

“New” techniques for more sensitive patch testing?

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Summary

The patch test is the mainstay of diagnosis in allergic contact dermatitis. In its nearly 120-year-long history, numerous efforts to standardize several methodical aspects proved successful. The aim was always to enhance the validity and reliability of patch testing. This article reviews modifications of the basic patch test technique for enhancing test sensitivity. We expand on the recently validated strip patch test, which is a modification that involves affixing tape strips to the skin and removing them prior to patch testing.

Although it was first described in 1953, we proposed the first protocol for standardized performance of the “strip patch test.” The protocol was then tested in studies. As a result, test sensitivity of standardized strip patch test versus patch test was increased. The protocol produced a uniform reduction in the thickness of the stratum corneum and a good inter-rater agreement. Additionally, there was evidence of a non-specific stimulation of the inflammatory epidermal system. With regard to patient history, the standardized strip patch test showed a vastly better sensitivity than the patch test with only marginally lower specificity. As a complementary measure to patch testing diagnostic accuracy could be improved significantly by performing the standardized strip patch test. Further studies are needed on the reproducibility of the standardized strip patch test.

Patch tests

At the congress of the German Society of Dermatology in Graz in 1895, Josef Jadassohn introduced the technique of “functional skin testing” [1]. Since then, the basic method of patch testing has remained largely unaltered [2].

Today, the patch test is considered the “gold standard” in the diagnosis allergic contact dermatitis, a form of delayed hypersensitivity [3]. Various studies have shown that early diagnosis of allergic contact dermatitis can have a positive influence on the costs as well as the course of disease and quality of life [4, 5].

Guidelines and recommendations

Over the years, continual efforts have been made to standardize patch testing

procedures, improve the reliability and validity of the method, and enable a comparison of test results; attempts include standardizing terminology, the test substances and concentrations used, the application site and duration of the test, time elapsed until test reading, and the evaluation of reactions [6, 7]. A comprehensive review of these and other patch testing methods has been recently published by Lindberg and Matura [8].

The German Contact Dermatitis Research Group (DKG) has published a set of guidelines on standardized methods for patch testing with contact allergens [9] which are now available in their revised form [10]. The basic method for performing a patch test consists of placing various test substances in small

chambers (the most common of which is Finn Chambers® on Scanpor® tape [3]) and affixing them to the untreated, normal skin of the back (2–4 cm from the spine) where they are left in place, under occlusion, for 24 or 48 hours. The first reading of results is done the day the patch is removed. The reaction should be assessed no earlier than 30 minutes after removing the patch. Further readings are done 72 or 96 hours after application of the patch. For some patients, another later reading may be advisable, e.g., after 168 hours. The DKG recommends scoring the results at 72 hours or later [11] as follows: “+”, “++” or “+++” (positive/allergic).

Although the sensitization profile does not differ significantly between children

and adults, for children between 6 and 12 years of age, it is advisable to restrict testing to 12 substances in a standard series (<http://www.ivdk.gwdg.de/dkg/dkgblo.html#a002>, September 2010) [12]; testing of additional substances is only recommended if warranted by the patient's medical history. In pediatric patients, the patch should be removed 24 hours after application in order to reduce the chance of irritation. Readings and evaluations of the test reaction are the same as for adults. Children under 6 years of age should only undergo testing if there is a strong suspicion of allergy and only with the suspected contact allergens. In children over 12 years of age, the procedures are roughly the same as for adults [10].

Reliability and validity

The patch test method has been the subject of numerous studies, mainly due to its controversially discussed reliability [13–15]. The reproducibility of results of simultaneous double-testing varies by study and test substance, but ranges between 40 and 92 % [16–19]. The reproducibility for consecutive patch tests is similar, also ranging between 40 and 92 % [16, 20–25]. There have been reports, for example, that positive patch test results serving as the basis for dermatologists' reports on the presence of an occupational disease could not be reproduced. In a study by Prott [26], out of 58 reported positive patch tests only 16 could be confirmed, which corresponds to a very low rate of 28 %.

The validity of patch tests is also limited [2, 13, 14]. Thus the results of patch testing are not always considered reliable, certain, or usable [27]. There are only a very few published, standard indices for measuring the validity of the patch test. The first of these was published in 1989 by Nethercott and Holness [28]. Based on the patient's medical history (external criterion), the authors assessed the validity of North American and international standard patch test series. The reported sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) was 77 %, 71 %, 69 % and 78 % versus 68 %, 77 %, 66 % and 79 %. The sensitivity and specificity of the patch tests was thus generally presumed to be between 70 % and 80 % [3, 29, 30]. In a 2005 multicenter study with 1 701 patients, Frosch and colleagues [31] tested two test substances

from the European standard series of allergens – fragrance mix I (FM I) 8 % pet. and fragrance mix II (FM II) 14 % pet. The reported sensitivity, specificity, PPV, and NPV were 25.2 % (FM I) and 13.5 % (FM II), 96.5 % (FM I) and 98.8 % (FM II), 45.1 % (FM I) and 55.6 % (FM II), and 91.8 % (FM I) and 90.8 % (FM II). Compared with the question-based external criterion, which was assessed for all patients prior to testing, the sensitivity and PPV for individual test substances were thus shown to be much lower than was originally believed.

Test-substance dependent reaction evaluation

Analyses of patch test results that have been done on large patient samples with the same test substance preparations have shown that the profile and dynamic development of skin reactions vary significantly depending on the substance being tested. Different substances produce different types of reactions of varying strengths, including irritant, equivocal, weak, and strong positive reactions. Such characteristics are assessed by the reaction index [32] and the positivity ratio [33]. Such indices enable a comparison of various test substances at a glance and are thus gaining wider use in Germany and internationally for describing and analyzing the results of patch tests [34, 35]. The results of such analyses clearly demonstrate that patch tests results must be evaluated in terms of the specific substance being tested. Broad generalizations on “patch testing” are of little use.

To help identify skin irritation, the DKG also recommends simultaneous testing of sodium lauryl sulfate (SLS) 0.25 % aq. as the reaction to this irritant can serve as a guide to the overall sensitivity of the skin and the type of response elicited [36].

Modifications in patch testing

The ability of the test substance to penetrate the skin depends on several factors which inevitably vary from test to test. Various attempts have been made at modifying the patch test, primarily in order to increase the sensitivity of the skin and thus enhance the reliability and validity of the results [37].

1. Modifications suggested at the beginning of the last century included “rubbing the skin with abrasive pa-

per”, “scarification”, or “scratching with a sharp spoon” prior to testing [38]. Given the risk of sensitization, however, these methods were quickly abandoned.

2. Haxthausen [39] proposed an “electrophoretic patch test” in 1947 which is one of the more difficult modifications. This method used galvanic electricity to introduce the test substance into the skin. The procedure never gained much recognition [40], but recently a study with nickel (II) sulfate showed that iontophoresis of nickel can eliminate the need for affixing test chambers [41]. The procedure is considered too technically complex for use in routine practice and its use with non-ionized haptens is uncertain.
3. In their study on chromate allergy and cement dermatitis, in 1953 Spier and Natzel [42] proposed the “strip patch test” using 0.1–0.001% potassium dichromate solution for early and uncomplicated diagnosis of even mild sensitization to chromate. Later Spier and Sixt [43] conducted systematic studies on the relationship between the results of patch testing and the thickness of the stratum corneum (SC). The authors suggested that increased sensitivity to the patch test was due to the reduced thickness of the SC after application and removal of tape strips as this diminished the inhibitory effect of the SC on the skin reaction [44].
4. In 1954 Fernström [45] proposed the “pressure-patch-test” in which a plastic sponge was used to apply pressure to the skin in order to enhance contact with the test substance and facilitate its penetration. This modification of the patch test increased the number of positive results. Yet the use of more than 5 mm thick plastic sponges, or even harder devices, also traumatized the skin and this in turn led to unspecific false-positive reactions [37, 46]. Hornstein and Kienlein-Kletschka [47] further modified the “pressure-patch-test” by placing a 10 kg sack of sand on the back of the prone patient for 15 minutes after applying the patch to the patient's back. In this small study, they reported that in 50 patients there were more positive reactions than on patch testing, and in 13 patients there were also

more positive test reactions than after the “strip patch test” method.

5. In 1964 Burckhardt and Schmid [48] suggested a relatively time-consuming open patch testing method involving repeated contact with the causative substance in solution. This is intended to simulate actual occupational contact. The procedure consisted of applying the test substance repeatedly to a round area of healthy skin measuring 5 cm in diameter using a cotton swab. Usually a concentration was used that was slightly more than the usual concentration used. The solution was applied every 30 seconds, and, depending on the substance the total test took 10, 20, or 30 minutes. If needed the test was repeated after 24 and 48 hours. The process of swelling, related to an increased penetration of the test substance, is probably the reason why the last application came directly in contact with the living epidermis in an effective concentration and thus elicited a positive test result. The limitation of the test is that open patch testing requires that the substance be suitable for use as a solution.
6. In 1977 Hannuksela and Lahti [49] introduced the “scratch patch test” (SCT; scratch chamber test) for testing primarily plain protein-based extracts (e.g., foodstuffs). To increase the sensitivity of the patch test, the lancet is used to produce a 0.5–1.0 cm long non-bleeding scratch prior to application of the test substance. This test is difficult to standardize and reproduce, however, and the sensitivity is low; given these drawbacks it is not advised in routine clinical practice [50, 51].
7. To avoid artifacts to due application of relatively high concentrations of test substances under occlusion, Hannuksela and Salo [52] developed the “repeated open application test” (ROAT) in 1986 which is supposed to approximate actual exposure conditions. The basic principle is to expose an area of the skin on the medial aspect of the proximal lower arm for 1–2 weeks or more – until there is an eczematous skin reaction – twice daily to the usual concentrations of the test substance, without occlusion [53]. ROAT can be very helpful in certain situations such as when the clinical

relevance of a patch test is questionable. Yet its use is impractical for rapid patch testing of numerous substances (e.g., as commonly done with the DKG standard series). In addition, a conclusive standardization and validation are not yet available [52, 53].

8. Another recently proposed method of increasing the sensitivity of patch tests is to apply the detergent SLS along with the test substance. In 1994 Seidenari [54] reported a 30-minute-long pre-treatment of the test site with SLS 5 % aq., followed by 24-hour pre-treatment of the test site with SLS 0.5 % aq. if a false negative result was suspected [55]. Later suggestions for simultaneous application of SLS 0.25 % or 0.5 % aq. with a test substance [56, 57] were based on an earlier study by Kvorning and Svendsen [58]. Based on the results, it seems that simultaneous application of SLS can reduce the test substance concentration needed for eliciting a reaction. Yet neither approach has been adequately standardized and the preparation of test substances is made much more complicated by simultaneous application with SLS.
9. Earlier suggestions by Jacques and colleagues [59] for using laser to reduce the SC thickness prior to patch testing and thereby increase the test substance penetration, were expounded upon by Veremis-Ley and colleagues [60] who first published results of the procedure in 2006. Although the test substances were applied for only one hour to the laser-treated area of the skin, known delayed hypersensitivity was confirmed in several patients and in once patient new, clinically relevant delayed hypersensitivity was diagnosed. The authors did not report any skin irritation or pain associated with the “laser patch test.”

Of all of the above-named modifications, the only one that is currently widely used in routine diagnosis of allergic contact dermatitis is the “strip” patch test [61–63]. It is not used routinely, however, mainly because it is more time-consuming than traditional patch testing given the need to apply adhesive tape strips before testing, and given lacking standardization [64].

The “strip” patch test

The basic method for tape stripping was first proposed in 1939 by Jan Wolf [65].

Wolf used the method as an aid to histological study of morphological details of the SC. Spier and colleagues [42, 43] later combined application and removal of adhesive tape with patch testing and then later applied this to studies on the relationship between patch test results and SC thickness. The authors recommended applying and stripping 10–15 adhesive strips to the test site prior to application of the test substance. Used on the back, this led to a reduced SC thickness of up to 50 %. The reaction-inducing concentration of the test substance could thus be reduced by more than a 30th of the standard test concentration (the amount varied by substance); in other words, test sensitivity was 30 times or more higher than with patch testing. Given the increased intensity of contact between the test substance and the epidermis due to the reduction of the SC, occlusion for more than 24 hours is unnecessary.

The use of this method is now advised if a false negative patch test result is suspected [64]. Yet the literature still does not contain any recommendations on practical and standardized methods for the “strip” patch test, but merely a few general tips on procedure. Differences in the method include using various types of adhesive tape and different numbers of tape strips in different patients.

Proposal for standardization

Under the auspices of the DKG, a pilot study was conducted which served as the basis for a proposal on standardized procedures for the “strip” patch test [66]. The proposal for standardization (Figure 1) involves first determining optically, i.e., without technical measurement, the number of adhesive strips needed to reach the individual endpoint (i.e., to reach the stratum lucidum – layer where the skin “glistens” – with complete removal of the SC) and then calculating the individual number of tape strips needed for the “strip” patch test. This method is largely independent of the skin features and surrounding conditions.

Test sensitivity

The increased test sensitivity of the standardized “strip” patch test compared with the traditional patch test has been shown by with dilution series of the test substances nickel (II) sulfate (1.0 %, 0.5 %, 0.1 %, 0.05 % and 0.005 % aq.)

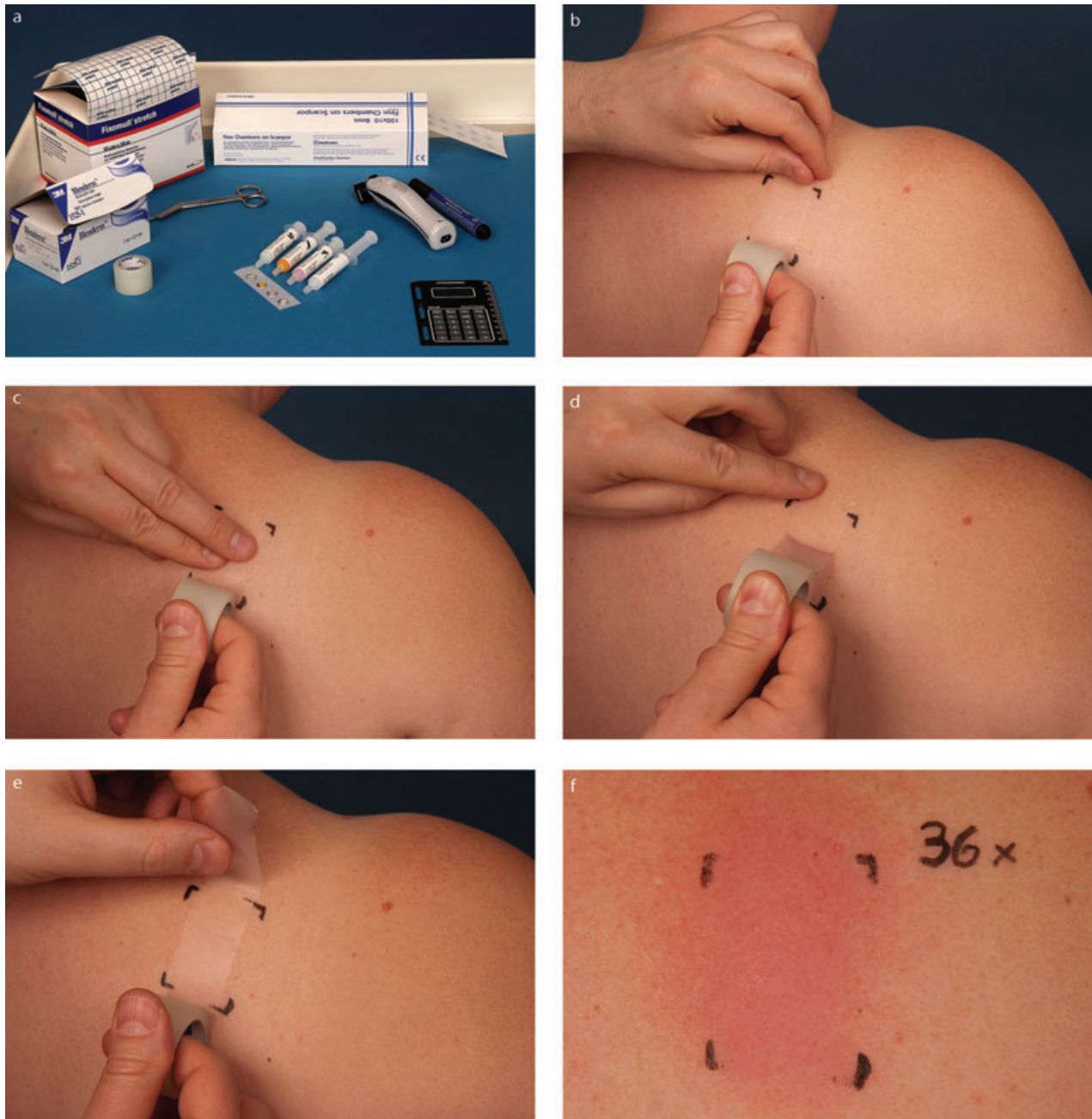


Figure 1: Protocol for standardized performance of the strip patch test [66]:

1. 3M™ Blenderm™ surgical tape (width 25 mm) is used. If hair removal is necessary, clippers are used (e. g., 3M™ surgical clipper). Set of needed test materials (a).
2. Stripping of the healthy skin at one upper part of the back is done until the stratum lucidum is reached, i. e., the surface shows three small glistening spots. Tape is vertically (parallel to the spine) applied onto the skin without tension (b). Tape is smoothed out and gently pressed downward by fingertips for about 2 seconds (c). Tape is removed in one quick movement at an angle of 45° in the direction of adherence (d). For each single strip a new tape cut is used and positioned on exactly the same skin area (e).
3. The number of tape strips required to produce three small glistening spots is multiplied by the tape-specific correction factor $cf = 11/26 \approx 0.42$ (f).
4. The calculated number of tape strips is applied contralateral and/or lateral to spine: Tape strips are performed as specified by the Items 2 (g). The filled patch test device (e. g., Finn Chamber® on Scanpor® tape) is then applied and fixed (h). Patch test is removed after 24 hours. Test results are read according to the guidelines of traditional patch testing [10].

and potassium dichromate (0.1 %, 0.05 %, 0.01 %, 0.005 % and 0.0005 % aq.) [67]. A lower concentration of the test substance necessary for eliciting a reaction was considered to be evidence of an increased sensitivity to the test for

each substance. Use of the standardized “strip” patch test allowed a median reduction in the necessary concentration for eliciting a reaction to nickel (II) sulfate by a factor of 10 and to potassium dichromate by a factor of 5.

The increased test sensitivity of the standardized “strip” patch test is primarily due to acute damage of the permeability barrier of the epidermis given the reduced thickness of the stratum corneum. The level of reduction of the SC strongly

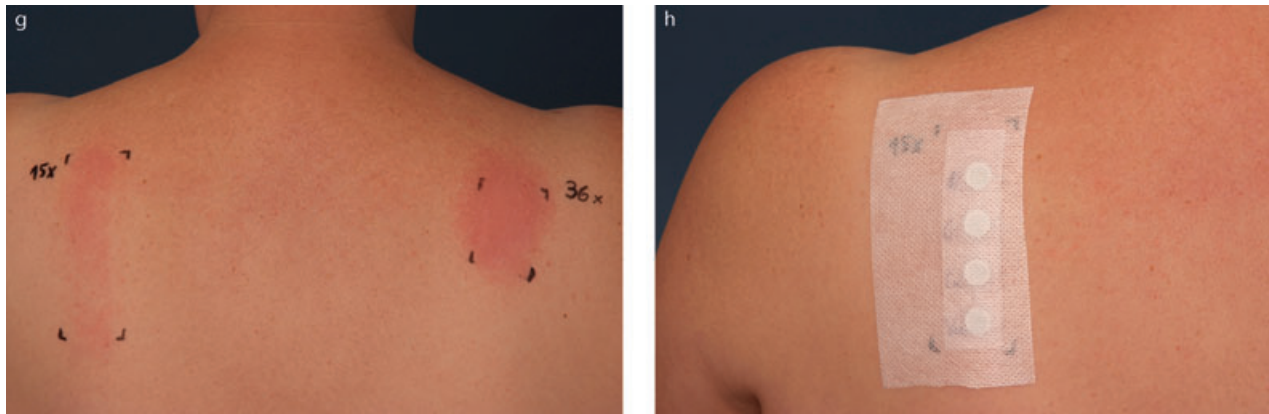


Figure 1: Continued.

determines how much of the test substance reaches the living epidermis [68]. The “strip” patch test method reduced the thickness of the stratum corneum on average by 31.3 %; 95 % of all SC thickness reduction values were between 22.5 and 40.0 % (normal distribution) [69]. This level of standardization and comparability of the SC thickness reduction is sufficient given the varying numbers of tape strips used in different patients in routine allergy diagnosis. This method also demonstrated a good level of “inter-rater reliability.”

The increased sensitivity of the “strip” patch test is not attributable only to the acute damage of the epidermal permeability barrier as a result of targeted reduction in SC thickness, but rather it is also the result of a direct influence on the epidermal immune barrier due to unspecific activation of keratinocytes [70]. In one study, epidermal mRNA expression of TNF- α , IL-33, Hsp70, Hsp90, and IL-8/CXCL8 at the test site 6 hours after the “strip” patch test was significantly increased compared with an untreated area; epidermal mRNA expression of CCL5/RANTES was significantly reduced [71]. Thus it appears that stripping of the adhesive tape “alarms” the keratinocytes which are responsible for various effector mechanisms that subsequently trigger a cutaneous inflammatory process, which in turn may be responsible for the increased reactivity of the skin or allergenicity of the test substance in the standardized “strip” patch test versus the patch test [72]. Yet whether these mediators more strongly mediate an irritant reaction or an allergic reaction is still unclear given lacking specificity [73].

Validity

In an investigator-blinded multicenter study with 787 patients with a history of sensitization (reference standard), the standardized “strip” patch test was compared with the patch test for sensitivity, specificity, PPV, and NPV for the test substances nickel (II) sulfate 5 % pet., potassium dichromate 0.5 % pet., and wool wax alcohol 30 % pet. The differences in the sensitivities of the standardized “strip” patch test and the patch test were 16.4 % (95 % CI: 8.7–24.1 %) for nickel (II) sulfate and 25.0 % (95 % CI: 8.9–41.0 %) for potassium dichromate [74]. The specificity of patch testing was slightly better: 3.3 % (95 % CI: 1.7–5.0 %) for nickel (II) sulfate and 2.9 % (95 % CI: 1.5–4.3 %) for potassium dichromate. This means that, bearing in mind that all patients had a history of delayed hypersensitivity (a reference standard that cannot be “perfectly” defined) [75], of 100 patients with nickel or chromate allergy, the patch test missed 16 (nickel) and 25 (chromate) more sensitized patients than were identified by the “strip” patch test, while among 100 patients with no sensitization to nickel or chromate, the “strip” patch test incorrectly identified 3 patients more than patch testing as being sensitized. The moderate to poor PPV for nickel (II) sulfate of about 54 % and potassium dichromate of around 26 % does not differ significantly between the two tests. This underscores the fact that for routine clinical allergology, patch testing, with or without the aid of prior application and stripping of adhesive tape, ultimately rests on a careful taking of the patient’s medical history [29, 76]. In some in-

stances it must be further augmented by determining the test concentration threshold and by use tests (ROAT and its variants [77]). Compared with patch testing, the standardized “strip” patch test had a very good NPV for nickel (II) sulfate of around 92 % and potassium dichromate of around 98 %. Compared with nickel (II) sulfate and potassium dichromate, the rate of reactions to wool wax alcohol is much lower [78]. A much larger patient sample would be needed in order to draw any conclusions on this substance. In summary, the standardized “strip” patch test was shown to be a safe method that produces valid test results.

Clinical uses

The standardized “strip” patch may be recommended in current routine allergy diagnosis in the following situations:

1. In patients with a prior diagnosis of delayed hypersensitivity in whom a patch test fails to confirm sensitization [79],
2. In a negative or questionably positive patch test with continued suspicion of delayed hypersensitivity [80],
3. For testing a contact allergen with low permeation of the SC (e.g., metal salts, heparins) [74, 81, 82] or with a low test concentration [67] (e.g., patient’s own eye make-up),
4. If the penetration ability of the contact allergen (e.g., dental metals, ophthalmic products) on the back (test site) is lower than on the organ with which it comes into contact [61, 83, 84],
5. When the use of repeated stripping of adhesive tape is intended to simulate extremely sensitive or damaged skin

(e.g., hands) using an area on the back (test site) [85, 86].

In-vitro tests

For nearly 120 years [1] the largely unchanged patch test has remained the “gold standard” for detecting delayed hypersensitivity in diagnosing allergic contact dermatitis. In-vitro tests have not yet been sufficiently evaluated for routine clinical use [87, 88].

According to our own research [89], e.g., even advanced flow cytometry analysis of CD69 expression on CD4+/CLA+ T cells [90] is not a viable alternative for diagnosing nickel allergy. Routine allergy diagnosis should still be done using a patch test and based on the patient's medical history; use of the standardized “strip” patch test may serve as an additional, complementary diagnostic aid [89].

Conclusions for clinical practice

For everyday clinical practice, the patch test remains the gold standard for the diagnosis of allergic contact dermatitis. As an “add-on” test, the “strip” patch test, based on a standardized protocol, can serve as a useful diagnostic aid for certain patients. To check allergic test reactions of equivocal clinical relevance, ROAT may be used following either test. <<<

Conflict of interest

None.

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