

ORIGINAL ARTICLE

Strip patch test vs. conventional patch test: investigation of dose-dependent test sensitivities in nickel- and chromium-sensitive subjects

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Summary

Background Tape stripping the skin prior to patch testing (strip patch test, SPT) may enhance percutaneous penetration of test substances and increase skin reactivity to test substances.

Objective To evaluate the test sensitivity of SPT in compliance with our recently presented practical method vs. conventional patch test (PT) in nickel- and dichromate-sensitive subjects.

Methods Thirty subjects participated in the prospective, randomized, controlled, investigator-blind study. Serial dilution tests of nickel sulphate (10 000–50 ppm aq.) and potassium dichromate (1000–5 ppm aq.) were used. Tests were applied in duplicate to both sides of the upper back of the subject, where one, randomly chosen side was tape stripped.

Results According to the estimated test sensitivities, SPT is more sensitive than PT in nickel- and dichromate-sensitive subjects, regardless of the interindividual different numbers of tape strips. The superiority of the SPT grows with diminishing concentrations of the allergens.

Conclusion Test sensitivity of SPT vs. PT is increased. If no positive results are obtained by PT, the SPT could help to detect hidden allergens in case of weak sensitization or low concentration and poor percutaneous penetration of the test substances.

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Keywords

contact sensitization, nickel sulphate, patch test, potassium dichromate, strip patch test, test sensitivity

Conflict of Interest

None declared.

Introduction

Adhesive tape stripping prior to patch testing (strip patch test, SPT) was first described in 1953 by Spier and Natzel.¹ Performing this modification of the conventional patch test (PT) in order to detect weak sensitizations to chromium and high-grade sensitizations by a reduced elicitation threshold, respectively, they tested aqueous solutions of potassium dichromate in concentrations from 0.001% to 0.1%. Spier and Sixt² later demonstrated the influence of the stratum corneum barrier on the results of the PT.

The SPT may enhance test substance absorption into the skin³ and increase skin reactivity to the test substance.^{4–6} Therefore, tape stripping is a common pre-treatment for patch testing

ophthalmic drugs,^{7–9} contact lens solutions,¹⁰ heparins,¹¹ and aeroallergens.^{12–14} Additional fields of application comprise patch testing of metal salts¹⁵ and photo patch test substances.¹⁶ Only recently, Fernandes *et al.*¹⁷ reported the results of a standard patch test tray, applied in duplicate to both sides of the back – on ‘abraded’ and ‘unabraded’ skin – of 65 patients.

However, in contrast to the PT,¹⁸ there is no standardized recommendation for a practical application of the SPT. The differences in SPT methods include differences in the source of test substances, vehicles, pretreatment procedures of the skin, application site, application period, and days of readings of the test. Due to these differences, large variations of SPT results exist.

In a multicentre pilot study, we recently presented a reproducible easy-to-handle first approach for carrying out a uniform SPT.¹⁹ In the meantime, this approach was adopted in 'Allergy Diagnostic Testing: An Updated Practice Parameter'²⁰ by the American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology.

The study reported here was undertaken to evaluate for the first time the test sensitivity of SPT in compliance with our suggested method¹⁹ vs. conventional PT in nickel- and dichromate-sensitive subjects, respectively. We hypothesized that there are different test sensitivities, depending on the patch test method and the dose of the test substance. Additionally, these differences might manifest themselves also in different elicitation thresholds. For this purpose, we used serial dilution tests of nickel sulphate and potassium dichromate, following the range of reported dilutions in literature.^{21,22}

Subjects and methods

Study design and study population

This prospective, randomized, controlled, investigator-blind, monocentric study took place from January to May 2008. Inclusion criteria were based on positive routine PT (+, ++, or +++; performed in the Department of Dermatology and Allergology of Bochum) to nickel sulphate 5.0% pet. and/or potassium dichromate 0.5% pet. in the recent past (henceforth called 'historical' PT), age (≥ 18 years), and clinically normal skin. Exclusion criteria were pregnancy, lactation, intake of immunosuppressive drugs, and topical use of glucocorticosteroids at the back and/or intense ultraviolet exposure on the back during 4 weeks prior to the study enrolment.

The study was approved by the ethics committee of the Medical Faculty of the Ruhr-University Bochum. All procedures were explained in detail to all subjects, who signed informed consent forms prior to participation. A total of 30 subjects participated in the study; 13 of them had been routinely patch tested positive to nickel sulphate, 3 were positive to nickel sulphate and potassium dichromate, and 14 positive to potassium dichromate. There was no dropout.

Test procedures

The serial dilution test of nickel sulphate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, EINECS no. 232-104-9, CAS no. 10101-97-0, purity $\geq 99.0\%$) used for PT and SPT comprised 1.0% (10 000 ppm), 0.5% (5000 ppm), 0.1% (1000 ppm), 0.05% (500 ppm), and 0.005% (50 ppm) in distilled water. The serial dilution test of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$, Sigma-Aldrich Chemie GmbH, EINECS no. 231-906-6, CAS no. 7778-50-9, purity $\geq 99.8\%$) used for PT and SPT comprised 0.1% (1000 ppm), 0.05% (500 ppm), 0.01% (100 ppm), 0.005% (50 ppm), and 0.0005% (5 ppm) in distilled water. As control, a sample of distilled water was also tested at the same time in PT and SPT. The

test solutions were freshly prepared every week and kept in a refrigerator below 5 °C when not in use. A 15- μL aliquot of each test solution was micropipetted onto a filter paper disc placed within a small, 8-mm Finn Chamber on Scanpor® (Epitest Ltd Oy, Tuusula, Finland).^{23,24} The diagnostic test concentrations of 5.0% (50 000 ppm, pet.) nickel sulphate and 0.5% (5000 ppm, pet.) potassium dichromate were not retested.

PT with serial dilution tests was performed according to standard methodology.¹⁸ SPT with serial dilution tests was performed according to our recently published first approach for a uniform and practical procedure.¹⁹ In so doing, tape stripping is performed with a 3 M™ Blenderm™ surgical tape (3 M™ Medica, 3 M Deutschland GmbH, Neuss, Germany; metric 25 mm $\times$ 4.5 m), which is an occlusive, transparent, latex-free and hypoallergenic plastic tape. Each piece of tape is applied vertically (i.e. parallel to the spine), onto the skin without tension, gently pressed downward by fingertips for about 2 s to cause it to adhere evenly, and then removed in one quick movement at an angle of 45° in the direction of adherence. For each single strip a new tape cut is used and positioned on exactly the same skin area. The patient first receives tape strips to the intact, non-inflamed skin at the upper back until the skin surface starts to glisten (i.e. shows three small glistening spots indicating that the stratum lucidum has been reached). If necessary, hair is gently removed before with clippers. The individual number of strips to the stratum lucidum is then multiplied by the tape-specific correction factor ($\text{cf} = 11/26 \approx 0.42$). The calculated number of strips for the SPT is finally performed as described on an untreated site. After that, test substances are applied for 24 h and reading of skin reactions is performed in the conventional way of patch testing.

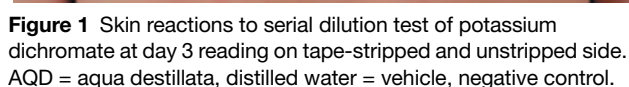
All tests were applied in duplicate to the left and right side of the upper back of the subject, where one, randomly chosen side was tape stripped (Fig. 1). Tests were uniformly removed after 24 h (day 1). Readings were done at day 1 and day 3. Positive PT results reported here are based on the day 3 reading and were graded according to the recommendations of the International Contact Dermatitis Research Group.²⁵ For statistical analyses, test response was dichotomized. All '+', '++', and '+++' reactions were summarized as positive responses, whereas '?', 'ir', and '-' reactions counted as negative responses.

The investigator (H.D.) was blinded with respect to which side of the subjects' back was stripped. Unblinding was done after the last reading.

Statistical analysis

For description, categorical variables were summarized by absolute and relative frequencies, continuous variables by median (minimum, maximum, Q1, and Q3).

As our primary response, we specified the test sensitivity for the different test substances, test methods, and test concentrations. According to standard nomenclature,²⁶ the test sensitivity is defined as the proportion of true positives that are correctly



All statistical analyses were performed with SAS® 9.2 (SAS Institute, Cary, NC, USA). For drawing figures, we used Microsoft® Excel 2000.

In the nickel group ($n = 16$), in median 20 months (min-max, Q1, and Q3: 0–49, 3, and 24 months) had elapsed since positive PT

In Figs 2 and 3, we report on the estimated test sensitivities (with 95% CI) of SPT vs. PT for both allergens. These estimates are adjusted for the previously defined covariates (test methods, days of readings, number of tape strips for the SPT, times elapsed since positive historical PT, reactions in the historical PT at day 3 reading, test concentrations of serial dilutions) and are displayed for an 'average' patient with all of these covariates set to their means. There were no differences between SPT and PT at day 1 reading (data not shown). However, the SPT is more sensitive than the PT at day 3 reading. This difference was highly significant for the test concentrations of 1.0–0.05% nickel sulphate and 0.1–0.05% potassium dichromate, shown by non-overlapping confidence intervals for the respective concentrations.

Table 1 PT and SPT results with serial dilution tests in 30 nickel- and/or dichromate-sensitive subjects at day 3 reading

Subject no.	Sex	Age (year)	Pre-sensitization/ Serial dilution test	Method	Taps strips (n)	Test concentration (aq.) and result at day 3							Quotient of elicitation thresholds (PT/SPT)
						1.0%	0.5%	0.1%	0.05%	0.01%	0.005%	0.0005%	
1	F	50	Ni	PT		++	+	+	–	NT	–	NT	2
				SPT	13	++	++	+	+	NT	–	NT	
2	F	40	Ni	PT		+	+	–	–	NT	–	NT	10
				SPT	17	+++	+++	++	++	NT	–	NT	
4	F	58	Ni	PT		+	–	–	–	NT	–	NT	2
				SPT	13	++	++	–	–	NT	–	NT	
5	F	36	Ni	PT		?	?	?	?	NT	–	NT	100*
				SPT	14	++	++	++	+	NT	–	NT	
6	F	62	Ni	PT		?	–	–	–	NT	–	NT	100*
				SPT	11	+	+	+	+	NT	?	NT	
7	F	47	Ni	PT		+	+	–	–	NT	–	NT	nc
				SPT	15	–	–	–	–	NT	–	NT	
9	F	38	Ni	PT		–	–	–	–	NT	–	NT	10*
				SPT	14	++	++	–	–	NT	–	NT	
11	F	45	Ni	PT		–	–	–	–	NT	–	NT	50*
				SPT	21	++	++	+	?	NT	–	NT	
14	F	39	Ni	PT		–	–	–	–	NT	–	NT	5*
				SPT	10	+	–	–	–	NT	–	NT	
18	F	43	Ni	PT		++	+	–	–	NT	–	NT	100
				SPT	15	++	++	+	+	NT	+	NT	
22	F	58	Ni	PT		+	?	–	–	NT	–	NT	20
				SPT	16	+	+	?	+	NT	–	NT	
25	F	50	Ni	PT		++	+	?	–	NT	–	NT	10
				SPT	10	++	++	+	+	NT	–	NT	
26	F	51	Ni	PT		+	?	–	–	NT	–	NT	20
				SPT	27	++	?	++	++	NT	–	NT	
3	M	71	Cr	PT		NT	NT	+	+	–	–	–	10
				SPT	10	NT	NT	++	++	+	+	–	
8	M	57	Cr	PT		NT	NT	+	?	–	–	–	10
				SPT	26	NT	NT	++	+	+	?	–	
10	M	60	Cr	PT		NT	NT	–	–	–	–	–	0
				SPT	11	NT	NT	?	–	–	–	–	
12	F	40	Cr	PT		NT	NT	–	–	–	–	–	0
				SPT	7	NT	NT	–	–	–	–	–	
13	F	55	Cr	PT		NT	NT	–	–	–	–	–	5*
				SPT	15	NT	NT	+	?	–	–	–	
15	F	58	Cr	PT		NT	NT	++	+	–	–	–	10
				SPT	17	NT	NT	++	++	++	+	–	
16	F	71	Cr	PT		NT	NT	–	–	–	–	–	5*
				SPT	16	NT	NT	+	–	–	–	–	
17	F	68	Cr	PT		NT	NT	–	–	–	–	–	0
				SPT	13	NT	NT	–	–	–	–	–	
19	M	51	Cr	PT		NT	NT	+	–	–	–	–	10
				SPT	17	NT	NT	++	++	+	?	–	
20	F	61	Cr	PT		NT	NT	+	–	–	–	–	2
				SPT	20	NT	NT	+	+	–	–	–	
21	F	48	Cr	PT		NT	NT	–	–	–	–	–	5*

Table 1 Continued

Subject no.	Sex	Age (year)	Pre-sensitization/Serial dilution test	Method	Taps strips (n)	Test concentration (aq.) and result at day 3							Quotient of elicitation thresholds (PT/SPT)
						1.0%	0.5%	0.1%	0.05%	0.01%	0.005%	0.0005%	
27	F	42	Cr	SPT	12	NT	NT	+	-	-	-	-	10*
				PT		NT	NT	-	-	-	-	-	
29	F	39	Cr	SPT	28	NT	NT	+	+	-	-	-	10*
				PT		NT	NT	?	-	-	-	-	
30	F	46	Cr	SPT	15	NT	NT	+	+	-	-	-	5
				PT		NT	NT	++	+	-	-	-	
23	F	64	Ni	SPT	13	NT	NT	++	++	+	?	-	10*
				PT		-	-	-	-	NT	-	NT	
			Cr	SPT	18	-	+	-	?	NT	-	NT	20
				PT		NT	NT	+	?	-	-	-	
24	F	52	Ni	SPT	18	NT	NT	++	++	+	+	-	5
				PT		++	+	-	-	NT	-	NT	
			Cr	SPT	12	++	++	++	-	NT	-	NT	5
				PT		NT	NT	++	+	-	-	-	
28	M	28	Ni	SPT	12	NT	NT	++	++	+	?	?	5
				PT		+	+	-	-	NT	-	NT	
			Cr	SPT	19	++	++	+	-	NT	-	NT	5*
				PT		NT	NT	-	-	-	-	-	
				SPT	19	NT	NT	+	-	-	-	-	

Ni, nickel sulphate; Cr, potassium dichromate; NT, not tested; nc, not counted, denominator with zero value.

*numerator with earlier positive result to diagnostic test concentrations of nickel sulphate 5.0% pet. and/or potassium dichromate 0.5% pet. as reference value.

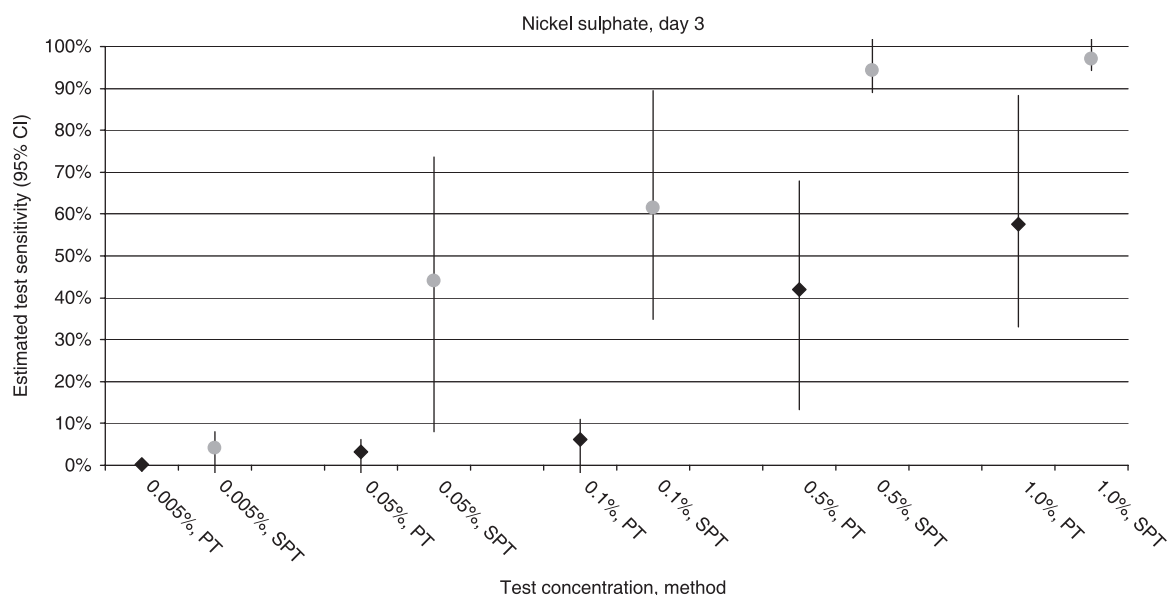


Figure 2 Adjusted estimated test sensitivities for serial dilutions of nickel sulphate at day 3 reading: PT (black square) vs. SPT (grey circle).

In Tables 2 and 3, we calculated the quotient between the estimated test sensitivity of SPT and the estimated test sensitivity of PT at day 3 reading. For each test concentration and for both test substances, the sensitivity of the SPT is always significantly larger than those of the PT, as

the CI never includes the 1. It can also be seen that the lesser the concentration of the test substance, the more superior SPT vs. PT becomes.

Considering the estimates for the influence of the other covariates in our logistic regression model, there were no significant effects

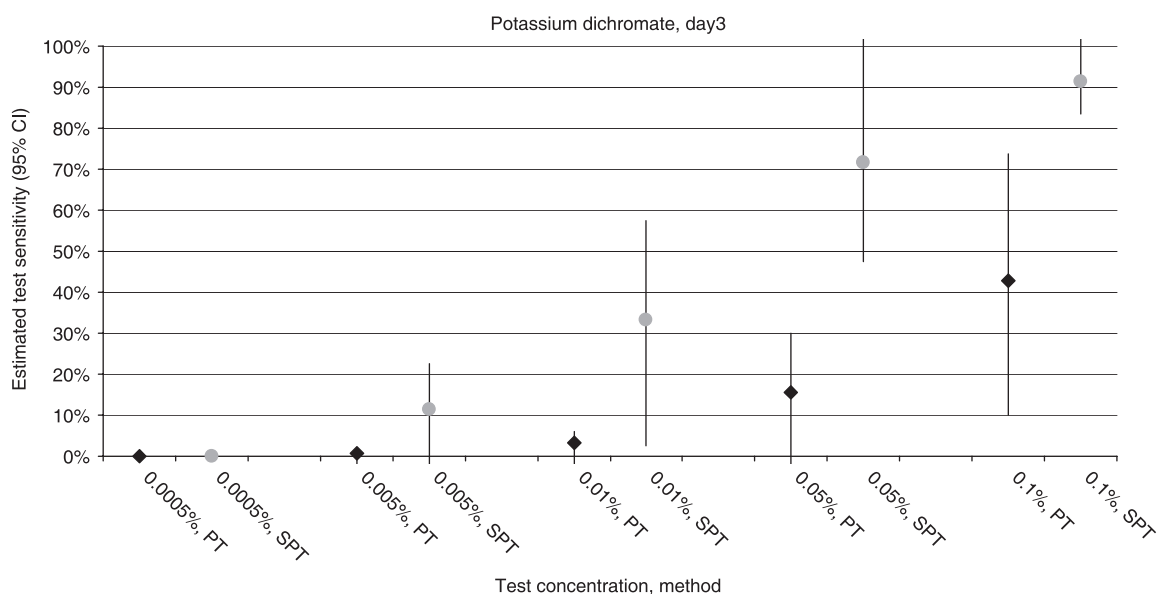


Figure 3 Adjusted estimated test sensitivities for serial dilutions of potassium dichromate at day 3 reading: PT (black square) vs. SPT (grey circle).

Table 2 Sensitivity quotient (estimated test sensitivity of SPT/estimated test sensitivity of PT) for serial dilutions of nickel sulphate at day 3 reading

Nickel sulphate, day 3		
Test concentration	Sensitivity quotient	95% CI
1.0%	1.7	1.2–3.6
0.5%	2.2	1.4–5.4
0.1%	9.6	3.4–46.5
0.05%	13.6	3.9–78.2
0.005%	23.6	5.7–159.2

Table 3 Sensitivity quotient (estimated test sensitivity of SPT/estimated test sensitivity of PT) for serial dilutions of potassium dichromate at day 3 reading

Potassium dichromate, day 3		
Test concentration	Sensitivity quotient	95% CI
0.1%	2.1	1.3–6.6
0.05%	4.6	2.3–37.3
0.01%	10.1	4.7–78.4
0.005%	13.0	5.9–114.3
0.0005%	14.7	6.4–138.4

to observe for either the number of tape strips for the SPT, the time elapsed since positive historical PT, or the final reaction in the historical PT (data not shown).

Discussion

In this study, we evaluated exemplarily differences in test results to serial dilutions of nickel sulphate and potassium dichromate between our proposed SPT procedure¹⁹ and conventional PT in subjects known to be sensitized towards the standard patch test substances nickel sulphate 5.0% pet. and potassium dichromate 0.5% pet., respectively. The basic idea of the SPT is to increase the bioavailability of a test substance in the deeper epidermal cell layers thus reducing the test concentration eliciting a skin reaction. This is said to be achieved by thinning the stratum corneum (mechanical reduction of corneocyte layers) via stripping the test site several times with an adhesive tape prior to the application of the test substance.^{3,30–32}

Using the 'subtotal' strip with an average strip thickness of 0.45 µm, Spier and Sixt² required 12–15 strips from the back to

reduce the stratum corneum by approximately less than or equal to 50%. Almost always, they were able to reduce the minimum test concentration eliciting a skin reaction of most allergens tested (chromate, mafenide, novocaine hydrochloride, benzocaine, and turpentine; but not mercuric chloride) to lesser than one thirtieth of the routine test concentration, or, in their words, increase the 'sensitivity' by a factor of 30 and more. However, the concentration eliciting a skin reaction additionally depends on the penetration characteristics of the allergen.³³

On the basis of patch tests with serial dilutions of different test substances (unspecified) on skin with a 50% reduced stratum corneum by tape stripping (half of tape strips that were necessary for reaching the stratum lucidum as the individual visual reference point), Frosch *et al.*⁷ reported an increased 'sensitivity' in SPT by a factor of as many as 10 with a range from 5 to 20. Skin reactions in SPT were thereby consistently stronger than in PT.

Unfortunately, elicitation thresholds and test sensitivities have been used interchangeably in these previous publications.^{2,7} According to our results, the elicitation thresholds in SPT vs. PT

at day 3 of nickel sulphate and potassium dichromate could be lowered in median by a factor of 10 and 5, respectively. In SPT, we observed more and comparably stronger skin reactions in both serial dilution tests (as an example Fig. 1). However, all these effects were not visible at day 1 (data not shown).

It is interesting to note that in SPT, the median number of tape strips was 15 in both serial dilution tests, with a broad interindividual variation of 10 to 27 tape strips in the nickel group and 7 to 28 tape strips in the dichromate group, respectively. This broad interindividual variation was similarly reported by Frosch *et al.*⁷ Given the fact that these interindividual different numbers of tape strips had statistically no effect on our estimated test sensitivity, we feel vindicated in our assumption that a fixed, patient-independent defined number of tape strips for the SPT might not be appropriate.⁶ The number of tape strips varies depending on the patient's skin and ambient conditions at the day of testing,³⁴ most likely even intra-individually. This highlights our proposed SPT procedure,¹⁹ in which we claim to be independent of ambient conditions, skin conditions, and technical equipment (except for type of adhesive tape).

As limitation of the study, we did not retest with the standard patch test substances nickel sulphate 5.0% pet. and potassium dichromate 0.5% pet. Deciding reason was to avoid boosting test reactions to the dilution series or increasing cumulative application rates over the recommended patch test concentrations because of possible irritant and toxic effects, respectively, particularly with regard to potassium dichromate. Therefore, we cannot make a point concerning the reproducibility of the historical PT results. The reliability of sequential patch testing is measured by its reproducibility, which cannot assumed to be 100%.³⁵ In patch testing with nickel sulphate 5.0% pet. and potassium dichromate 0.5% pet., respectively, reproducibility is reported to be $\geq 80\%$ independent of test intervals from 9 months to 13 years.³⁶ In our study, all nickel-sensitive subjects reacted at day 3 at least to one of the serial dilutions in SPT and PT, respectively, whereas three dichromate-sensitive subjects reacted not to any of the serial dilutions neither in SPT nor in PT. With the serial dilution test in the nickel group, we observed 94% of the sensitizations by SPT vs. 63% by PT and in the dichromate group, respectively, 82% of the sensitizations by SPT vs. 47% by PT. However, it is worth noting: As we only included truly sensitized subjects, every estimated test sensitivity smaller than 100% (cf. Figs 2–3) indicates a failure of the test at the respective test concentration because sensitized patients would be missed by the test. Finally, in our statistical model, time elapsed since positive historical PT in the overall range from 0 to 5 years showed likewise no effect on the estimated test sensitivity.

In conclusion, we could hereby show an actual increase of test sensitivity of SPT vs. PT with the help of serial dilution tests of nickel sulphate and potassium dichromate, respectively. We believe that stripping the skin of the back according to our proposed uniform and practical procedure¹⁹ may have the potential to be a

second step after PT in practice. If no positive results are obtained by PT, the SPT could help to detect hidden allergens; especially when a weak sensitization is present or substances with a low concentration and poor percutaneous penetration, respectively, need to be tested. However, it is yet unknown if this enhanced sensitivity of the SPT comes at the cost of a diminished specificity. Therefore, in an ongoing multicentric validation study, we determine and compare sensitivity, specificity and the corresponding predictive values of the SPT and the PT to detect type IV sensitization in patients with a suspected allergic contact dermatitis by using 'anamnestically present sensitization: Yes/No' as the reference standard.⁶

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