

Patch testing with neomycin sulfate

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The recommended patch test concentration of neomycin sulfate is 20% in petrolatum applied occluded for 48 h (2 days [D]). In the current study, the efficiency of such a test is compared to results with other techniques using lower allergen dose than obtained by approximately 20 μ l of the 20% substance. Efficiency of petrolatum and demineralized water as vehicles are compared. 16 neomycin-sulfate positive subjects were retested with serial doses ranging from 0.4 to 0.0085 mg/cm² neomycin sulfate in cellulose printed on polyester squares and applied by both tapes and plastic foils. Additionally, tests were performed with the 20% petrolatum substance in Finn Chambers[®]. Tests containing the 20% substance in petrolatum and in water were applied directly with transparent foils. Readings ranged from 3 to 264 h (11D) following applications. Results show that foil applications of a polyester square dose, that is 7% of the dose obtained with the 20% substance, distinguished between perfusion of subjects who were visually positive and not with any test in the study. With the same test technique and visual assessments, 2 subjects were false-negative but developed doubtful test responses with the Finn Chambers[®].

Key words: allergic contact dermatitis; patch testing technique; tape; bioengineering methods; laser Doppler perfusion imaging; laser Doppler perfusion scanning; dose; application time; visual assessment; medicaments. © Munksgaard, 2000.

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Neomycin is a stable and water-soluble antibiotic (1) not considered to sensitize excessively (2–4). Sensitization appears to be associated with length of use (5, 6), but has been reported down to a 5-week-old infant (7). Apart from localized dermatitis, anaphylactic reactivity caused by contact with neomycin (8), response to ingestion (9), cross-reactivity with drugs (1, 10–17) and a fixed drug eruption (18) have all been reported. Neomycin patch tests are among allergens in test series that most frequently generate positive tests (19). In a Portuguese study on children, neomycin was the most common positive allergen, with 18.4% of all positive tests, compared to 5.7% with nickel sulfate (20). Even though neomycin sensitivity appears to be an active problem, an optimal test technique with neomycin is still unknown. A study used 11 different ways to test with neomycin but found no single way to generate 100% test sensitivity (21).

In a recent study, we compared visual assessments of neomycin sulfate patch tests to assessments made with a laser Doppler perfusion imaging technique, and investigated the effect of application time and dose on test results (22). The current study is based on the same subjects, with tests carried out with a wide variety of neomycin

sulfate patch test substances and control patches, applied by different application devices. Assessments of test responses are made both visually and with the laser Doppler technique (22–30).

Objectives

(A) To follow tests over time and investigate: (i) when reactions appear and disappear visually; (ii) which is the shortest application time for detection of visually positive reactions at different serial dose levels; (iii) whether vehicles may affect perfusion or visual test results.

(B) To use all patch test results to find out which subjects are positive with any test technique, and which are not, to investigate in terms of perfusion and visual assessments: (i) whether 20% neomycin sulfate in petrolatum tests applied with Finn Chambers[®] and tapes for 48 h is a sensitive way to test; (ii) how low a dose can be used to detect all positive subjects.

Subjects, Materials and Methods

For a detailed description, the reader is referred to our recent study (22). In short, 16 white subjects

had reacted with at least erythema and infiltration when tested with 20% neomycin sulfate in petrolatum in Finn Chambers® and tapes for 48 h (2 days [D]). 6 of these, (nos. 3, 5, 9, 11, 13 and 16), had clinical relevance between their contact allergy and a product containing neomycin, while the others were uncertain. In current study, retests were carried out on the backs of these subjects as follows.

(i) All subjects were tested with approximately 20 µl of 20% neomycin sulfate in petrolatum in Finn Chambers® and tape for 48 h (2D).

(ii) All subjects were tested with 0.23, 0.0766, 0.0255 and 0.0085 mg/cm² neomycin sulfate in a methylcellulose vehicle printed on polyester squares. 1 dose of each patch was applied with both tape strips and transparent adhesive foils (Tegaderm™). Each subject was applied with the strips for 3, 6, 24 (1D), 48 (2D), 72 (3D) and 96 h (4D) and the foil for 168 h (7D). In addition, a dose of approximately 0.4 mg/cm² in a carboxymethylcellulose vehicle was tested on all strips and foils of subjects nos. 8–16.

(iii) Subjects nos. 8–16 were tested with approximately 20 µl of 20% neomycin sulfate in petrolatum applied directly to application foils, and with the same concentration and volume in demineralized water for 168 h (7D).

(iv) Approximately 2 months after application of above materials, subject no. 8 was tested with a polyester patch printed with 0.69 mg/cm² neomycin sulfate in polyvidone and a repeated application with the 0.23 mg/cm² neomycin sulfate patch above. Both were applied with a strip for 48 h (2D).

All tests were controlled with 2 identical control patches, except for allergens on the same application device, with the exception that all controls containing cellulose were controlled with carboxymethylcellulose. The Finn Chambers® and 96 h (4D) strip applications on subjects nos. 1 and 16 were removed earlier than planned, as was the 72-

h strip on subject no. 16. Test areas were read both visually with a standard accepted by the North American Contact Dermatitis Group (31) and with a laser Doppler perfusion imager® PIM 1.0 (LDPI). Mean perfusion value of control patches is subtracted from patches containing allergen on the same test device, with the resulting value termed "adjusted perfusion value" (APV) indicated in V. Reading sessions were at 3 h, 6 h, 24 h (day [D] 1), 48 h (D2), 72 h (D3), 96 (D4), 168 (D7) and 216 h (D9) on all subjects, and additionally at 264 h (D11), except at 240 h (D10) on subjects nos. 1 and 8–16. At each session, readings were taken of all patch test areas of detached strips. Tests of foils were read regardless of detachment and at the session when they were detached, both prior to and following the detachment. Readings of the 0.69 mg/cm² patch and the concomitantly tested 0.23 mg/cm² patch were 1-time readings at 72 h (D3). 1 subject had a 2nd reading of tests of tape strips applied for 48 h (2D), 72 h (3D) and 96 h (4D) at the session when the strips were detached.

Ethical approval was obtained from the Ethical Committee of the Karolinska Institute, Stockholm (no. 95/130).

Results

14 of the 16 subjects investigated developed visually positive reactions with at least 1 of the test techniques, while the remaining 2 subjects (nos. 10 and 15) developed only reactions with papules, not fulfilling criteria for positive reactions.

Appearance and disappearance of tests

Time of appearance and disappearance of visible reactions is shown in Table 1. Results of subjects nos. 1, 4, 8 and 12 are based on single positive reactions and, in the case of subject no. 8, on a single reading. Subject no. 6 had 20 ?/?+ reactions

Table 1. Reading time intervals at appearance and disappearance of at least 1 visually positive reaction (hours)

	Subject no.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
time of earliest detection	216	96	96	72	72	48	48	72	72	No reactivity	72	168	168	216	No reactivity	48
time of latest detection	216	216	216	72	216	216	168	72	168		168	168	216	240		72
time of earliest disappearance	216	96	216	96	216	168	168	72	96		96	168	240*	240*		72
time of latest disappearance	216	264*	264*	96	264*	216	264*	72	240*		240*	168	240*	240*		240*

* End of follow-up.

Table 2. Shortest application time required to detect a visually positive reaction

Dose (mg/cm ²)	Subject no.															
	1 ^{a)}	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16 ^{b)}
Approximately 0.4	NP	NP	NP	NP	NP	NP	NP		24 (24)		24 (3)			48 (24)	48 (48)	6 (3)
0.23		6 (3)	24 (24)		24 (3)	6 (3)	3 (3)		24 (24)		24 (3)				48 (24)	24 (3)
0.0766		48 (6)	24 (24)		6 (3)	6 (3)	6 (3)		24 (24)		24 (6)				48 (24)	6 (6)
0.0255		48 (6)	24 (24)		24 (6)	6 (6)	6 (3)		72 (24)		48 (6)					24 (6)
0.0085		72 (6)	24 (24)		48 (6)	24 (3)	24 (6)		96 (6)		96 (48)					24 (6)

Parentheses indicate shortest application time required to detect reactivity not positive with the assessment scale used in current study. Empty spaces indicate no response to any application with the dose concerned. Applications are with tapes for 3–96 h (4D).

^{a)} 96 h application not performed. ^{b)} 72 and 96 h applications not performed. NP: Not performed.

and 4, negative reactions at the end of follow-up (264 h (D11)).

Shortest application time for detection of positive tests

Table 2 shows the shortest application time required for detection of a visually positive reaction on each subject with each of the serial doses.

Vehicles

Results of foil applications with 20% neomycin sulfate in 2 different vehicles are shown in Table 3. With these vehicles, only the LDPI allows a distinction between positive subjects during the study and subjects nos. 10 and 15 who are not. Of single reading times, 96-h (4D) readings with the water vehicle best serve that purpose, where the lowest APV of a visually positive subject is 1.02 V while subject no. 15 has an APV of 0.00 V. Subject no. 10 was not read at 96 h (D4), but all other readings with that vehicle yielded an APV of 0.00 V. With the petrolatum vehicle, the single best reading interval was at 240 h (D10), when the difference between visually positive subjects during the study and others was very narrow, and where 0.06 V is the lowest APV of a visually positive subject.

Allergy of subject no. 8 is detected visually with neither vehicle in Table 3, but that subject developed a + test with the 0.69 mg/cm² neomycin sulfate dose tested in a polyvidone vehicle. APV of the reaction was 0.61 V (one time reading). On that subject, the highest serial dose of approximately 0.4 mg/cm² neomycin sulfate in a carboxymethylcellulose vehicle yielded a negative reaction with an APV of 0.10 V despite multiple readings, and no

positive test was elicited with the methylcellulose vehicle used to test serial doses of 0.23 mg/cm² and lower.

Tests with 20% neomycin sulfate in petrolatum in Finn Chambers®

Test results with the 48-h (2D) tape applications of the 20% test substance in petrolatum in Finn Chambers® are shown in Table 4. The left side shows the highest assessments with readings performed at 72 h (3D) and 216 h (9D) with each reading technique. These reading time intervals were the best of any 2 intervals for each assessment approach, to distinguish between positive subjects of the study and others. Additional visual readings did not detect positive reactions in subjects nos. 8 and 12. The right side in Table 4 shows the highest assessment results when readings are followed over

Table 3. Difference in test results with different vehicles used for tests containing 20% neomycin sulfate applied by foils for 168 h

Subject no.	APVs Vehicle		Visual assessments Vehicle	
	demineralized water	petrolatum	demineralized water	petrolatum
8	1.85	3.40	??+	??+
9	2.19	2.13	++	++
11	5.09	4.46	++	++
12	1.46	0.27	++	??+
13	5.90	5.22	++	++
14	6.46	5.59	++	++
16	5.31	4.40	+++	+++
15	0.08	0.17	??+	??+
10	0.00	0.18	–	–

Highest test assessments are shown of readings from 3 h until end of follow-up.

Table 4. Test results of 20% neomycin sulfate in petrolatum applied in Finn Chambers® and tape for 48 h (2D)

Subject no.	Readings (h)			
	72 and 216		48 through 168	
	APV (V)	Visual assessment	APV (V)	Visual assessment
7	4.37	++	4.37	++
5	4.27	+++	5.08	+++
6	2.72	+	2.77	++
11	2.26	+	2.88	++
14	2.13	++	2.13	??+
8	2.13	??+	2.13	??+
9	2.10	++	2.10	++
4	1.52	++	1.52	++
2	1.46	+	5.05	++
13	1.31	++	1.43	??+
3	1.20	+	1.68	+
12	0.39	??+	1.09	??+
10	0.17	—	0.36	??+
15	0.10	??+	0.36	??+
16	application strips removed before 48 h			
1	because of subjects' discomfort			

The left side of the table shows highest assessments using 2 reading times at the most, while the right side shows highest assessment values of reactions when followed up to 168 h (7D).

Table 5. Test techniques with the lowest test dose possible that best distinguish between visually positive and negative subjects for each reading method if maximally 2 readings are performed; highest assessment values are shown

Assessment technique:	Superficial perfusion	Morphology
dose of neomycin sulfate:	approximately 0.4 mg/cm ²	
application device and time of test-material application:	foils for 168 h	
reading time(s) (h):	24 and at 168 h following detachment of foils	216 h
Subject no.	APVs	Visual assessments
11	3.14	+
13	2.52	++
9	1.70	++
16	1.69	+
14	0.73	++
8	0.30	—
12	0.23	—
15	0.11	??+
10	0.05	—

time, from the time that strips were detached at 48 h (D2) until 168 h (D7).

Low-dose distinction between positive and other subjects

No test technique detects all subjects who are positive in the study. The best technique is shown in

Table 5 for each reading method if 2 reading times are allowed. That technique detects 1 more positive subject than detected with all tape applications in Table 2.

Tests with papules only

1 subject who does not elicit positive reactions with any test develops papules alone when tested for 48 h (2D) or longer with the approximately 0.4 mg/cm² serial dose with tape strips.

If tests with papules only are considered positive, then all subjects tested with the approximately 5.35 mg/cm² neomycin sulfate patches (20 µl of the 20% test substance) applied in Finn Chambers® with tape for 48 h (2D) would be detected with a single reading at 96 h (4D).

Discussion

Appearance and disappearance of tests

The slow appearance of neomycin reactions (13, 32, 33) and their long persistence, often for weeks, is well known (13). We do not always find this to be the case, as in Table 1, where reactions may start as early as between 24 h (D1) and 48 h (D2) (detected at 48 h) and disappear as early as between 48 h (2D) and 72 h (3D). Of further interest in the table is the inter-individual variation of the time of earliest appearance of reactions and the fact that at the same reading session some reactions on the same subject may be discovered while others have disappeared.

Where the disappearance of reactions is concerned, all subjects who develop positive reactions in the study are positive at the end of follow-up, except the 4 subjects (nos. 1, 4, 8 and 12) who had only single positive reactions in the study, and the subject who had 20 ??+ and 4 negative reactions at the end of follow-up (subject no. 6). It is possible that the 2 subjects who do not develop positive reactions in the study regardless of test techniques may require longer follow-ups (nos. 10 and 15). This may be the case even though we showed in a recent study that highest reactivity of reactions of all other subjects than those 2 are at 96 h (4D) or 168 h (7D) reading intervals, with minor exceptions for an adjacent reading time interval, except in a single subject who had highest reactivity at 72 h (D3) (22).

Shortest application time for detection of positive tests

At each of the serial doses in the table, there is an inter-individual variation concerning the shortest possible application times that can be used to de-

test positive reactions. Some subjects require longer applications with decreased dose for elicitation of positive tests in Table 2. A reason why the test technique in Table 5 detects more positive subjects than any method in Table 2 may be due to a longer application (168 h (7D) versus 3–96 h (4D)).

Vehicle

Comparison of test results with 2 different vehicles, petrolatum and water, is based on tests with foils. The foils allow assessment of tests for a longer period of time than opaque application devices, as foils allow readings while tests are applied (24, 30). It is interesting that both vehicles make it possible to distinguish in terms of APVs between positive subjects with any test and others (nos. 10 and 15) (Table 3).

Neither vehicle in Table 3 detects a visually positive reaction on subject no. 8. This is also the case when the petrolatum vehicle is used in a Finn Chamber[®]. However, when a polyvidone vehicle is used, and at a much lower dose (0.69 mg/cm²), a positive reaction is elicited. Despite different test techniques, this finding is interesting, but it must be kept in mind that the polyvidone vehicle was only used in that single polyester square patch test to obtain a chemically stable test substance with a higher dose than the approximately 0.4 mg/cm² dose on polyester patches.

It has been suggested that false-negative tests (34) and a long reaction time (35) are caused by slow penetration of the allergen. It is possible that neomycin in the form of a sulfate salt may decrease the penetration rate (13). Sodium dodecyl sulfate may not be appropriate to use as it has been reported to increase precipitation of neomycin (36). Contaminants in vehicles constitute another problem because they may be irritant, as experienced by Epstein using 40% and 20% cetyl alcohol in petrolatum as a vehicle for neomycin patch tests (37). Bajaj et al. have compared the use of yellow petrolatum, propylene glycol in varying concentrations, lanolin, olive oil, polyethylene glycol 400 and plastibase in tropical areas; of these, they recommend the last 2 vehicles for neomycin (38).

Ointments have been suggested to be the major cause of sensitization to neomycin (39). They may have an occlusive effect enhancing penetration (40). This may be reflected by a higher number of positive neomycin tests with ointments compared to creams (41). Schorr et al. (11) have found stronger test responses with concentrations below 30% in petrolatum, and consider the 30% level in petrolatum to form a semisolid matrix with a paste-like consistency with a poorer release of

allergens. In the current study, subject no. 12 elicits a positive reaction (++) when tested with the water but not with the petrolatum vehicle (Table 3). This may suggest a better release of allergens from the water vehicle than the petrolatum vehicle, the latter of which may be expected to have a more pasty consistency. Better release from the water vehicle is also supported by both a higher APV with that vehicle, when results with both vehicles of subject no. 12 are compared in Table 3, and by a bigger difference between positive subjects and others (nos. 10 and 15) in terms of APVs in the same table with the water vehicle. Also in favor is an earlier distinction between positive and other subjects with the water vehicle at a single best reading (96 h (4D) versus 240 h (10D)).

Tests with 20% neomycin sulfate in petrolatum in Finn Chambers[®]

If the LDPI is used for test assessments with the test technique in Table 4, it is possible to distinguish between subjects who are visually positive with any technique, and who were also tested with that specific technique, and the 2 other subjects who did not develop positive tests (subjects nos. 10 and 15). The time period between the optimal reading times with the LDPI, at 72 h (D3) and 216 h (D9), is quite interesting and also that the use of both reading times increases test sensitivity with the visual assessment approach. With the visual assessment technique, 4 positive subjects in the study do not develop positive reactions, even though tests are read at 48 h (2D), 72 (3D), 96 (4D) and 168 h (7D). With the 2 optimal reading times (Table 4), 2 subjects still do not become positive. These findings reveal the importance of long follow-ups of neomycin patch tests. The low sensitivity with the visual assessment approach and the 2 best readings (Table 4) is interesting as the 13× lower dose per square area used for the technique in Table 5 yields the same number of subjects who are not positive; however the low-dose technique is based on a more limited number of subjects.

Tests with papules only

The results of the subject (no. 15) who does not develop positive reactions with any test technique in the study, but who develops papules alone with 48 h (2D) or longer applications at the highest serial dose (approximately 0.4 mg/cm²) is interesting. The application-time dependency may suggest such reactions to be weak positive tests. This may seem to be borne out by a recent study with neomycin sulfate where such tests were found at the shortest application of only 3 h while ++ or even

+++ reactions were elicited with longer applications of identical patches and application device (22). However, some findings in that study do not confirm that such reactions are particularly weak, e.g., when an identical patch on the same subject yields a + reaction at a shorter application than eliciting papules only, and a +++ reaction at a longer application.

Given that tests with papules alone are indeed positive tests, it is interesting that all subjects tested with the 20% substance in Finn Chambers® for 48 h (2D) are detected if tests are read at 96 h (4D), but not if tested with the same substance with foils for an even longer period of time (168 h (7D)). The reasons may be increased bioavailability by the chambers due to better occlusion and/or a higher dose of allergen per square area tested. As the test technique with the chambers is the only technique that detects at least some reactivity in all subjects tested in that way, if followed for sufficiently long, it must be considered the safest one even though some retesting may be required.

Low-dose distinction between positive and negative subjects

With the technique in Table 5, 1 more subject is detected compared to results of all the tape applications taken together (Table 2). Even though the test technique in Table 5 uses a $13\times$ lower dose per square area than the one in Table 4, a comparison between these 2 techniques is affected by fewer subjects tested with the low-dose technique. Also, it is not clear if reduced bioavailability is secured with the low-dose technique, as it is unknown.

The 0.69 mg/cm^2 dose elicits the only positive reaction detected on the subject so tested (subject no. 8). This is interesting and may imply that a higher dose than the highest serial dose of approximately 0.4 mg/cm^2 should be investigated further for tests with polyester squares. However, the difference in the test results may be affected by the use of a polyvidone vehicle with the 0.69 mg/cm^2 dose and a carboxymethylcellulose vehicle with the other dose, and the fact that the tests were performed sequentially.

One study uses 11 different combinations of application methods and concentrations ranging from 1 to 50% (21). Even though accepting a few papules alone for positive tests, no single test technique detected all positive subjects. The standard neomycin sulfate patch-test concentration long recommended is 20% in petrolatum applied occluded for 48 h (2D) (42). It appears that such recommendations must take into account environmental factors that may affect test responses, as supported by a study where only 0.35% neomycin

sulfate was required in a hot, humid climate for testing (43). We find a dose of approximately 0.4 mg/cm^2 to be equally effective as the dose of $20\text{ }\mu\text{l}$ of the 20% test substance, even though being only 7% of the latter (tested on 9 subjects). However, the difference between the test techniques needs to be considered in this context, as well as interpretation of tests with papules only. Before suggestions can be made, a larger number of subjects needs to be investigated.

With the optimal test technique in Table 5, it is possible that an application time shorter than 168 h (7D), which was the only application time used with the foils, would be possible. It can be argued that such a long application time may be irritant and explain increased perfusion with the LDPI, such as the 0.30 and 0.23 V APVs of subjects nos. 8 and 12 in Table 5, while visual assessments of these tests are negative. However, irritancy does not seem to be a problem with neomycin sulfate patch tests. Some authors have used up to 50% neomycin in petrolatum and do not mention in their papers (21, 44), or have not encountered, false-positive reactions (40). Obviously perfusion may increase if traumatic application methods are used, such as the chamber-scarification technique of Frosch and Kligman, which causes irritation in all subjects tested with 20% aqueous neomycin sulfate (5). Another example is the scratch-patch technique, which may cause difficulty in distinguishing between irritant and allergic reactions, regardless of whether a 20% neomycin sulfate test substance in water or a 0.5% neomycin sulfate petrolatum test substance is used (45).

Alternatives

Of concern are subject nos. 10 and 15 who develop no more than +/- reactions with all the test techniques in the current study, as these subjects had positive reactions with 1 of the techniques prior to the study and which was repeated in current study. Even though the clinical relevance between earlier test results and reactivity to a product containing neomycin has been unclear in these subjects, allergy cannot be ruled out. Factors that may explain the results in these subjects include the fact that current study requires at least discrete papules, in addition to erythema and infiltration, for a positive test on the initial testing. Another explanation may be a change in the allergy state of the subjects between the 1st and the 2nd testing. For these subjects, alternative techniques must be considered.

Better test results have been reported with intradermal tests than with patch tests (45–47). Such results may encourage the use of intradermal tests directly without further patch tests in subjects with

doubtful or negative patch test responses and a suspicion of allergy. 0.1 to 1% dilutions have been suggested for the intradermal tests (47) which respond more rapidly than patch tests, when comparing test results with serial dilutions ranging from 1% to 0.001% neomycin in saline intradermally to results with 20% neomycin in petrolatum patch tests (35). 1 disadvantage with the use of high concentrations of neomycin in intradermal tests is the risk of an anaphylactoid inflammatory response that may be provoked by solutions exceeding 0.1% (0.05 or 0.1 ml tests) (3, 48). However, this view is not held by all investigators (11, 49). Another disadvantage of intradermal tests is a transient traumatic reaction that results from injury of injecting needles. Such reactions are usually less than 5 mm in diameter and disappear or fade after 24 h (1D) to 48 h (2D) (50).

The + reaction elicited only with the polyvidone vehicle and the comparison between the water and the petrolatum vehicles in Table 3 suggest the importance of the vehicle for neomycin patch tests. In cases where a clinical suspicion of allergy remains after a negative or a doubtful patch test, it may be logical to consider a water vehicle for retesting, if not used initially, before invasive methods are considered. This view is supported by a study using 20% neomycin sulfate in water and 0.05 ml of 1% neomycin sulfate intradermally, where, at a 7-day reading, 7 subjects became positive with the patch test approach, while only 1 became positive with the intradermal approach (4). However, it is our view that tests with papules alone should be interpreted as positive tests, and best test results in our population of all test techniques used are thus obtained with the 20% test substance in petrolatum tested in Finn Chambers® and tape for 48 h (2D), with a single reading at 96 h (D4), thus making alternative methods unnecessary.

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