Comparison of three different techniques for application of water solutions to Finn Chambers®

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Background: With regard to contact allergy, the dose of a sensitizer per unit skin area is an important factor for both sensitization and elicitation, and therefore a known amount/volume of test preparation should be applied at patch testing.

Objectives: To compare three different techniques for the application of aqueous solutions to Finn Chambers, in order to determine the precision and accuracy of each technique when the recommended 15 μl volume is applied.

Methods: Four technicians applied formaldehyde 1.0% aq. (wt/vol) and methylchloroisothiazoline/methylisothiazolinone 200 ppm (wt/vol) in sets of 10 onto Finn Chambers, with three different techniques: (i) micro-pipetting; (ii) dripping the solutions; and (iii) dripping the solutions followed by removal of excess solution with a soft tissue. Assessment of the variations was performed with the use of descriptive data. The ability to apply the exact amount was assessed by Fisher’s exact test by categorizing each application as in or out of the range 12–18 μl.

Results/Conclusions: The micro-pipette technique had the best accuracy and precision, as well as the lowest inter-individual variation. The technique in which excess solution was removed had good precision, but failed in the application of the defined amount, i.e. 15 μl.

Key words: patch test, appropriate amount, dose, application technique, liquid test preparation © John Wiley & Sons A/S, 2010.

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The elicitation of a positive patch test reaction in a given individual depends on: (i) the dose, i.e. the number of molecules of the sensitizer applied per unit skin area; (ii) the patch test technique, i.e. the vehicle used and the type of occlusion; and (iii) the occlusion time (1–5). The dose is determined by the concentration and volume/amount of the test preparation applied. Thus, if the same volume/amount is applied all the time with the same test technique and occlusion time, concentration can be used as a dose parameter.

For most sensitizers used in commercial test series, petrolatum (pet.) is used as a vehicle, as it is stable and seems to prevent/diminish degradation of the incorporated allergen as well as oxidation and polymerization (6–8). One disadvantage of using pet. as a vehicle is that there has not been a recommended quantity of pet. preparation that is universally accepted for the various patch test techniques. However, a recent study performed on the behalf of the European Society of Contact Dermatitis recommended 20 mg of pet. preparation as the optimal dose for the Finn Chamber with internal diameter 8 mm (9). However, for liquid vehicles, i.e. solutions, there have been generally accepted amounts to be applied in each chamber when testing with the Finn Chamber technique and the van der Bend chamber® technique, i.e. 15 and 20 μl, respectively (10, 11). Recently, Isaksson et al. further investigated whether the recommended volume of solution in these two test systems was adequate. It was concluded that water solutions...
should preferably be tested in Finn Chambers, whereas acetone and ethanol solutions were better tested in the van der Bend chambers (12).

For solutions, as opposed to pet. preparations, it is easy to apply the same amount repeatedly if using a micro-pipette. At our department, we have applied solvent preparations with micro-pipettes since the 1980s. However, micro-pipettes are not used everywhere when applying solutions to patch test chambers. This study was performed in order to comparatively compare different techniques for application to Finn Chambers with respect to water solutions, in order to determine both the precision and the accuracy of each technique, with respect to applying the volume 15 μl. The definitions of the terms precision and accuracy, as used in this article, are as follows.

A set of applications can be:

- Accurate, but not precise, i.e. the mean volume is the correct/set (in this case 15 μl) volume, but the separate applications differ from the set volume.
- Precise, but not accurate, i.e. there is no variation between the separate applications (same volume applied each time), but the mean volume differs from the set volume.
- Accurate and precise, i.e. the mean volume is the set volume (15 μl) and there is no variation between the different applications.

Materials and Methods

Study design

Different application techniques used when applying solutions to patch tests chambers were investigated by allowing four technicians, experienced in patch testing, to use three different techniques when applying the two liquid preparations present in the European baseline series (13), i.e. formaldehyde 1.0% aq. (wt/vol) (Chemotechnique Diagnostics, Vellinge, Sweden) and methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) 200 ppm aq. (wt/vol) (Chemotechnique, Diagnostics), on a set of 10 Finn Chambers (internal diameter 8 mm) on Scanpor® tape (Epitest Ltd Oy, Tuusula, Finland). The latter preparation was not in the concentration used in the Europeans baseline series, i.e. 100 ppm, but the concentration in the Swedish baseline series was used. In total, each technician applied each solution 10 times with each application technique. The liquid preparations were kept in 5 ml polyethylene bottles (personal communication, Chemotechnique Diagnostics), which were squeezeable.

The drop technique. Drop application was conducted by placing a set of 10 Finn Chambers on Scanpor with a filter paper placed in each chamber on a scale, which was then set to zero. A drop of solution was placed on the filter paper by squeezing the plastic bottle containing the solution. Thereafter, the scale was read and then set to zero to enable a new application to be performed. Applications were performed by the four technicians to all 10 chambers for each solution investigated.

The drop and wipe technique. The drop and wipe application was conducted by placing a set of 10 Finn Chambers on Scanpor with a filter paper placed in each chamber on a scale, which was then set to zero. To enable each application, the filter paper was removed from the chamber with tweezers and a drop of solution was placed on the filter paper by squeezing the container. Before the filter paper was replaced on the chamber, the excess solution was removed by gently placing the filter paper edge against a soft tissue. After the filter paper was replaced, the scale was read and then set to zero to enable a new application to be performed. Applications were performed by the four technicians to all 10 chambers for each solution investigated.

Statistical calculations

All statistical calculations were carried out using spss version 17.0 (SPSS, Chicago, IL, USA). Assessment of the variations was performed with descriptive data such as mean, median, standard deviation, and coefficient of variation (CV).

Results

The descriptive data show that the micro-pipette technique has the lowest intra-individual and inter-individual variation (Table 1; Fig. 1). This is also illustrated in Fig. 1, where the box plots of the different application techniques for formaldehyde reveal that the micro-pipette applications are fairly concentrated, with small boxes and whiskers, indicating that each technician is able to apply about the same amount of solution each time; that is, the precision is good. Furthermore, with the micro-pipette technique, each technician has about the same mean as well as a relatively small difference between maximum and minimum amount applied, and the calculated mean and CV for all technicians.
Table 1. Descriptive data for the series of applications made, for each technique and solution, by each technician. All four technicians made 10 applications with each solution for all of the three techniques investigated; that is, all presented data are calculated on the basis of series of 10, with the exception of the columns ‘All technicians’, where the presented data are based on a series of 40, i.e. the sum of all applications made by all four technicians.

<table>
<thead>
<tr>
<th></th>
<th>Pipette technique</th>
<th>Drop technique</th>
<th>Drop and wipe technique</th>
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<tr>
<td></td>
<td>Technician 1</td>
<td>Technician 2</td>
<td>Technician 3</td>
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<td>1.78</td>
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<td>MCI/MI</td>
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<td>CV (%)</td>
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CV, coefficient of variation; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone.

Discussion

With regard to contact allergy, the dose of a sensitizer per unit skin area is important both for sensitization and when performing multicentre studies including studies in humans such as the elicitation threshold measured in studies such as elicitation threshold variation. On the other hand, the drop and wipe technique has poor precision and accuracy; hence, the micro-pipette technique, has good precision, but also a failure in detecting contact allergy leading to a false-negative reaction, i.e. an irritant reaction that shows poor accuracy.

When investigating dermatitis patients, a dose of a sensitizer per unit skin area is important both for sensitization and when performing multicentre studies including studies in humans such as the elicitation threshold measured in studies such as elicitation threshold variation. On the other hand, the drop and wipe technique has poor precision and accuracy; hence, the micro-pipette technique, has good precision, but also a failure in detecting contact allergy leading to a false-negative reaction, i.e. an irritant reaction that shows poor accuracy.
is morphologically indistinguishable from a positive reaction. Furthermore, there is a risk of active sensitization when the dose applied is too high (4).

This study shows the importance of using the micro-pipette technique when applying the recommended amount of water solutions to Finn Chambers. This technique is by far the most accurate for applying the recommended dose of 15 μl, and it is also the technique that generally shows the lowest inter-individual and intra-individual variation; that is, with this technique, almost the same dose is applied each time regardless of who applies it (Tables 1 and 2; Fig. 1). Even though this technique was by far the best of the three techniques studied, it should be noted that the precision and accuracy were poorer than should be expected when using micro-pipettes. According to the ISO 8655 standard, the accuracy when pipetting 15 μl with a 2–20 μl micro-pipette should not be larger than ±0.2 μl, and the precision, presented as CV, should not be larger than 0.67% (personal communication, WVR International). The inter-individual variation in precision and accuracy could possibly be explained by the fact that different and uncalibrated micro-pipettes were used by the different technicians, and the scale was also not calibrated. Furthermore, the four technicians did not perform the applications at the same time, and therefore the indoor temperature and hence the pipetted volume might differ, depending on the temperature on the day of application. With regard to the rather high intra-individual variation for some of the technicians, it should be recognized that, although the use of micro-pipettes can appear easy, it is necessary to master a certain technique. Therefore, it is important to regularly use calibrated micro-pipettes as well as to learn the correct pipetting technique in order to minimize the inter-individual and intra-individual variation when using the micro-pipetting technique. However, if the same margin of error is used for the dose as the manufacturers of patch test allergens use for the concentration given on the label, i.e. ±20% (7), the accuracy and precision shown by the four technicians when using the micro-pipette technique are acceptable. This was exemplified by categorizing each application as in or out of the range 15 ± 3 μl (i.e. ±20%) and thereafter comparing the techniques with Fisher’s exact test (Table 2).

With the drop technique, the amount applied was far higher than the recommended amount for all of the technicians. It was also the technique that showed the highest inter-individual and intra-individual variation. One way of exemplifying the inter-individual variation is illustrated in Table 1, where technician 1 had a range from 11.4 to 46.6 mg when applying formaldehyde; that is, for 10 applications, the lowest and the highest dose differed by a factor of 4. This could have clinical implications, especially for the two solutions investigated in this study, as they both have steep dose–response curves; that is, they have a narrow concentration range in which a dose that is too low may result in a doubtful reaction, although the patient is truly allergic (4). Formaldehyde has previously been tested at 2.0% but, because of a high frequency of irritant reactions, it is now tested at 1.0% (4). Furthermore, MCI/MI was previously patch tested in 300 ppm, but as this resulted in some cases of active sensitization, the recommended patch test concentration was lowered. In Sweden, the recommended concentration is 200 ppm, whereas 100 ppm is used internationally (4). It has been shown that lowering the test concentration by a factor of 3, e.g. either from 300 ppm or from 100 ppm, means that approximately half the number of contact allergic persons will fail to react to the low concentration (14). However, the examples above are based on the assumption that the same amount/volume is applied at each time, and as the dose is determined not only by concentration but also by the amount applied, it is obvious that, in the case of formaldehyde and MCI/MI, there is a significant risk of adverse reactions with the drop technique, as the result from this study shows that the amount applied can be as much as four times higher than intended.

With the drop and wipe technique, the amount applied is approximately 40% higher than that recommended, with calculated means based on all four technicians of 20.75 mg and 20.86 mg for formaldehyde and MCI/MI, respectively. The intra-individual and inter-individual variation are fairly low (Table 1; Fig. 1), implying that the technique might enable quite stable applications when the different technicians apply the studied solutions. However, an analysis with Fisher’s exact test for the drop and

| Table 2. The number of applications in and out of the range 12–18 μl, and the P-value calculated with Fisher’s exact test, when comparing the micro-pipette test with the other two tests in 2 × 2 contingency tables |
|---------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                                | Pipette technique | Drop technique | Drop and wipe technique | | Pipette versus drop | Pipette versus wipe | |
|                                | In | Out | In | Out | In | Out | P-value | P-value |
| Formaldehyde                   | 39 | 1   | 2  | 38 | 1  | 39 | <0.001  | <0.001  |
| MCI/MI                         | 40 | 0   | 1  | 39 | 0  | 40 | <0.001  | <0.001  |

MCI/MI, methylchloroisothiazoline/methylisothiazolinone.
wipe technique failed to show that the applied dose equals the recommended amount of 15 μl (Table 2). It should be mentioned that the applications were not performed entirely blinded. The scale was read and the weight was noted by an observer, but the technician performing the applications could also read the scale, and there is a theoretical possibility that the result could been affected by this, as knowing the last volume applied might have made the technicians change their application technique slightly during the applications, in order to correct for any volumes interpreted as out of range.

Conclusion
The recommended volume and patch test system for liquid solutions is 15 μl in a Finn Chamber. This study clearly shows that the micro-pipette technique is preferable when applying solutions in general, as it is the only technique that enables application of a known amount. It is also the most stable technique; that is, it has the best precision and accuracy.

References

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