

Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing*

MAGNUS BRUZE, MARLÉNE ISAKSSON, BIRGITTA GRUVBERGER AND MALIN FRICK-ENGFELDT

Department of Occupational and Environmental Dermatology, Lund University, University Hospital, Malmö, Sweden

Background: No recommendation exists on the amount of petrolatum (pet.) preparation to be applied in a Finn Chamber[®] or van der Bend Chamber.

Objectives: The aim of this study was to determine the appropriate amount of pet. preparation to apply in the Finn and van der Bend chambers.

Methods: 12 volunteers were patch tested with green-coloured pet. in Finn and van der Bend chambers on the back on 3 occasions. Doses were 10, 15, 20, 25, and 30 mg initially and on second and third occasion, 10 mg was removed in van der Bend Chambers and 35 mg was added. On day 2, the test sites were scored. A negative test equalled a test area not covered to 100%. A positive test meant a completely green-coloured test area. Minor and major spreading was noted. The amount of pet. preparation yielding at most 5% negative reactions and having the least number of reactions with major spreading equalled the appropriate amount.

Results: For van der Bend Chambers, all doses yielded more than 5% negative reactions. For the Finn Chamber[®], 30, 25, and 20 mg pet. yielded less than 5% negative reactions and with major spreading in 60%, 40%, and 25% of test sites.

Conclusions: 20 mg pet. preparation was the optimal dose for the Finn Chambers[®].

Key words: appropriate amount; different doses; Finn Chambers[®]; petrolatum test preparations; van der Bend Chambers. © Blackwell Munksgaard 2007.

Accepted for publication 3 January 2007

The elicitation of a positive patch test reaction in a given individual depends upon (i) the dose, i.e. the number of molecules of the sensitizer applied; (ii) the patch test technique, i.e. the vehicle used and type of occlusion; and (iii) the occlusion time (1–5). The dose is determined by the concentration and volume/amount of test preparation applied. Thus, if the same amount/volume of a test preparation is applied all the time with the same test technique (same area of skin) and occlusion time, it is appropriate to use concentration as a dose parameter. For most sensitizers, petrolatum (pet.) is an appropriate vehicle as it is stable and seems to prevent/diminish degradation as well as oxidization and polymerization of the incorporated allergen (6–8). However, with pet. as the vehicle, it is impossible to repeatedly apply an exact volume/amount. An experienced and trained person can, however, keep the variation within a limited range (9). This statement is sup-

ported by the results of a recent study performed at our department (10). All test strips mounted with Finn Chambers[®] (Epitest Ltd Oy, Tuusula, Finland), to which 49 pet. preparations had been applied, were removed from 3 test cubicles, where 3 technicians were patch testing (10). The weighing of the pet. preparations showed that the 3 technicians had the equivalent precision in their weighing, i.e. they had about the same coefficient of variation and standard deviation. However, there was a significant interindividual variation between the 3 technicians. One major reason for this is that there have not been any amounts of pet. preparation recommended that is of universal acceptance for the various patch test techniques used. This study was therefore performed on behalf of the European Society of Contact Dermatitis to determine the appropriate amount of pet. test preparation to apply at patch testing with the Finn Chamber[®] (diameter 8 mm) and van der Bend Chamber (van der Bend, Brielle, the Netherlands) techniques. The study was divided into 3 parts and was performed in July and November 2005 and February 2006.

*The study was conducted on behalf of the European Society of Contact Dermatitis.

Materials and Methods

Test substance

A green caramel colour was purchased from a grocery in Malmö, Sweden. The colour was mixed with pet. resulting in an evenly green-coloured preparation.

Patients

12 healthy hospital employees participated in the study. There were 10 women (mean age 41.9 years, range 23–58 years) and 2 men (25 and 47 years, respectively). Individual weight, height, body mass index and subjective perspiration tendency were recorded.

Patch testing

All participants were patch tested with one test strip mounted with Finn Chambers[®] and one test strip mounted with van der Bend Chambers. For the respective test technique, 5 pet. preparations containing green caramel colour were applied using 5 different doses, i.e. 30, 25, 20, 15, and 10 mg on the first test occasion. On the second and third test occasion, the 10 mg dose was removed when testing with the van der Bend Chambers and 35 mg was added. The test strips were randomly allocated to one of 3 different areas on the left side of the upper back (outer, central and middle, respectively) and secured with Scanpor[®] tape (Alpharma AS, Norgesplaster, Norway). After 2 days, the strips were removed by the investigators and the test sites were read visually. Photos were taken from the back after 2 days, before and after the removal of the test strips as well as of all test chambers. The test sites were categorized into negative and positive reactions with subdivision of the positives, based on an evaluation according to the following scale: (i) negative reaction, the test area is not completely green coloured, i.e. there are uncoloured gaps or only the middle is green coloured, (ii) positive reaction, the test area is completely green coloured and with the green colouring confined to the test area, and (iii) besides a complete green colouring of the test area, there is a green colouring outside this area (spreading). The spreading was evaluated as minor or major (arbitrarily chosen to be less or more than 2.5 mm, respectively). For the test chambers, the evaluation focused on (i) absence of green colouring outside the test chamber and (ii) presence of green colouring outside the test chamber (i.e. green colouring of the adhesive tape). In February, the same individual had the tests placed in the same position as in November.

Definition of appropriate amount of pet. preparation

The amount of pet. preparation yielding at the most 5% negative reactions in the 12 individuals and at the same time having the least number of reactions with major spreading was designated as the appropriate amount.

Ambient conditions

The first patch testing was undertaken in the summer with day temperatures varying between 25 and 30°C. The second test occasion in November had a day temperature of 10–15°C, while the temperature in February 2006 was around 0°C.

Results

The body mass index showed 9 individuals to be within the normal range, 2 were overweight and 1 was obese. The body mass index, the subjective perspiration tendency, the age of the individuals, the time of the year the testing was performed, and the placing of the test strips on the back did not significantly influence the results, even if there was a tendency to a higher number of positive scores in the central part of the back. The results of patch testing with 5 different amounts of pet. preparations using either the Finn Chamber[®] system or the van der Bend system in 12 individuals are given in Tables 1–3. Whenever there was green-coloured pet. spreading outside the test site, there

Table 1. Number of reactions from the 3 separate test occasions and from all test occasions combined with petrolatum preparations in Finn Chambers[®] in 12 subjects

Test occasion	Results	Doses (mg)				
		30	25	20	15	10
July 2005	Negative	0	0	1	2	5
	Positive	12	12	11	10	7
	No spreading	3	3	5	5	2
	Minor spreading	3	4	3	2	2
	Major spreading	6	5	3	3	3
November 2005	Negative	0	0	0	1	6
	Positive	12	12	12	11	6
	No spreading	1	2	6	6	5
	Minor spreading	5	6	4	3	1
	Major spreading	6	4	2	2	0
February 2006	Negative	0	0	0	0	1
	Positive	12	12	12	12	11
	No spreading	2	1	5	7	8
	Minor spreading	1	5	3	2	2
	Major spreading	9	6	4	3	1
All 3 occasions	Negative	0	0	1	3	12
	Positive	36	36	35	33	24
	No spreading	6	6	16	18	15
	Minor spreading	9	15	10	7	5
	Major spreading	21	15	9	8	4

Table 2. Results from all 3 test occasions with petrolatum preparations in Finn Chambers[®] and depending on the localization on the back

Placing of test strips	Results	Doses (mg)				
		30	25	20	15	10
Outer (to the far left)	Negative	0	0	0	1	5
	Positive	12	12	12	11	7
	No spreading	1	1	2	4	2
	Minor spreading	2	6	6	3	4
	Major spreading	9	5	4	4	1
Central (left half of back)	Negative	0	0	0	1	5
	Positive	12	12	12	11	7
	No spreading	3	3	9	9	6
	Minor spreading	3	4	3	1	0
	Major spreading	6	5	0	1	1
Middle (next to spine)	Negative	0	0	1	1	2
	Positive	12	12	11	11	10
	No spreading	2	2	5	5	7
	Minor spreading	4	5	1	3	1
	Major spreading	6	5	5	3	2

was presence of green colouring outside the test chamber on the adhesive tape. For the Finn Chamber[®] technique, 30, 25, and 20 mg pet. yielded less than 5% negative reactions and with spreading of pet. more than 2.5 mm outside the chamber in approximately 60%, 40%, and 25% of the test sites. 20 mg pet. was thus shown to be the optimal dose to apply in a chamber, both when looking at a separate test occasion and when taking all 3 test occasions together. Also, for the Finn Chamber[®] technique, there were no intraindividual significant differences between the 3 test occasions with respect to the results (number of positive reactions and tendency to spreading).

For van der Bend Chambers, all doses yielded more than 5% negative reactions. With 35 mg, 19 of 24 (79%) test sites were covered to 100%

Table 3. Results from all 3 test occasions with petrolatum preparations in van der Bend Chambers

Test occasion	Results	Doses (mg)					
		35	30	25	20	15	10
July 2005	Negative	NA	10	11	10	11	12
	Positive	2	1	2	1	0	0
	No spreading	2	1	1	1	0	0
	Minor spreading	0	0	0	0	0	0
	Major spreading	0	0	1	0	0	0
November 2005	Negative	3	9	12	12	12	NA
	Positive	9	3	0	0	0	0
	No spreading	8	3	0	0	0	0
	Minor spreading	0	0	0	0	0	0
	Major spreading	1	0	0	0	0	0
February 2006	Negative	2	11	12	12	12	NA
	Positive	10	1	0	0	0	0
	No spreading	10	1	0	0	0	0
	Minor spreading	0	0	0	0	0	0
	Major spreading	0	0	0	0	0	0

NA, not applicable.

with 1 major spreading and with 30 mg, 6 of 36 (17%) test sites were covered to 100%.

Discussion

The prerequisite for a patch test to have the possibility to fulfil the International Contact Dermatitis Research Group (ICDRG) criteria of a positive allergic test is the requirement that the whole test area is covered with the allergen in question. Therefore, the amount of pet. preparation that coloured the whole test area green without any tendency of spreading of the green colour outside the test area could be defined as the appropriate amount of pet. preparation to be applied onto an individual test chamber. A test site that is not sufficiently covered with the test preparation will not fulfil the prerequisites stated above and thus will not without demur have the possibility to give an eczematous reaction covering the whole test area, i.e. it will be a false negative test.

The ideal test situation is a test area completely covered with the test preparation without any spreading outside the test area. This can be achieved by placing a string of pet. intentionally across the chamber (11 and our own experience) (Fig. 1). Due to the physical condition of the pet. preparation, this can be achieved for almost all preparations. However, the viscosity of some preparations makes it difficult to obtain a string, and in that case, the preparation must be placed in the middle of the chamber like a pile (Fig. 2). Furthermore, a completely covered test area with spreading still will contain the prerequisites for a positive test and can be accepted provided the spreading is minor and does not interfere with surrounding test sites. Thus, the only situation generally not acceptable is the one with insufficient coverage.



Fig. 1. A 20 mg petrolatum test preparation placed as a string across the Finn Chamber[®].

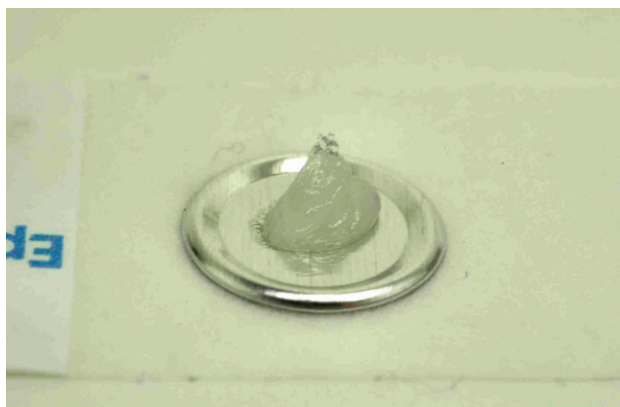


Fig. 2. A 20 mg petrolatum test preparation placed in the middle of the Finn Chamber[®] as a pile.

In this study, a failure (a test area not covered to 100% = a negative test area) of 5% or less was accepted. As this was not seen for the van der Bend Chambers, we cannot, from this study, say anything about the appropriate amount of pet. to use in those chambers. Only the highest dose, 35 mg pet. preparation, covered 19 of 24 of the test sites, which is a failure of 21% (Table 3). Therefore, the following discussion only pertains to the Finn Chambers[®]. For the Finn Chambers[®], 20 mg pet. preparation was the dose that fulfilled at the most a 5% failure and had the lowest number of major spreading. However, also 30 and 25 mg showed less than 5% failure, but when comparing the number of major spreading in these 2 doses, substantial differences were noted compared to 20 mg (Table 1). Surprisingly, interindividually, obesity, perspiration tendency, and time of season when testing had no significant influence on the outcome of the results. Moreover, the results from each of the 3 separate test occasions did not differ significantly intraindividually. Furthermore, the placing of the test strips on the back had no significant influence on the outcome, even if there was a tendency to a better result in the central part of the back. In 1984, Fischer and Maibach concluded that the optimal volume semi-solid test substance was 12–15 μ l when testing in a Finn Chamber[®] with 1 day occlusion (12). This corresponds to 10–12 mg and is almost half the amount to what we have found. However, they had the patch occluded on the back for 1 day and not 2 days.

For every patch test technique, concentration can be used as a dose parameter provided that the optimal test dose and inner area of the test chambers are known. However, the concentration of the respective allergen will differ depending on which concentration the allergen has in the pet. preparation and depending on the chamber area. For example, if 20 mg pet. preparation is dispersed

in a Finn Chamber[®], which has an inner area of 0.5 cm², then for nickel sulphate hexahydrate 5.0%, the corresponding test concentration in a Finn Chamber[®] will be 2.0 mg/cm², while *Myroxylon pereirae* 25.0% will correspond to 10.0 mg/cm² and colophony 20.0% to 8.0 mg/cm².

Returning to the van der Bend Chambers, we noted some peculiarity. When we removed the test chambers after 2 days, we saw that the green-coloured pet. preparation seemed to have stuck to the paper in the bottom of the chamber and it had not spread out from where it had been placed to begin with. This was noted irrespective of dose. We have as yet no certain explanation as to why the spreading of the pet. preparation was hampered, but the filter paper most certainly has a decisive impact.

To conclude, 20 mg pet. preparation was the weight that in a majority of test sites was the optimal dose for the Finn Chambers[®] system. If aiming at 20 mg pet. preparation in a Finn Chamber[®] in a test situation (Figs 1 and 2), there will be a variation both encompassing higher and lower doses, as it is impossible to repeatedly apply an exact volume/amount, as stated before. However, the variation of a few mg up or down from 20 mg will still be sufficient. To show that the recommended dose of 20 mg holds true also for sensitizers when patch testing a sensitized individual, testing at the individual threshold level is needed, which would be very difficult.

For van der Bend Chambers, it is impossible to recommend a defined amount from this study.

References

1. Upadhye M R, Maibach H I. Influence of area of application of allergen on sensitization in contact dermatitis. *Contact Dermatitis* 1992; 27: 281–286.
2. Bruze M. Patch testing with nickel sulphate under occlusion for five hours. *Acta Derm Venereol* 1988; 68: 361–364.
3. Friedmann P S, Moss C, Shuster S, Simpson J M. Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects. *Clin Exp Immunol* 1983; 53: 709–715.
4. Bruze M, Conde-Salazar L, Goossens A, Kanerva L, White I R. Thoughts on sensitizers in a standard patch test series. The European Society of Contact Dermatitis. *Contact Dermatitis* 1999; 41: 241–250.
5. Webster R C, Maibach H. Percutaneous absorption relative to occupational dermatology. In: *Occupational and Industrial Dermatology*, Maibach H (ed.): Chicago: Yearbook Medical Publishers Inc., 1987: 241–257.
6. Bruze M, Fregert S. Studies on purity and stability of photopatch test substances. *Contact Dermatitis* 1983; 9: 33–39.
7. Isaksson M, Gruvberger B, Persson L, Bruze M. Stability of corticosteroid patch test preparations. *Contact Dermatitis* 2000; 42: 144–148.
8. Andersen K E, Rastogi S C, Carlsen L. The Allergen Bank: a source of extra contact allergens for the dermatologist in practice. *Acta Derm Venereol* 1996; 76: 136–140.

9. Moffitt D L, Sharp L A, Sansom J E. Audit of Finn Chamber patch test preparation. *Contact Dermatitis* 2002; 47: 334–336.
10. Bruze M, Frick-Engfeldt M, Gruvberger B, Isaksson M. Variation in the amount of petrolatum preparation applied at patch testing. *Contact Dermatitis* 2007; 56: 38–42.
11. Fischer T, Rystedt J. Cobalt allergy in hard metal workers. *Contact Dermatitis* 1983; 9: 115–121.
12. Fischer T, Maibach H. Finn chamber patch test technique. *Contact Dermatitis* 1984; 11: 137–140.

Address:

Magnus Bruze

*Department of Occupational and Environmental Dermatology
Malmö University Hospital*

SE-205 02 Malmö

Sweden

Tel: +4640331760

Fax: +4640336213

e-mail: magnus.bruze@med.lu.se