Diagnostic Methods for Contact Allergy to Metals

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Abstract: The epicutaneous patch testing is regarded as the best method of diagnosis for allergic contact dermatitis (ACD). Patch tests can be used to confirm a suspected allergic contact dermatitis and either to recommend avoidance of particular products or to recommend alternative products in a particular patient. It is based upon re-exposing the skin of the patient to suspected allergens under controlled conditions. Different test sites and test tapes can be used and different variables such as intrinsic penetration capacity, concentration, exposure time and vehicle can be changed to obtain an optimal bioavailability of the haptens. The ideal patch test should cause as few adverse reactions as possible, and be reproducible and specific. In this paper, the application fields, the advantages and the disadvantages of the patch tests are reviewed. Other diagnostic methods as the open test, the provocative test, the repeated open application test and the photopatch test are also discussed.

Keywords: Contact dermatitis, patch test, open application test, provocative test, repeated application test, photopatch test.

1. PATCH TEST

Allergic contact dermatitis (ACD) is a delayed type of hypersensitivity of the skin, for which epicutaneous patch testing is regarded as the best method of diagnosis. It is based upon re-exposing the skin of the patient to suspected allergens under controlled conditions. It is a bioassay that reproduces contact dermatitis. The first epicutaneous tests were carried out by Jadassohn in 1895 [1]. Although it is more than 100 years since the method of application was introduced, it is still the method of choice to establish contact allergy [2, 3].

The present patch test technique is the result of a continuous process of development and improvement since its first application in the late 19th century [4]. Patch testing is only indicated if after history taking and clinical examination, allergic contact dermatitis is suspected. Epicutaneous patch testing is especially indicated if: a) a clear relationship is evident between the dermatitis and certain professional or other activities, b) the dermatitis is confined to the hands or the feet, peri-orbital, around ulcera cruris or peri-anal dermatitis, c) acute and wetting dermatitis of any localization and d) any dermatitis that is therapy resistant, exists for over 3 months or worsens during topical treatment [5].

1.1. Patch Test Tapes and Test Sites

Different patch test units are now commercially available; such as the Finn Chambers or van der Bend square chambers. These test chambers are filled manually. The modern adhesive tapes are acrylate and not colophony based, so the problem of colophony allergy has been eliminated.

Standard patch test allergens are commercially available and have to be chemically defined and pure. The suppliers' recommendations on storage are to be followed to minimize the risk of degradation due to humidity, air or light. Most preparations should be kept in a refrigerator and in the dark; those in diluted liquid preferably in dark bottles. The allergens should not be stored vertically, to prevent sedimentation and concentration changes of the allergens. The test preparation in petrolatum, kept in syringes, is applied directly onto the test chamber. Liquid test preparations are applied *via* a digital pipette to allow exact dosing.

Ready-to-use patch test systems are also available, preloaded by the manufacturer. They eliminate some of the variability in patch testing.

The preferred test site is the upper back, but the outer sides of the upper arms are also acceptable, especially when retesting. Only areas covered by clothing should be used, because some positive reactions may persist for several weeks and occasionally produce hypo- or hyperpigmentation. Removal of hair on the back is sometimes recommended for practical reasons, but it can contribute to the skin irritation. Oily skin can be degreased with a mild solvent, which must evaporate before applying the test strips.

The skin of the back should not be treated with topical corticosteroids for one week before testing. Preferably oral corticosteroids should also be avoided during testing, because they can suppress positive test reactions. The same goes for cytostatics and cyclosporin. During one week before testing the skin should not be irradiated by the sun or artificial ultraviolet sources.

Each test site can be easily delineated with a marking paint, such as gentian violet, felt pens or the nearly colorless "ultraviolet" paint, which shines bright yellow when exposed to black light. Patients should be informed about avoiding excessive exercise, showers, etc. to keep the test system dry.

1.2. Reading of the Patch Tests

The patch test system is usually removed after 48 hrs, as recommended by the International Contact Dermatitis Research Group guidelines [6], and readings are done 20 min

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after removal of the strips (day 2) and after 72 (day 3) or 96 hrs (day 4). For some test series it is preferable to read the tests once more after 7 days, not to miss the late reactions. Gold and certain therapeutic agents such as dermatocorticosteroids and neomycin have the tendency to appear later than reading day 2 or 4 [7, 8]. Patients should be asked to note new positive reactions that arise after the readings at 96 hrs and report them promptly. Occasionally some severe reactions can cause itching and burning, in which case, that patch can be prematurely removed without disturbing the others.

1.3. The Interpretation Method Recommended by the International Contact Dermatitis Research Group

-	Negative reaction
?+	Faint erythema only: doubtful reaction
1+	Nonvascular erythema, infiltration, possibly papules: weak positive reaction
2+	Vesicular erythema, infiltration, papules: strong positive reaction
3+	Intense erythema and infiltration, coalescing vesicles, bullous reaction: extreme positive reaction
IR	Irritant reaction of different types
NT	Not tested

The ?+ reaction is meaningful for an allergic reaction in 1-5%, the 1+ reaction in 20-50% (depending upon the allergen), the 2+ reaction in 80-90% and a 3+ reaction is almost always allergic. This method was developed to make the interpretation standard and easy, but not all types of reactions fit this outline. Irritant reactions are said to be characterized by fine wrinkling ("silk paper"), erythema and papules in follicular distribution, petechiae, pustules, bullae or even necrosis and with minimal infiltration [9]. Irritant reactions are frequent, even to the standard series, because some of the concentrations have been chosen close to the irritancy threshold to diminish the risk of obtaining false-negative reactions. The morphology can differ from mild erythema to bullae. Sometimes it is indistinguishable from an allergic reaction and can be the cause of false-positive test reactions.

1.4. The Allergens

Different variables can be influenced to obtain an optimal bioavailability of the haptens: intrinsic penetration capacity, concentration, exposure time, vehicle and occlusivity of patch test systems.

The penetration capacity depends upon the salt used. For example, there is a significant difference between the penetration of nickel achieved by nickel sulphate and nickel chloride [10]. It is important to find the ideal test concentration. Too high concentrations can cause irritation; too low concentrations are responsible for the false-negative test reactions. False-negative test reactions can also be due to the failure to duplicate the conditions present in the real dermatitis situation. The concentration of an allergen is normally given as a percentage, but in comparative studies with different salts of a substance it is essential to use the same molality [11]. Mostly an exposure time of 48 hrs is chosen and all test strips are removed at the same time.

Although the history and examination of a patient with suspected allergic contact dermatitis give clues to the allergens responsible, it is not enough to test only the initially suspected sensitizers, because the unsuspected frequently turn out to be the real cause of the dermatitis. There are approximately 3,700 currently known allergens [12]. That's why a small number of substances, considered to account for the majority of delayed hypersensitivity reactions, are grouped into a standard patch test series. Bruze *et al.* [4] discussed the requirements to be fulfilled by a sensitizer for inclusion in the standard series. Demands on a sensitizer in the standard series are, being common in the environment, contact allergy rate above 0.5-1.0% in routinely tested dermatitis patients, reliable patch test results, high degree of clinical relevance and minimal adverse effects, particularly patch test sensitization. Generally 20-30 test preparations are grouped in a standard test series, which consist of chemically defined compounds, mixes of allergens, both natural and synthetic. These series are revised frequently to adapt to changes in exposure and the introduction of new allergens onto the market and one should always remain critical of the contemporary composition of the standard series. Minor variations are due to differences in culture, industrialization and use in different countries [13]. Testing with the test preparations in a standard series is said to detect 70 to 80% of all contact allergies [14]. The European Environmental and Contact Dermatitis Research Group detected by the standard series hypersensitivity with a range from 37 to 73% of all contact allergies [6].

To evaluate the significance of specific exposures, different specific screening series are available. They can be divided into different categories based upon the occupation of the patient (e.g. hairdressing or bakery series), the localization of the dermatitis (e.g. shoe series), series of chemically related compounds (e.g. the acrylates or epoxy series) or functionally related compounds (e.g. corticosteroid series, cosmetics series). Overlapping occurs since many chemicals are present in several unrelated compounds.

Mixes of four or five closely related chemicals are used to save time and space while patch testing. In the past several different mixes of allergens have been tried in patch tests, but at present the most standard series contain mixes of "caine" anesthetics, parabens, fragrances and rubber chemicals. Caution must be observed in the exact composition of the mixes. The concentration of each chemical has to be sufficient. A sensitive patient can show a negative reaction to a mix, but a positive reaction to one of its ingredients tested separately, because the concentration of the ingredient in the mix is insufficient. The combination of the substances in the mix may not cause chemical reactions deactivating one of the ingredients or induce irritation to the skin. In cross-sensitivity, contact allergy caused by a primary allergen is combined with allergic reactions to other, chemically closely related substances.

Products or materials brought by the patient and suspected of causing dermatitis should be tested with great caution. First of all it should be stated that totally unknown products should never be applied to human skin. Therefore it is recommendable to start with an open test, to minimize the risk of severe irritancy. If this is negative, occlusive patch

testing can take place, usually starting at the lowest concentration and rising if the preceding test is negative. Depending on the likely irritant or sensitization potential of the product, it is recommended to start with concentrations of 0.001% or 0.01%. The ideal vehicle and test concentration for each product or chemical compound is difficult to discover, but help can be found in the literature.

When solid products such as textiles, paper, rubber, plants or synthetics are suspected, it can be tested as thin, regular-sided, smooth sheets or extracts can be obtained by placing a sample of the material in water, synthetic sweat, ethanol or ether, and heating it up to 50 °C [15]. For most products intended for use on normal or damaged skin such as cosmetics, detergents, topical medicaments, etc. open tests and use test give probably more information on the pathogenesis of the patient's dermatitis than an occlusive patch test does [16].

1.5. Vehicles

The skin is directly in contact with environmental molecules which are present in the air or directly in contact with the epidermis. Despite the assumption that it has a barrier role which could prevent the penetration of molecules, the skin is permeable to a lot of substances. The degree of permeability varies depending on the physiological state of the skin and the chemical properties of molecules [17].

Each allergen has its own optimal vehicle. Generally, enhanced thermodynamic activity increases percutanous absorption, hence improving the solubility of a substance in its vehicle may render results in hitherto negative or unclear patch tests [18]. White petrolatum is the most widely used. It gives a good occlusion and keeps the allergens stable, but it can retain the allergen and irritate and even sensitize the skin [19]. Liquid vehicles such as water or solvents facilitate the penetration, but they evaporate, and this interferes with exact dosing. Most test solutions with liquid vehicles must be freshly made. When using other, more sophisticated vehicles, containing alkalis, anionic detergents, etc., the vehicle itself must also be patch tested to exclude the possibility that the vehicle is irritant.

The *in vitro* experiments performed by D'Arpino *et al.* [20] on enhancing results in the local lymph node assay suggest ways of possibly improving the outcome of patch testing and avoiding false negative results. This may be achieved by supplying petrolatum with additives such as Transcutol P, sodium lauryl sulphate or phtalates.

1.6. Reproducibility, Sensitivity and Specificity

The reproducibility of patch tests remains controversial. In the literature we can find reproducibility percentages varying from 48% up to 96% [21]. Brasch *et al.* found that non-reproducibility of patch tests seems to be strongly allergen dependent. In their synchronous left-versus right-sided patch test study, the likelihood of non-reproducible allergic reactions increased when more than four positive reactions were seen at the same time, and with another positive reaction located in close proximity to an allergic reaction. Other factors such as age, sex, atopy, sleeping habits, lipogenic skin activity, systemic medication, inflammatory dermatoses outside the back and internal medication (excluding corticosteroids) were of minor importance for patch test reproducibility [22]. Weaker patch test reactions seem to be less reproducible. Some of the variability is eliminated by the use of ready-to-use patch test systems. Gollhausen *et al.* found that such a system (TRUE test) eliminated about half of the non-reproducible reactions [23] and Lachapelle *et al.* found that another preloaded system (Epiquick) was 95% reproducible in a left-to-right comparison [24].

The ideal patch test should give no false-positive or false-negative reactions. A false-positive reaction is an irritant reaction with the same morphology as an allergic patch test reaction and therefore cannot be separated from reactions caused by sensitization. It can be caused by too elevated test concentrations, impure or contaminated test substances, irritant test substance or vehicle, current or recent dermatitis at the test site, current dermatitis at distant skin sites, pressure effects or mechanical irritation.

False-negative reactions in the presence of a contact allergy can be due to too low test concentrations, test substance in insufficient amount or not released from the vehicle, test panels removed too soon, reading taken too early, inappropriate co-medication such as corticosteroids or due to a compound allergy.

Inappropriate co-medication during patch testing includes topical and oral corticosteroids and immunomodulators. Treatment of the test site with topical corticosteroids can mitigate the responses obtained to a high degree [25].

Testing patients on oral corticosteroids is not recommendable. Comparison of the intensity of the reaction before and during treatment with corticosteroids has suggested that an important allergy cannot be missed under corticosteroids up to 20 mg. However, it is usually advisable to defer the test until after corticosteroids have been stopped. The use of antihistamines as a contraindication for patch testing is not universally accepted. Some studies show that it is useless to stop antihistamines before patch testing, since clinical evaluation of tests is not hampered by a potent antihistamine [26]. The influence on patch testing of other immunomodulators such as orally or parenterally administered cytostatic drugs has not yet been clarified.

Several studies have reported on the suppressive effect of ultraviolet B (UVB), UVA sunlight and psoralen and ultraviolet A light (PUVA) therapy on contact dermatitis. However, studies that have tested the hypothesis that patch tests reactions have a seasonal variation due to the suppressive influence of sunlight, have had conflicting results [27].

1.7. Relevance of Positive Patch Test Reactions

Patch tests can be used to confirm a suspected allergic contact dermatitis and either to recommend avoidance of particular products or to recommend alternative products in a particular patient.

The true rate of clinically relevant hypersensitivity in positive patch test reactions remains for a great part unknown. To know that a patient has been exposed to a sensitizer is insufficient to conclude that the positive patch test is relevant. There is always the risk of over- or underestimating the significance of positive patch test reactions.

When a positive patch test reaction is found, an attempt must be made to fit it into the information obtained from the history and clinical examination.

The major prerequisites for a contact allergy to be clinically relevant are: 1) exposure to the sensitizer and 2) presence of a dermatitis which is understandable and explainable with regard to the exposure on the one hand and type, localization and course of the dermatitis on the other [28]. The problem is that one sensitizer causing the entire clinical picture of the dermatitis is rare. Mostly the cause is multifactorial, including irritant and constitutional influences. It is important to obtain sufficient information on the exposure to the suspected sensitizer, by questioning the patient's own experience, analyzing data sheets on packages of used products, chemical analyses, etc. Just as a positive reaction does not always mean that the primary cause of the dermatitis has been found, so a negative reaction does not always mean that the dermatitis is not caused by contact allergic hypersensitivity. Standard series include only statistically common allergens; one must be constantly alert to the possibility of rare, exotic or new sensitizers.

1.8. Adverse Reactions

The ideal patch test should cause as few adverse reactions as possible.

1. Irritancy itself can be considered as an adverse effect, especially the more severe reactions such as a chemical burn. Irritant patch test reactions are usually sharply demarcated, confined to the area covered by the patch.
2. The "excited skin syndrome" or "angry back" means that there are many patch test reactions of which some are false-positive [29]. The cytokines released by inflammatory skin may enhance other patch test reactions. It is a regional phenomenon caused by: a) subclinic dermatitis in an atopic patient or b) the presence of a strongly positive patch test reaction, which produces a state of skin hyperreactivity in which other patch test sites become reactive, especially the marginal irritants. To confirm or deny the significance of the individual reactions, each substance should be tested again individually.
3. The "edge effect" is often an irritant reaction, with a more intense reaction at the periphery of the patch than in the center, due to an increased concentration of the irritant liquid at the margin. Sometimes a reaction with edge effect can be a false negative or doubtful patch test reaction to a corticosteroid. This is an eczematous reaction only apparent on the edge of the patch test site, particularly at the first reading. Probably the inflammation is still suppressed in the middle of the site where the concentration of the corticosteroid is the highest, while around the edges of the site, the corticosteroid diffuses through the skin, and the low concentrations allow the allergenic effect to prevail [8].
4. Pustular patch test reactions are sometimes a manifestation of irritancy. Pruritus is often minimal or absent and the reactions usually disappear promptly, although they can occasionally persist for some days [30].
5. Pressure reactions can occur, especially with the use of solid test substances and in patients with a tendency to dermographism.
6. A temporary flare of the existing dermatitis elsewhere on the skin can be due to a positive patch test reaction.
7. Numerous substances can cause contact urticaria, most frequently patients' own materials brought from home or work to test; especially common are penicillin, balsam of Peru and phenylmercuric compounds.
8. Hyperpigmentation may result from the inflammation alone, independent of the chemical response in certain patients [31]. Sometimes a severe reaction causes hyperpigmentation or total depigmentation.
9. Most dermatologists will not patch test pregnant women, although the teratogenic capacity of the patch test substances is probably nil. The rationale is to avoid problems if there should be perinatal or congenital abnormalities due to other causes. However, before introducing new compounds into the test series, the teratogenic potential should be considered.
10. Patch test sensitization is considered to be the most serious adverse reaction of patch testing. It is detected by a flare-up reaction at the test site at least 10 days after the application [32]. On being repeated, the test is already positive at day 2-4. It is more likely that the flare-up reaction represents patch test sensitization than does the finding of a positive reaction to a substance that has previously been tested negatively. In the latter case, the patient may have been sensitized in the interval between the performances of the patch tests due to environmental exposure to the antigen [33]. Active sensitization is less likely to happen by using the lowest concentration of test substance required to cause a reaction. That is another reason why one should beware of patch testing substances brought by patients. Nevertheless there is a risk of active sensitization from the standard series. *para*-Phenylenediamine is an example of a compound in the standard series that has a strong capacity to sensitize and therefore it is still subject of continuing debate whether it belongs in a standard series [34].

A lot of adverse reactions have been summarized above, but it has to be noted that the overall risk-benefit equation of patch testing is in favour of the benefit, if performed correctly and with the proper indications.

2. OTHER TESTS

2.1. Open Test

Open tests are recommended as the first step when testing poorly defined or unknown substances, brought by the patient. This concerns especially gels, liquids or creams, suspected of producing irritant reactions if occluded. Cosmetics such as perfumes, aftershave lotions and hairsprays are the prototypes in this kind of testing. It is applied undiluted to the normal skin twice a day for at least two days. The outer aspect of the upper arm or the retroauricular area is the recommended site for open tests. It should be left uncovered and the application has to be discontinued if any irritation arises. The test is read after 15 to 30 min to detect contact urticaria. Otherwise the readings are done as with the closed patch tests. A negative open test indicates that an oc-

clusive patch test can be preformed with the substance without expecting severe irritant reactions.

2.2. Use tests: Provocative and Repeated Open Application Tests

Use tests such as the provocative use test (PUT) or repeated open application test (ROAT) have been created to better understand the clinical significance of patch test results. It has been suggested that since these tests typically utilize only one substance at a time and avoid occlusion, they minimize the occurrence of irritation and false positives and, thus, are more reflective of real-life exposure to an allergen.

The provocative use tests are performed to confirm positive patch test reactions. The suspected agent is used on normal skin as it was before, to evaluate if a relapse occurs. If no reaction occurs, the test may be considered negative. It is important to evaluate the clinical significance of the ingredients of a formulated product found positive by patch testing.

The ROAT is a use test, performed on the outer aspect of the upper arm, the antecubital fossa or the scapular area of the back over an area of approximately 3 cm in diameter. The substances are applied twice daily for 7 days. A positive response usually appears on day 2 to 4. The patient is instructed to stop the application of the test substances when a reaction is noticed.

2.3. Photopatch Test

Photopatch testing should be used to investigate patients with clinically suspected photoallergic contact dermatitis (PACD). PACD is caused by photochemical conversion of a certain agent into a contact allergen, mainly induced by UVA. Particularly plant derivatives, fragrances, antiseptics and sunscreen agents are known for photosensitization. The latter have now become the most common photoallergens, due to extensive use during recent decades. The differential diagnosis of PACD includes: airborne allergic contact dermatitis, phototoxic reactions, chronic actinic dermatitis, seborrhoeic dermatitis, polymorphic light eruption, variants of systemic lupus erythematosus and cutaneous porphyrias. The photopatch test procedure can vary, but in general goes as follows. The test materials are applied to the back in a duplicate set for 24 hrs. One test site is irradiated with UVA (320-400 nm) and the other serves as an unirradiated control. The tests are read immediately and 24, 48 and 72 hrs after UVA irradiation.

ABBREVIATIONS

ACD	=	Allergic contact dermatitis
UVA	=	Ultraviolet A
UVB	=	Ultraviolet B
PACD	=	Photoallergic contact dermatitis
PUT	=	Provocative use test
PUVA	=	Psoralen and ultraviolet A light
ROAT	=	Repeated open application test

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