unopened container stored under its manufacturer guarantees the stability of the product. Noted that the expiration date is the date to which the rations from three different patch test clinics. It must be from both 'in use' in-date and expired allergen preparations from two different manufacturers were utilized. It was stated that reagents were properly stored; however, time to expiration was not reported. One reagent had non-detectable methyl methacrylate levels, and the other only 25% of the 'declared' concentration. Goon et al. (10) compounded several acrylates, including methyl methacrylate in pet., in their laboratories, and analysed the concentrations of the preparations when stored in syringes and IQ™ chambers. They observed that syringes containing methyl methacrylate in pet. stored at room temperature or at 4°C lost > 20% of the methyl methacrylate within 2 weeks. When they were stored at −16°C, the methyl methacrylate concentration was > 80% of the initial concentration at day 128, but had dropped below 80% of the initial concentration by 6 months. More rapid loss of methyl methacrylate was observed with storage in IQ™ chambers under all conditions. They also noted losses during compounding and lower than expected concentrations of methyl methacrylate in patch test preparations obtained from commercial sources. Possible causes of the loss of methyl methacrylate were noted to be evaporation or spontaneous polymerization.

A potential problem with commercial methyl methacrylate 1 patch test preparations was initially reported by Kanerva et al. (15). False-negative or questionable methyl methacrylate patch test preparation results were noted in two cases in which methyl methacrylate preparations from two different manufacturers were utilized. It was stated that reagents were properly stored; however, time to expiration was not reported. One reagent had non-detectable methyl methacrylate levels, and the other only 25% of the ‘declared’ concentration. Goon et al. (10) compounded several acrylates, including methyl methacrylate in pet., in their laboratories, and analysed the concentrations of the preparations when stored in syringes and IQ™ chambers. They observed that syringes containing methyl methacrylate in pet. stored at room temperature or at 4°C lost > 20% of the methyl methacrylate within 2 weeks. When they were stored at −16°C, the methyl methacrylate concentration was > 80% of the initial concentration at day 128, but had dropped below 80% of the initial concentration by 6 months. More rapid loss of methyl methacrylate was observed with storage in IQ™ chambers under all conditions. They also noted losses during compounding and lower than expected concentrations of methyl methacrylate in patch test preparations obtained from commercial sources. Possible causes of the loss of methyl methacrylate were noted to be evaporation or spontaneous polymerization.

Compounding of methyl methacrylate into pet. requires melting the pet. (> 65°C) to obtain a uniform distribution of the allergen throughout the pet. Like Goon et al. (10), we observed loss of methyl methacrylate during compounding, and attempts to use alternative methods for compounding methyl methacrylate in pet. failed (data not shown). In our study, we consistently observed lower concentrations of methyl methacrylate at the tip of the syringes, regardless of source, amount remaining in the syringe, or expiration date. The concentration at the tip of the syringes averaged 42% (range 30–55%) less than in subsequent aliquots taken from the syringe. This, along with the tip to plunger gradient from a reagent...
Given that the initial concentrations of methyl methacrylate in the syringes obtained from a patch test supplier/manufacturer were less than the industry-acceptable 80% of the labelled concentration, it was suggested that the major reason for loss of methyl methacrylate is volatility. It should be noted that the initial concentrations of methyl methacrylate in the syringes obtained from a patch test supplier/manufacturer were less than the industry-acceptable 80% of the labelled concentration.

In practice, a reagent syringe may be removed from the refrigerator for use many times during its shelf-life, with the potential for increase losses of methyl methacrylate resulting from uncapping and temperature changes. These analyses suggest the possibility of false-negative patch test results being caused by reagent instability/volatility. Possibly, smaller, single-use sealed containers of methyl methacrylate in pet. may be an option to ensure consistent methyl methacrylate patch test preparation concentrations.

NiSO₄₃ is insoluble in pet., and a few studies have questioned whether it is the most appropriate vehicle. Wahlberg et al. (16) compared the threshold values for NiSO₄ in pet. and in water. They reported that pet. had a lower threshold (0.27% in distilled water versus 0.31% in pet.) and was a slightly more reliable vehicle. van Ketel (17) compared water and pet. as NiSO₄ vehicles in 20 nickel-allergic patients. When pet. was used as the vehicle, all 20 patients had a positive patch test reaction, whereas only 16 of 20 had positive reactions when pet. was used as the vehicle. They speculated that the reason was uneven distribution of the insoluble particles within the test reagent. Microscopic and chemical examination of the NiSO₄ in pet. preparations in our study showed that the particulate was uniformly distributed in the vehicle. Our results also suggest that NiSO₄ stability is not an issue, and that false-negative patch test results are not attributable to allergen deterioration or distribution. Thus, false-negative patch test results may be attributable to bioavailability (dissolution and penetration of the stratum corneum).

We also measured the concentrations of two aldehydes, formaldehyde and glutaraldehyde, in their respective patch test preparations. The formaldehyde concentration measured in reagents obtained from one participating clinic and directly from another supplier was consistent with that stated on the label; however, upon re-assay after refrigerated storage without being reopened for 1 year, the reagents had lost 67% and 41% of their formaldehyde content, respectively (assay run in triplicate). The formaldehyde patch test preparations from a different manufacturer supplied in opaque plastic dropper containers sealed with a screw-top lid were re-assayed 11 months post-expiration, and were found to have lost 31% of formaldehyde (data not shown; three containers from the same lot, one never opened; each assayed in triplicate). These had also been assayed 1 year earlier, and been found to contain 1% formaldehyde, as labelled. The chemistry and instability of formaldehyde in water are well known. Dilute formaldehyde and water exist, in equilibrium, mainly as methylene glycol. Over time, this is subject to air oxidation and conversion to formic acid, and it may also potentially polymerize to paraformaldehyde. Although the opaque, screw-top containers seemed to preserve the formaldehyde content better, additional storage studies directly comparing containers are required to verify this preliminary observation.

Patch test preparations of glutaraldehyde are dispersed in pet. with 5% sorbitan sesquioleate as the emulsifying agent. Sorbitan sesquioleate is a known contact allergen, and is available commercially as 20% sorbitan sesquioleate in pet. The use of a known allergen as an emulsifier in a test reagent for another allergen may confound the interpretation of the patch test result.

A major finding of the present study was the instability of glutaraldehyde in the pet. patch test preparation. As noted in Table 1, the lowest concentration measured in the three reagent syringes obtained from participating clinics was in a commercial test reagent assayed 0.8 years prior to the stated expiration date. This syringe had only 20% of the labelled concentration of glutaraldehyde. Preliminary results obtained during method development from glutaraldehyde in pet. purchased by the laboratory directly from a supplier indicated that the concentration upon receipt was ~70% of the labelled concentration (data not shown). We did not observe a concentration gradient across the syringes assayed. Initial measurements of glutaraldehyde in pet./sorbitan sesquioleate compounded within our laboratory indicate that the compounding process does not contribute to the loss of glutaraldehyde. Loss of glutaraldehyde was not observed when it was added directly to sorbitan sesquioleate (data not shown). The laboratory-compounded pet./sorbitan sesquioleate reagents lost >20% of the glutaraldehyde content within 1 week when stored at room temperature. It is possible that loss of glutaraldehyde over time in the patch test preparation may be attributable to polymerization. Dehydration of glutaraldehyde through evaporation or introduction into a hydrophobic solvent leads to polymerization (18, 19). It is also possible that plastic chemicals leached from the syringe into the vehicle react with the glutaraldehyde. Aqueous solutions of glutaraldehyde are usually acidic (pH 3–4). Glutaraldehyde is subject to (acid-catalysed) nucleophilic attack by OH groups (nucleophilic addition reaction), resulting in the formation of hemiacetals or acetals.
Glutaraldehyde is relatively stable in water, although its structure in water is not limited to the monomeric form, as multiple forms have been reported to exist in water (20). Most of the early studies of allergic patch test reactions to glutaraldehyde used concentrations from 0.1% to 1% glutaraldehyde in water (21). Irritant patch test reactions to 1% glutaraldehyde in pet. are also common. Hansen and Menné (21) reported that 9 of 13 patients patch tested with 1% glutaraldehyde showed irritant reactions, whereas no irritation was observed in 844 patients patch tested with 0.1% glutaraldehyde.

However, they questioned the diagnostic efficacy of using such a low concentration of glutaraldehyde. In the absence of analytical confirmation of the glutaraldehyde concentration in pet., it is not possible to confidently establish optimal glutaraldehyde test concentrations for the diagnosis of glutaraldehyde contact allergy. Possibly, re-evaluation of the use of aqueous glutaraldehyde as the standard patch test preparation should be performed, in light of the instability of glutaraldehyde in pet.

In conclusion, the results of our study showing problematic methyl methacrylate, formaldehyde and glutaraldehyde commercial patch test preparations are consistent with those reported for other allergens, such as diphenylmethane-4,4′-diisocyanate (4) and triglycidyl isocyanurate (8). The reliability of commercial diagnostic reagents continues to be of concern, as it impacts not only on diagnostic accuracy in clinical practice, but also on the reliability of comparative, trend and prevalence data reported in the literature.

References
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