Chapter 22

Patch Testing

22

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22.1 Introduction

22.1.1 The Purpose of Patch Testing

Patch testing is a well-established method of diagnosing contact allergy– a delayed type of hypersensitivity (type IV reaction). Patients with a history and clinical picture of contact dermatitis are re-exposed to the suspected allergens under controlled conditions to verify the diagnosis. Also testing patients with hand (dyshidrotic, hyperkeratotic), arm, face or leg eczema (stasis dermatitis), testing of other types of eczema (atopic, seborrheic dermatitis, nummular eczema), including patients with chronic psoriasis, vulval disorders or drug reactions (Chap. 24), is sometimes indicated, especially when they are recalcitrant to prescribed treatment and the dermatologist suspects contact allergy to prescribed topical medicaments and their vehicles.

Apart from its use to confirm a suspected allergic contact dermatitis, the patch test procedure can also be used before recommending alternative medicaments, skin care products, cosmetics, gloves, etc. in a particular patient. If the patient does not react to the alternatives tested, it is unlikely that he or she will react to the products in ordinary use.

Early classic publications on patch testing are reviewed in Chap. 1. More recent, often quoted, guidelines are presented by Malten et al. [1], Fregert [2] and Bandmann and Wohn [3].

Several studies (e.g. [4–6]) have shown that detailed patch testing is beneficial for patients and improve their quality of life (QoL). However, it has also been claimed that random patch testing with a standard series should be discouraged due to low pretest probability [7].

When performing patch testing it has to be remembered that the patch test is a biological provocation test and as such the outcome is dependent on multiple factors including the test system and test material, the biological/functional status of the tested person, and the responsible dermatologist. Most of theses aspects will be discussed in this chapter.

Core Message

- Indications for patch testing:
  - Cases of contact dermatitis
  - Other types of eczema and dermatoses, where a superimposed contact allergy is suspected, particularly if recurrent and nonresponsive to treatment
  - Suspected contact allergy to topical medicaments and their vehicles
  - "Predictive testing" of alternative products such as gloves, skin care products, medicaments

22.1.2 Standardization

The first patch tests according to present principles were carried out in 1895 [8], but were preceded by some preliminary experiments [9] (see Chap. 1). During the last few decades much effort has been put into standardization of allergens, vehicles, concentrations, patch test materials, tapes, and the scoring of test reactions, and the method today is considered accurate and reliable. A series of papers has demonstrated good reproducibility of patch test results [10–18]. Standardization has facilitated comparisons of contact allergy frequency in and between clinics, geographical areas, and areas with various degrees of industrialization but some questions still remain, especially concerning the reading and scoring of test reactions. This will be discussed in detail below.

22.1.3 Bioavailability

To obtain optimal bioavailability of a hapten one can influence the following five variables:

- Intrinsic penetration capacity
- Concentration, dose
- Vehicle
- Occlusivity of patch test system and tape
- Exposure time

Since it is desirable to remove all test strips at the same time – usually at day 2 (48 h) – four factors remain and can be varied and optimized by the manufacturers of patch test materials and allergen preparations and by the dermatologist responsible for the testing. The penetration capacity can depend upon the salts used; for example, there is a big difference between the penetration of nickel achieved by nickel sulfate and nickel chloride [19]. The higher penetration of nickel from the chloride is probably explained by the partition skin/vehicle of the salts, when applied in the same vehicle in equimolar concentration and under occlusion.

22.2 Test Systems

One can distinguish two test systems: the original one, where the allergens, patches, and tapes are supplied separately, and the modern ready-to-use system, where only a covering material has to be removed before the test is applied.

22.2.1 Original System (Allergen–Patch–Tape)

22.2.1.1 Patches

Some of the patch test units available are depicted in Fig. 1. In Finn chamber (Epitest, Finland) the test area is circular and in van der Bend (van der Bend, Netherlands) and IQ chambers (Chemotechnique Diagnostic, Sweden) they are square. The latter is claimed to facilitate distinguishing allergic from irritant reactions, since an irritant reaction tends to look square, while an allergic reaction tends to look round [1]. Based on a comparative study with ordinary (8 mm) and large (12 mm) Finn chambers, it was found that the larger chambers may be useful for detection of weak sensitization to some contact allergens [20–22]. However, the larger chambers are usually recommended for experimental studies when testing for irritancy.

22.2.1.2 Allergens

The standard patch test allergens sold by Chemotechnique Diagnostics [21] and Trolab Hermal [23], for example, can, according to the suppliers' product catalogues, be considered chemically defined and pure. However, the dermatologist responsible for patch testing is recommended repeatedly to request the manufacturers to provide results of chemical analyses.

The test preparations are presented in plastic syringes or bottles of inert material to prevent degradation or other chemical changes due to air, humidity, and light. The suppliers' recommendations on storage must be followed in order to minimize these risks. It is suspected that several of the contact allergies reported earlier were due to impurities or degradation products [24]. It has not been possible to confirm the allergenic potential of some claimed "allergens."

22.2.1.3 Vehicles

Each allergen almost certainly has its own optimal vehicle; it is improbable that just one vehicle (e.g., petrolatum) could be optimal for all allergens. White petrolatum is the most widely used vehicle, but its general reliability can be questioned. It gives good occlusion, keeps the allergens stable and is inexpensive. On the other hand, it can retain the allergen (see Sect. 22.5.6.1, Common Causes), irritate the skin, and even give rise to allergic skin reactions [25]. Liquid vehicles such as water and solvents (acetone, ethanol, methyl ethyl ketone) are recommended since they facilitate penetration of the skin, but they also have some drawbacks. Solvents may evaporate, which does not favor exact dosing, and most test solutions must be freshly prepared. Liquid vehicles are used mainly when testing chemicals and products brought by patients (see Sect. 22.13, Tests with Unknown Substances), and in research projects.

In the present standard series water is used for formaldehyde and for 5-chloro-2-methylisothiazol-3-one plus 2-methylisothiazol-3-one (MCI/MI). By using buffer solutions for acid and alkaline products,

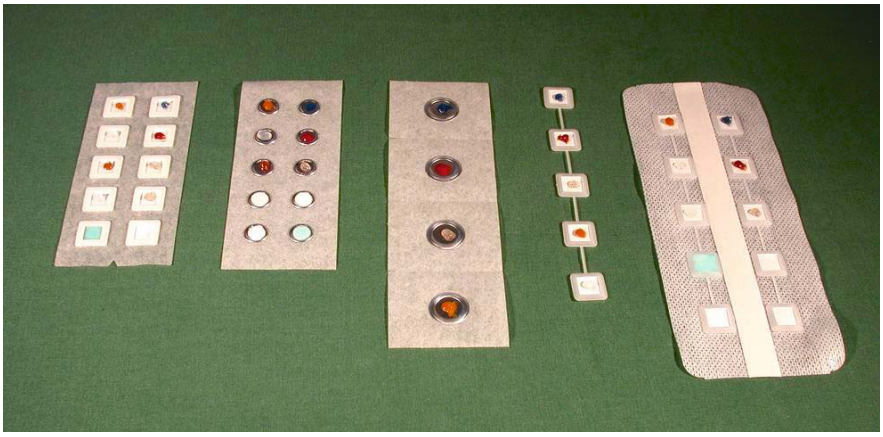


Fig. 1.
IQ square chambers (*left*), Finn chambers, ordinary (diameter 8 mm) and large (diameter 12 mm) (*middle*) and van der Bend square chambers with and without tape (*right*). Different test preparations applied for illustration. (Photo by Gunnel Hagelthorn)

the test concentration can be raised [26]. A filter paper must be used for liquid allergen preparations when using Finn chambers. Modern vehicles are hydrophilic gels (cellulose derivatives), used for example in the TRUE test (Mekos Laboratories, Denmark) [27].

When using more sophisticated vehicles containing salicylic acid, anionic detergents, solvents and others than those mentioned above (e.g., dimethylsulfoxide, DMSO), alkalis, etc. to increase penetration (see Sect. 22.1.3, Bioavailability), an extra patch with the vehicle, as is, must be applied to exclude the possibility that the vehicle is irritant. Since the number of test sites is limited, these vehicles cannot be recommended for routine use. However, they might be valuable where the standard preparation has given a negative reaction but the clinical impression of an allergic contact dermatitis remains.

Core Message

- White petrolatum is used as the vehicle in the majority of patch test preparations. However, in cases of unexpected, negative patch test results alternative vehicles have to be considered.

22.2.1.4 Concentrations

In textbooks on contact dermatitis and patch testing, and in suppliers' catalogues, the concentration of an allergen is given as a percentage. In one catalogue [19] molality (M) is given together with percentage (weight/weight) and in the TRUE Test concentration is given in milligrams or micrograms per square centimeter. The traditional method of presenting concentrations as a percentage is simple and probably practical, but has been questioned [28, 29], as we do not know if this means weight/weight, volume/volume, volume/weight or weight/volume. Especially when comparing substances and in research projects, it is the dose, the number of molecules delivered, that is of interest [30]. The concentration of Ni ions is 20.9% in nickel sulfate ($NiSO_4 \cdot 7H_2O$) compared to 24.7% in nickel chloride ($NiCl_2 \cdot 6H_2O$) [31]. Thus in comparative studies with these salts it is essential to use the same molality [32].

Core Message

- Test concentrations should preferably be expressed as weight per area, e.g., milligrams or micrograms per square centimeter (mg/cm², µg/cm²).

22.2.1.5 Tapes

Previously, most tapes were based on colophony and could cause severe and lasting reactions in patients for whom such a sensitivity was not anticipated.

By introducing modern acrylate-based adhesive tapes, for example Scanpor (Norgeplaster, Alpharma, Norway), the problem has almost been eliminated. Finn chambers on Scanpor tape are commercially available. In cases where loosening can be anticipated (oily or hairy skin, sweating, high humidity), some reinforcing tapes are recommended. Methods for studies on conformability and irritancy of tapes have been published [33, 34].

22.2.1.6 Application of Test Preparations to the Patches

Commercial test preparations – allergens in petrolatum and kept in syringes – are applied directly into the test chambers, or onto the filter paper discs of the other patches (Fig. 2a) and a small amount, "a snake" (approx. 5 mm long) [23], of the mixture is applied across the diameter of the disc. The orifice of the syringe is adjusted to facilitate this.

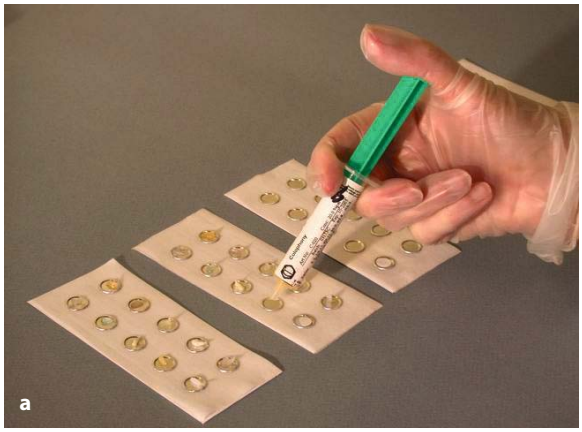
Liquid test preparations are preferably applied via a digital pipette with disposable plastic tips to allow exact dosing (15 µl calculated for ordinary Finn chambers) (Fig. 2b).

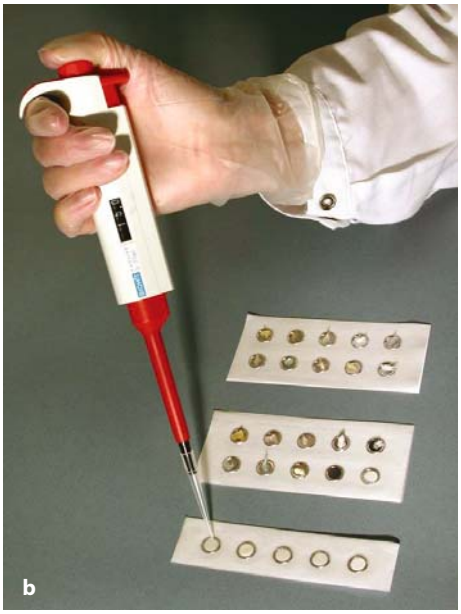
22.2.1.7 Some Practical Suggestions

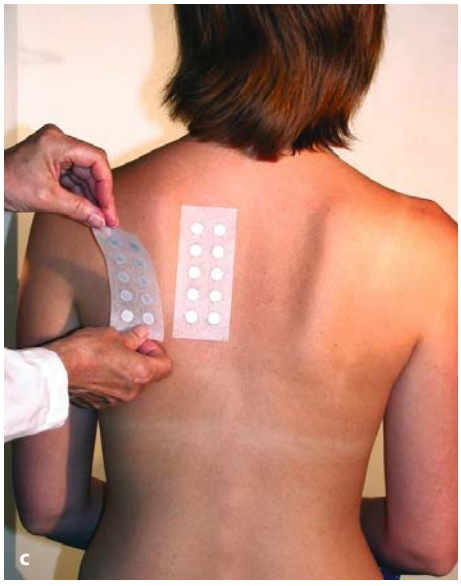
Storage

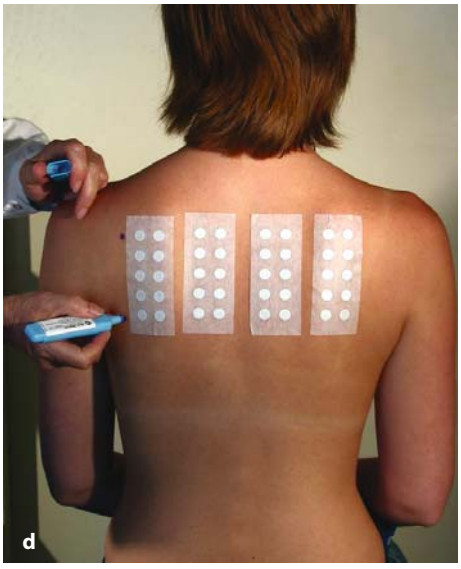
The allergens should be kept in a cool, dark place (refrigerator) to minimize degradation. Those diluted in liquids (water, solvents) should be kept in dark bottles. Allergens should be renewed according to their expiry dates.

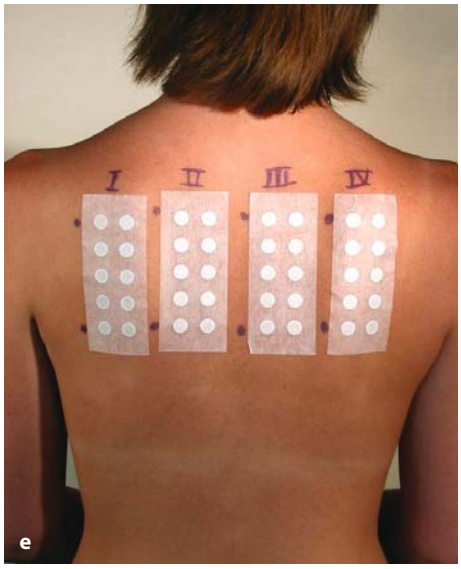
Fig. 2a–e. Patch testing. **a, b** Application of allergens to test patches (**a** allergen in petrolatum, **b** allergen in liquid test preparation using digital pipette with disposable tip). **c** Application of patch tests on the upper back. **d** Marking the test area. **e** Test applied to the upper back. (Photo by Gunnel Hagelthorn)











Sequence of Allergens

Adjust the sequence of the allergens so that those frequently causing strong, cross or concomitant reactions are not adjacent. In a study [35] using the TRUE Test system it was found that positive tests to nickel did not intensify reactions to dichromate (distance 1, 3, and 7 cm between the patches) while another [36] concluded that substances with a tendency to cross-reaction or co-sensitizing substances should be tested distant from one another, thus preventing the occurrence of false-positive results. The order given in the catalogues [21, 23] can usually be followed.

Testing in Pregnancy

We usually do not test pregnant women. There are no indications that the minute amounts of allergens absorbed in patch testing could influence the fetus, but in cases of miscarriage or deformity it is natural to blame several things, including medical investigations.

Test Sites

The preferred site is the upper back. For a small number of allergens, for example at retesting, the outer aspect of the upper arm is also acceptable. False-negative test results can be obtained when testing on the lower back or on the volar forearms (see Sect. 22.5.6.1, Common Causes).

Removal of Hair

On hairy areas of the back it is difficult to get acceptable skin contact, and for this reason clipping is recommended. However, a combination of clipping, petrolatum, and tapes sometimes contributes to the irritation seen, which makes reading somewhat difficult.

Degreasing of Test Site

In cases of oily skin, gentle treatment with ethanol or other mild solvents is recommended. The solvent must evaporate before the test strips are applied.

Application of Test Strips

Test strips should be applied from below with mild pressure to remove air pouches, followed by some moderate strokes with the back of the hand to improve adhesion [37] (Fig. 2c).

Skin Markers

Several solutions, inks or marking pens are available [2, 21, 23, 37, 38] Fig. 2d, e). If test strips with constant distance between the discs are used, only two marks are needed.

Positive Control

To exclude hyporeactivity, an impaired inflammatory response, and the possibility that the test patches do not adhere properly, sodium lauryl sulfate and nonanoic acid have been suggested as positive controls [39–42].

Instructions

We have found it valuable to inform our patients as to the aim of the test; about avoidance of showers, wetting the test site, irradiation, and excessive exercise; and about symptoms such as itch, loosening of patches, and late reactions. Examples of such written instructions and guidelines for patients are available [1, 38].

Reading

The light should be good (side lighting may be of help) and adjustable. A magnifying lamp or lens is often helpful. To facilitate reading, most test systems have a special reading plate with punched-out holes corresponding to the test sites.

22.2.2 Ready-to-use Systems

In the ready-to-use patch test system, all necessary material is prepared in advance and the dermatologist, nurse or technician only has to remove the covering material, apply the test strips and mark. In the TRUE Test system (Mekos Laboratories, Denmark) [27] the allergens are incorporated in hydrophilic gels and the patches are 9 mm by 9 mm (Fig. 3). At present, this system is commercially available for the standard series.

Some comparative studies have been carried out with TRUE Test versus Finn chambers [43–47], demonstrating good concordance. The accuracy, reliability, simplicity, and costs of the ready-to-use system must be balanced by the costs, including personnel, of the original systems [see Sect. 22.2.1, Original System (Allergen–Patch–Tape)].

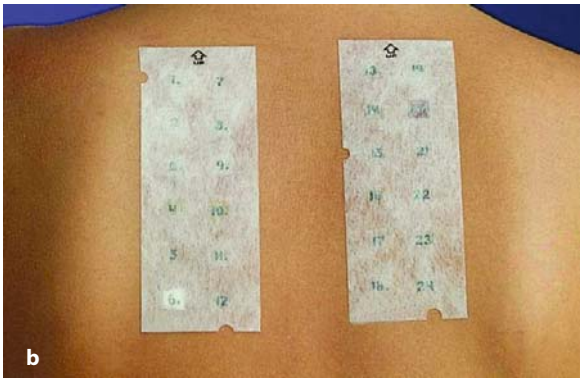


Fig. 3a, b. TRUE Test ready-to-use system. **a** Tests delivered in ready-to-use packages; **b** tests applied to the upper back. Note the square patches. Courtesy by Mekos Laboratories, Denmark.

22.3 Allergens

22.3.1 Numbers

There are 3,700 chemicals described that can cause allergic contact dermatitis [48], and data on new ones are published every year. In de Groot's first book [49], 2,800 allergens were reviewed, indicating that 900 additional substances were identified as sensitizers between 1986 and 1994. The new ones are identified when carrying out predictive testing and when examining and testing patients with contact dermatitis.

22.3.2 Suppliers

The catalogs from the suppliers (e.g., Chemotechnique Diagnostics [21] and Trolab, Hermal [23]) contain lists of approximately 350–400 test preparations in alphabetical order, allergens in the European and International standard series, tables of mixes, and lists of screening series. The catalogs also contain information on the occurrence of allergens and cross-reactivity, as well as some service items such as test sheets, guides to patch testing, skin markers, questionnaires, and advice to patients.

22.3.3 Screening Series

To evaluate the significance of special exposures – mainly occupational – a number of screening series are available (Table 1). They are compiled from the experience gathered at departments of occupational dermatology, and from the literature [50, 51]. Newly defined allergens are added regularly and these series can be considered to cover the present exposure situation.

However, the allergens are pure chemicals and, if the original offending agent was an impurity, a metabolite, a degradation product etc., the cause will be missed. A supplementary test with the patient's own working materials should be done in those cases where the test with the screening series was negative but the suspicion of allergic contact dermatitis remains. A matter of dispute is the ethical question: "should a patient be tested with a number of well-known contact allergens to which he or she has never been exposed?" Is there a risk of patch test sensitization? (see Sect. 22.8, Complications).

22.3.4 Variations Concerning Concentration and Vehicle

Slight differences in recommendations on concentrations and vehicles can be found in catalogs [21, 23] and textbooks on contact dermatitis and patch testing [2, 38, 52–54]. There are thus no ultimate test preparations that are optimal in all clinics or geographical areas. Patch and tape occlusion, humidity, temperature and other climatic factors (see also below Sect. 22.7.4, Irradiation), local experience, and tradition can motivate deviations from these recommendations. Test concentrations for children are presented in Chap. 43. However, the test preparations offered in catalogs are based on tests of several thousands of patients and must be considered very useful

Table 1. Examples of commercially available screening series and number of allergens in each series (*n*)

Chemotechnique [19]	*n*	Trolab Hermal [21]	*n*
Bakery	19	Antioxidants	6
Corticosteroids	8	Bakery allergens	14
Cosmetics	48	Cosmetics	13
Dental	30	Cutting oils (current)	26
Epoxy	9	Cutting oils (historical)	13
Fragrance	24	Dental materials	20
Hairdressing	26	Disinfectants	6
Isocyanate	6	Hairdressing	8
Medicaments	13	Industrial biocides	15
(Meth)acrylate:		Medicaments I Antibiotics	9
Adhesives, dental and other	15	Medicaments II Antiseptics, antimycotics	5
Nails – artificial	13	Medicaments III Miscellaneous	5
Printing	24	Medicaments IV Local anesthetics	5
Oil and cooling fluid	35	Medicaments V Corticosteroids	8
Photographic chemicals	16	Medicaments VI Ophthalmics	5
Plant	13	Metal compounds	7
Plastic and glues	25	Perfumes, flavors	24
Rubber additives	25	Photoallergens	17
Scandinavian photopatch	20	Photographic chemicals	16
Shoe	22	Plants	8
Sunscreen	10	Plastic, glues	30
Textile colors and finish	32	Preservatives	20
Various allergens	59	Shoe allergens	9
		Rubber chemicals	17
		Sunscreen agents	9
		Textile and leather dyes	13
		Vehicles, emulsifiers	8
		Miscellaneous	5

guidelines when setting up and running a patch test clinic.

22.4 Standard Series

The present European standard series contains 25 items, but 6 of them are *mixes*, so in fact at least 24 additional allergens are applied. Balsam of Peru, colophony, and lanolin are examples of natural mixes, where much effort has been spent identifying the allergens [55–58]. The basic idea of using mixes instead of single allergens is to save time and space. Also, the patients are tested with a number of closely related substances, among others rubber chemicals. The screening capacity of the standard series is thereby greatly increased. However, the value of these mixes is sometimes questioned [59]. It is difficult to find an optimal concentration for each allergen in a common vehicle (usually petrolatum) and to determine whether the allergens metabolize or interact to potentiate or decrease reactivity [60, 61].

At our clinic we use the mixes for screening purposes, positive cases being retested with the ingredients. Not unusually, these tests are negative and we then have to ask ourselves whether the initial reaction was an expression of irritancy and/or whether the ingredients have interacted. The opposite has also been noticed. The patient may be negative to a particular mix, but react when retested with its ingredients.

The advantages and disadvantages of using a standard series of patch tests were recently discussed by Lachapelle and Maibach [62]. They pointed out that it can be considered a limited technical tool, representing one of the pieces of a puzzle, to be combined with other means of diagnosis, and that it also compensates for anamnestic failures. The allergens of the standard series are presented in detail in Chap. 29 and the test concentrations in Chap. 49.

22.4.1 Deciding What to Include in the Standard Series

The original standard series was based on the experience of the members of the International Contact Dermatitis Research Group and mirrored the findings and current situation in different parts of Europe and the United States. The series is evaluated regularly by national and international contact dermatitis groups. Each test clinic is recommended to

compile its patch test results yearly. If the frequency of positive reactions to a particular allergen is less than 1%, its presence in a standard series can be questioned and it should probably be replaced by another compound. In these ways, the standard series continually changes in composition and in the total number of substances included.

The new allergens introduced are often preservatives. 5-Chloro-2-methylisothiazol-3-one (MCI) plus 2-methylisothiazol-3-one (MI) can be mentioned as a typical example. The first cases were observed in Southern Sweden in 1980 [63] and isothiazolinone then became an almost universal allergen, with local epidemics in Finland, the Netherlands, Italy, and Switzerland [64]. It was included in the Swedish standard series in 1985 and in the European standard series in 1988 [65].

A scheme [66] for identification of new contact allergens includes:

- Clinical
  - Positive patch test reaction to a product
  - Test with ingredients of the product
  - Serial dilution test to define a threshold of sensitivity
  - Control tests for irritancy
  - Cross-reactivity – equimolar concentrations
  - Use tests – repeated open application test (ROAT), provocative use test (PUT)
- Experimental
  - Structural formula
  - Chemical analyses – test material, product, purity, stability
  - Animal testing – allergenic potency, cross-reactivity pattern

The choice of patch test concentrations is initially decided by the dermatologist studying a suspected allergen in an index case of contact dermatitis. Most allergens are tested in the concentration range 0.01–10% and by analogy with similar chemicals the dermatologist will probably start within this range and then continue with a serial dilution test (Table 2). The threshold of sensitivity defined must be checked for irritancy by tests in controls [66]. If these control tests are negative, information on the case and on the test preparation, where allergen, concentration, and vehicle are stated, will be published as scientific reports and also disseminated to suppliers of patch test allergens. An instructive example of the procedure of defining a new allergen – the preservative iodopropynyl butylcarbamate – was recently presented [67, 68]. The issue is further discussed in Sect. 22.13.3, Test or Not?.

Table 2. Results of a serial dilution test with nickel sulfate in a patient who previously reacted to 5.0% (+++)

Dilution step (%)	Score
1. 1.0	+++
2. 0.3	++
3. 0.1	+
4. 0.03	+
5. 0.01	A few papules
6. 0.003	?
7. 0.001	–
8. 0.0003	–

Nowadays, following local epidemics, conference reports, and communications in scientific journals, several patch test clinics may choose to include a newly identified allergen in their standard series to investigate the frequency in their geographical area. If the initial reports can be confirmed and the allergen is diffused in many and various products, it is then recommended for inclusion in the standard series [69].

At a joint meeting of the International and European Environmental and Contact Dermatitis Research Groups [65], it was recommended to include MCI/MI and at the same meeting some other changes in the tray were made: carba mix (three rubber chemicals) was removed and replaced by another rubber chemical (mercaptobenzothiazole); *p*-phenylenediamine hydrochloride was replaced by the corresponding free base and the concentration was raised from 0.5% to 1.0%. In 1995 [70] the introduction of the sesquiterpene lactone mix in the standard series, replacement of ingredients in the PPD black rubber mix and the quinolone mix by single components, and the dropping of ethylenediamine dihydrochloride and a *p*-hydroxybenzoate from the paraben mix took place. More recently budesonide and tixocortolpivalate were recommended for inclusion in the European standard series [71]. A comparison of the European standard series with the North American and Japanese series shows some differences in composition, concentrations, and vehicles [70, 72]. An extended international standard series was suggested [72]. The groups' official recommendations for changes can be read by all interested parties, which also gives opportunities for questions and discussion.

22.5 Reading and Evaluation of Patch Tests

The diagnosis allergic contact dermatitis is based on patch testing and quantitative and qualitative expo-

sure assessment. The frequency of patch testing in national health care systems varies considerably around the world. In Denmark with a population of 5 million, approximately 25,000 new patients are patch tested yearly. Patch testing is a medical technology that has developed over the last 100 years and is now of major significance in the evaluation and classification of dermatitis. In cases of allergic contact dermatitis a clear outcome of the patch test can be obtained in most cases with a significant impact on clinical diagnosis and prognosis. Difficulties in discriminating weak allergic and irritant reactions will undoubtedly occur. Such gray zones need to be handled by supplementary tests such as dose–response, serial dilution, and ROATs and in the final conclusion related to the clinical history. Reading of patch tests is based on morphological criteria only. Reading of a patch test, as with all other tests in medicine, is a question of strictly following objective criteria. The interpretation of test results and the relevance depend on a global evaluation including the history of the patient, clinical observations, and exposure assessment.

22.5.1 Reading – When and How

The reading should be done by the dermatologist him- or herself, after adequate training.

22.5.1.1 Exposure Time

Most authors advocate an exposure time of 48 h. A few comparisons of 1-day (24 h) and 2-day (48-h) allergen exposure show some reactions positive only at day 1 (24 h) and some positive only at day 2 (48 h) [73]. A 1-day exposure would reduce the number of questionable reactions [74]. No definite conclusions can be drawn from the studies published so far [75].

It would be convenient for the patient, and probably for the dermatologist, if the exposure time could be reduced with retained accuracy. Preliminary studies with exposure to $NiSO_4$ for 5 h [76], to $K_2Cr_2O_7$ for 6 h and 48 h [77], and *para*-phenylenediamine (PPDA) for 15, 30, and 120 min [78] demonstrated that some patients will react at these brief exposures, but also showed great variability. To achieve reduced exposure time while retaining accuracy, the penetration capacity of the hapten must be increased, among other things by using higher concentrations or doses, more efficacious vehicles and optimal occlusion (see Sect. 22.1.3, Bioavailability). Working out these parameters for all existing allergens, however, would be an overwhelming task.

22.5.1.2 Reading When?

Wherever possible, it was strongly recommended that two readings be carried out, the first after removal of the patches (usually day 2) and the second 2–5 days later [79]. In a study, paired readings on days 4 and 7 were found to be more reliable than those on days 2 and 4 [80]. The readings must be related to the exposure times (see Sect. 22.5.1.1, Exposure Time) and if the patches are applied for only 1 day, readings should be at days 1 and 3.

If they are removed at the dermatologist's clinic or office, it is possible to check that they have adhered properly and that the marking is adequate. However, this procedure must be balanced by the great(er) value of later readings for the patients (see below). One should wait at least 15–30 min after the removal, since the combination of allergen, vehicle, patches, and tape causes a transient increase in skin blood flow, a sign of irritation [81]. At later readings it is possible to record which reactions have turned negative and which reactions have become apparent and/or increased (crescendo) or decreased (decrescendo) in intensity. From studies with repeated readings it is obvious that the same patch test preparation can produce lost as well as found reactions [82, 83]. Neomycin, corticosteroids, and gold are often-quoted examples of allergens with late appearance ("slow" allergens) while others (fragrance mix, Balsam of Peru) are classified as "early" allergens. When readings were carried out on days 2, 3, and 7, 3% and 8.2% respectively of the reactions first appeared on day 7 [84, 85]. However, some of the positive late reactions proved negative when retested [84]. Long-lasting reactions persisting weeks or months after the initial readings are increasingly attended [86]. However, the clinical significance is not yet settled.

A reaction positive on day 2 and a negative one on day 4 has been suggested to indicate irritancy. There are some examples where such a pattern has been found to be clinically relevant, but the frequency is not known. To contribute to the confusion, a few substances are known to cause "delayed irritancy" [1].

22.5.1.3 Compromise

Multiple readings are thus highly justified and the importance of readings beyond day 2 is stressed [87]. If practical or geographical circumstances permit only one reading, the present accepted compromise is at day 3 (72 h), i.e., 24 h after removal of the patches. However in recent papers [88, 89] it was stated that a single reading on day 4 would have been most useful. Patients are instructed to report any late reactions.

Table 3. Multiple readings – options and recommendations

Option	No. of visits	Day 0 Application	Day 2 Removal, reading	Day 3/4 Reading	Day 5/7 Reading	Comment
1	2	×	×			Not recommended
2	3	×	×	×		Recommended
3	3	×		×	×	Recommended
4	4	×	×	×	×	Highly recommended

Options and recommendations concerning multiple readings are presented in Table 3. Options 2–4 enable discrimination between crescendo and decrescendo reactions. When comparing options 2 and 3 – both with three visits – we slightly prefer option 3 since it gives an opportunity to do a late reading (day 5/7).

The value of repeated readings must be balanced by the discomfort, costs, and practical problems (e.g., travel) the repeated visits will cause the patients. However, it is our firm belief that repeated readings will increase the accuracy of our only method of establishing contact allergy.

Core Message

- Late-appearing positive patch test reactions can appear for most allergens and are common for some. These reactions are missed if only early readings are carried out. Multiple readings are thus encouraged and if one wants to restrict the number of visits to three we consider that a reading at day 5/7 is more valuable than at day 2 – just after the removal of the patches.

Table 4. Recording of patch test reactions according to the International Contact Dermatitis Research Group (ICDRG) [36]

?+	Doubtful reaction; faint erythema only
+	Weak positive reaction; erythema, infiltration, possibly papules
++	Strong positive reaction; erythema, infiltration, papules, vesicles
+++	Extreme positive reaction; intense erythema and infiltration and coalescing vesicles
–	Negative reaction
IR	Irritant reactions of different types
NT	Not tested

22.5.2 Recording of Test Reactions

The common method of recording patch test reactions, recommended by the International Contact Dermatitis Research Group [2], is presented in Table 4. These recommendations are followed worldwide and are referred to in most scientific reports. Typical examples are shown in Fig. 4.

However, this recording system is somewhat simplified and not all types of reaction fit this outline. While experienced patch testers rarely disagree concerning the reading of the obvious irritant (IR), ++ and +++ reactions, the reading of the +? and + reactions and some of the IR may cause difficulties.

For documentation of patch test results it is recommended that forms are used with space for additional notes on the morphological appearance of the test reactions. It should be mentioned that some investigators record any changes from normal skin and others might ignore a very weak follicular reaction and record it as negative. Especially when repeated readings are taken, or lesser-known or new substances have been applied, it is essential to follow the appearance and disappearance of the various components of the reactions. Pictures can be of value for documentation, but can rarely replace our traditional aids: inspection and palpation. Instruction and supervision by an experienced patch tester is recommended for the *novice*. Each test site should be inspected and palpated and daily readings in selected cases would enable her/him to follow the dynamics of test reactions.

22.5.3 Interpretation of Reactions at Test Sites

A reaction at a test site merely indicates some kind of change compared to adjacent, nontested skin: it is not synonymous with "allergic" or "relevant"! Some important and somewhat controversial issues on the interpretation of patch test reactions will now be discussed.

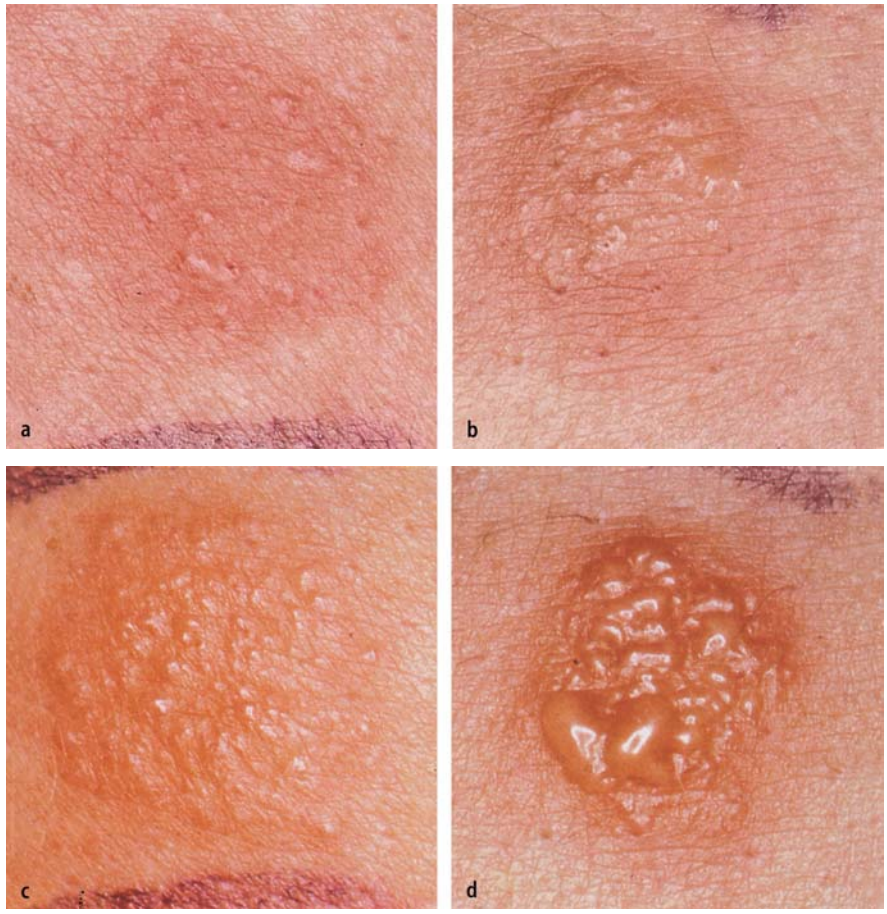


Fig. 4a–d. Allergic patch test reactions (all day 3) of increasing intensity. **a** + Reaction to nickel sulfate; **b** still a + reaction to *para*-phenylenediamine (*PPDA*); **c** ++ reaction to PPDA; **d** +++ reaction to PPDA. (Courtesy of P.J. Frosch)

Core Message

- Patch test reactions should be recorded according to the scheme presented in Table 4.
  Repeated readings would enable the reader – especially when under training – to follow the appearance and disappearance of various components of a reaction.
  A reaction at a test site merely indicates some kind of change compared to adjacent, nontested skin: it is not synonymous with "allergic" or "relevant."

22.5.3.1 Discrimination Between Allergic and Irritant Reactions

To distinguish allergic (Fig. 4) reactions from irritant (Fig. 5) reactions on morphological grounds alone is difficult. Irritant reactions (IR) are said [1, 37, 52] to be characterized by: fine wrinkling ("silk paper"), erythema, and papules in follicular distribution, petechiae, pustules, bullae and necrosis and with minimal infiltration. Typical examples are shown in Fig. 5. Extension beyond the defined area exposed to the allergen is used to discriminate between allergic and irritant reactions [1]. Fisher [38] frankly states: "There is no morphological way of distinguishing a weak irritant patch test from a weak allergic test." Examples are benzalkonium chloride and MCI/MC, where there has been some discussion concerning the somewhat peculiar features of the test reactions.

In Table 2 the results from a serial dilution test with nickel sulfate are shown. At dilution step 5 (0.01%), a few papules have been recorded and in this case we know that the reaction is relevant and that this patient is highly sensitive. However, if "a few papules" are noticed in another patient, where only *one* concentration of an allergen has been applied, the interpretation is much more difficult. Usually, we have to repeat the test and probably raise the concentration and/or carry out a serial dilution test.

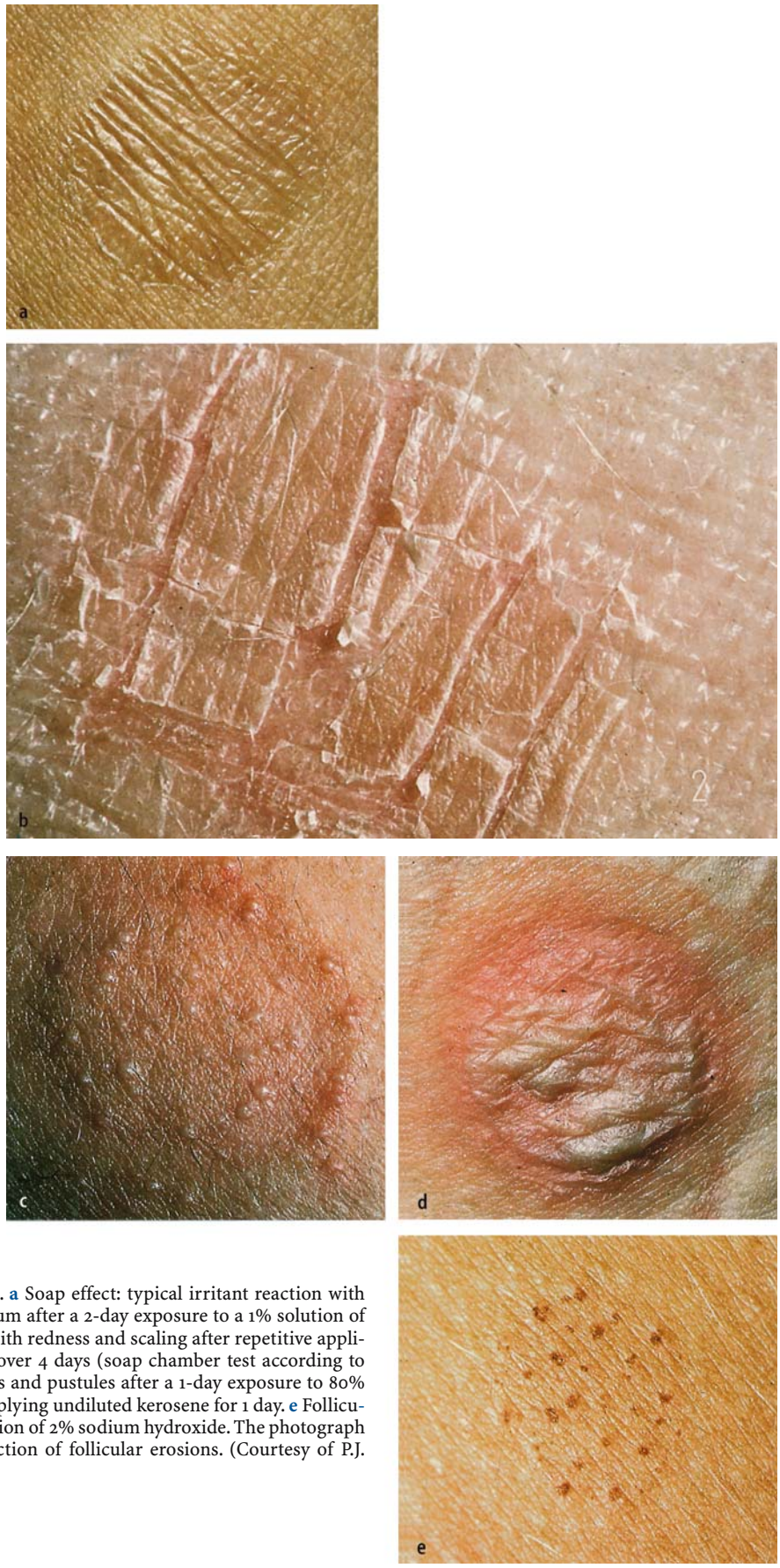


Fig. 5a–e. a, b Irritant reactions. **a** Soap effect: typical irritant reaction with glistening of the stratum corneum after a 2-day exposure to a 1% solution of toilet soap. **b** Irritant reaction with redness and scaling after repetitive application of an 8% soap solution over 4 days (soap chamber test according to Frosch and Kligman). **c** Redness and pustules after a 1-day exposure to 80% croton oil. **d** Full blister after applying undiluted kerosene for 1 day. **e** Follicular crusts after a 15-min application of 2% sodium hydroxide. The photograph was taken 1 day after the induction of follicular erosions. (Courtesy of P.J. Frosch)

22.5.3.2 Ring-Shaped Test Reactions

The somewhat peculiar ring-shaped test reactions (the "edge effect"), observed with – among other allergens – formaldehyde and MCI/MC in liquid vehicles, are in most cases an expression of contact allergy [90]. A special type can be seen with corticosteroids where the margins of the positive test are red, whereas the central area is whitish.

22.5.3.3 Ultrastructure

For distinguishing between allergic and irritant patch test reactions, traditional light or electronic microscopy has been of minimal help (see Chap. 8). Studies with monoclonal antibodies (e.g., [91]) and newer molecular techniques have not yet provided methods for clinical use to separate the two types of patch test reactions.

22.5.3.4 Doubtful and One Plus Reactions

When vesicles are present there is rarely any discussion of the allergic nature of the reaction, but the presence or absence of papules is more controversial [62, 92]. However, observed +? and + reactions may cause difficulties. As can be seen from Table 4, "possibly papules" is included in the + reaction. This expression can be interpreted in different ways: to be classified as a one plus (+) reaction – is erythema plus infiltration enough? What about erythema and papules, but no infiltration? According to Cronin [52], + is a palpable erythema. Historically the reading criteria for +? and + have not developed in parallel in all geographical areas. These differences in the interpretation of the objective skin changes explain some of the differences seen between departments and geographical areas.

When such a weak reaction (+? or +) has been obtained we recommend – as discussed in Sect. 22.5.3.1, Discrimination Between Allergic and Irritant Reactions – repeating the test, increasing the concentration by a factor of 5 or 10, and carrying out serial dilution (Table 2) and Use tests (see below).

Consensus on the denomination and interpretation of doubtful and weak reactions would be of great value and would facilitate comparisons between clinics and geographical areas.

Core Message

- Doubtful (+?) and weak test reactions (+) are hard to interpret. In those cases repeating the test, increasing the test concentration, serial dilution tests or Use tests are recommended.

22.5.3.5 Cross-Sensitivity

In cross-sensitivity, contact allergy caused by a primary allergen is combined with allergy to other chemically closely related substances. In those patients who have become sensitized to one substance, an allergic contact dermatitis can be provoked or worsened by several other related substances. A patient positive to *para*-phenylendiamine not only reacts to the dye itself, but also to immunochemically related substances that have an amino group in the *para* position, e.g., azo compounds, local anesthetics, and sulfonamides. When studying cross-reactivity it is essential to use pure test compounds [24].

22.5.4 Relevance

Evaluating the relevance of a reaction is the most difficult and intricate part of the patch test procedure, and is a challenge to both dermatologist and patient. The dermatologist's skill, experience, and curiosity are crucial factors.

For standard allergens, detailed lists are available that present the occurrence of each in the environment. The patient and the dermatologist should study the lists together, in order to judge the relevance of a positive patch test reaction, in relation to the exposure, site, course, and relapses of the patient's current dermatitis. A positive test reaction can also be explained by a previous, unrelated episode of contact dermatitis (past relevance).

Sometimes, the relevance of a positive reaction remains unexplained ("unexplained positive") until the patient brings a package or bottle where the allergen in question is named on the label. In other cases, chemical analyses demonstrate the presence of the allergen, or the manufacturer finally – after many inquiries – admits that the offending substance is present in the product. Methods for increasing the accuracy of the relevance of positive patch test reactions were recently presented [59, 93]. See also below, Sect. 22.10, Use Tests.

In cosmetics, skin care products, detergents, paints, cutting fluids, glues, etc., it is common that new ingredients are added or replace previous ones, but the product keeps its original trade name. Alternatively, well-known allergens are included in new products but with other fields of application than the original. To discover the cause of the patient's dermatitis the dermatologist must sometimes be obstinately determined!

The relative importance of different exogenous and endogenous factors to a given case of dermatitis might be hard to evaluate.

Core Message

- Evaluating the relevance of positive test reactions is the most difficult and intricate part of the test procedure and in this process the dermatologist's skill, experience, and curiosity are crucial factors. Clinical examination, repeated checking of history and exposure, Use tests, chemical analyses, and work-site visits ("the patient's chemical environment") can be of great help.

22.5.5 False-Positive Test Reactions

A false-positive reaction is a positive patch test reaction in the absence of contact allergy [94]. The most common causes can be summarized as follows:

1. Too high a test concentration for that particular patient
2. Impure or contaminated test preparation
3. The vehicle is irritant (especially solvents and sometimes petrolatum)
4. Excess of test preparation applied
5. The test substance, usually as crystals, is unevenly dispersed in the vehicle
6. Influence from adjacent test reactions (see above "Sequence of Allergens")
7. Current or recent dermatitis at test site
8. Current dermatitis at distant skin sites
9. Pressure effects of tapes, mechanical irritation of solid test materials, furniture and garments (brassiere)
10. Adhesive tape reactions
11. The patch itself has caused the reactions
12. Artifacts

Some are self-evident and can be predicted and monitored by the dermatologist carrying out patch testing, while others cannot.

22.5.5.1 The Compromise (Item 1)

While the current recommendations on allergen concentrations in relation to vehicle, patch, and tapes are based on long experience, they are nevertheless a compromise! The general problem is that if you lower the concentration to avoid irritancy you will also lose some cases that will be of special occupational and medicolegal importance. Well-known examples are dichromate, formaldehyde, tars, fragrance-mix and, previously, carba mix. It is probably better to have a (weak) false-positive reaction than a false-negative reaction because at least with a potentially false-positive reaction one is *alerted* to the possibility of allergy, which one can then confirm or deny, whereas with a false-negative reaction one is never alerted at all and may altogether miss a true allergy. Therefore, most dermatologists seem to prefer the higher concentrations of these marginal irritants, even though they know that nonspecific reactions from them are not uncommon.

Core Message

- Current recommendations on allergen concentrations in relation to vehicles, patches, and tapes are based on long experience but are nonetheless a compromise. If you lower the concentration to avoid irritancy you will also lose some cases. It is probably better to have a weak false-positive reaction than a false-negative reaction because the dermatologist is then alerted.

22.5.5.2 Excited-Skin Syndrome – "Angry Back" (Items 7 and 8)

Patients with current eczema may show cutaneous hyperirritability which can cause problems in patch testing. In the excited-skin syndrome, the presence of a strong positive reaction will influence the reactivity at adjacent test sites. When more than one site shows a reaction, this phenomenon must be considered, and retesting of the items one at a time is the usual recommendation (Fig. 6). Thanks to Björnberg's [95]

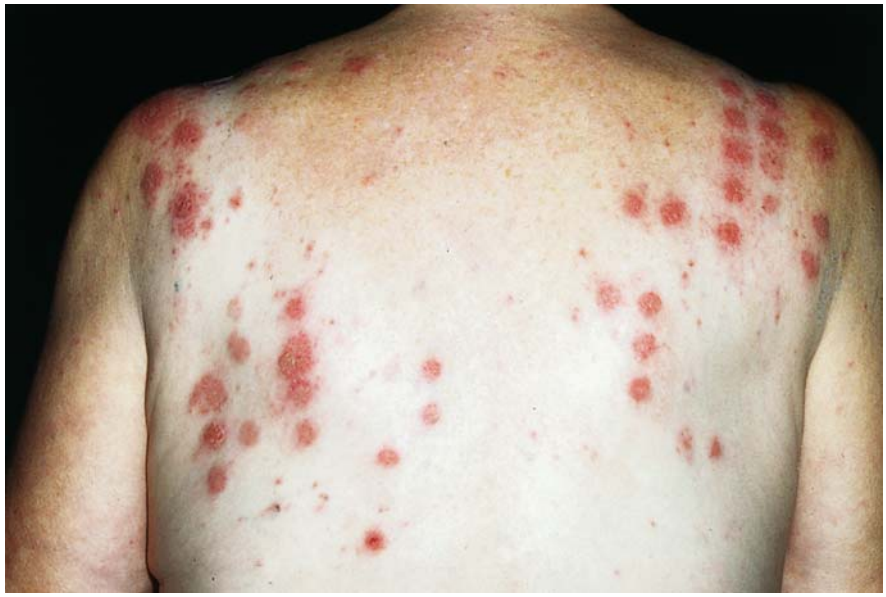


Fig. 6. Patients with multiple sensitizations do exist. This leg ulcer patient was allergic to numerous allergens. The strong reactions have been reproduced and were clinically relevant (wool wax alcohols, propylene glycol, parabens, *para*-phenylenediamine, MCI/MI, imidazolidinyl urea, thimerosal, thiuram mix, triamcinolone acetonide, amcinonide, and bufexamac). (Courtesy of P.J. Frosch)

important observations, we have always avoided patch testing a patient with current eczema and labile skin, and the excited-skin syndrome is seldom seen in our latitudes [96]. There is extensive literature on this syndrome [97, 98].

22.5.5.3 The Patch (Item 11)

After receiving intradermal allergen extracts due to pollen allergy, a few patients will develop sensitivity to aluminum. They will then react to an Al-test as well as to Finn chambers. Mercury-containing test preparations can react with aluminum, but nowadays plastic-coated Finn chambers are available.

22.5.5.4 Artifacts (Item 12)

Sometimes strong, necrotic reactions are seen and an artifact is suspected. In medicolegal cases, control patches (empty or containing water or petrolatum) are recommended for application simultaneously and in random order.

22.5.6 False-Negative Test Reactions

22.5.6.1 Common Causes

A false-negative reaction is a negative patch test reaction in the presence of contact allergy [94]. The most common causes can be summarized as follows:

1. Insufficient penetration of the allergen
   a. Too low a test concentration for that particular patient
   b. The test substance is not released from the vehicle or retained by the filter paper
   c. Insufficient amount (dose) of test preparation applied; patch test concentration lower than declared [99]
   d. Insufficient occlusion
   e. Duration of contact too brief – the test strip has fallen off or slipped
   f. The test was not applied to the recommended site – the upper back
2. Failure to perform delayed readings; e.g., neomycin and corticosteroids are known to give delayed reactions (see Table 3)
3. The test site has been treated with corticosteroids or irradiated with UV or Grenz rays
4. Systemic treatment with corticosteroids or immunomodulators
5. Allergen is not in active form, insufficiently oxidized (oil of turpentine, rosin compounds, D-limonene) or degraded
6. Compound allergy

Some of them are self-evident and can be predicted and monitored by the dermatologist, while others cannot. Examples of the latter category may arise in the following situations: when testing has been carried out in a refractory or "anergic" phase [97]; when the test does not reproduce the clinical exposure to reach the critical elicitation level (multiple applica-

tions), where some adjuvant factors are present (sweating, friction, pressure, damaged skin); or penetration at the test site (see Sect. 22.1.3, Bioavailability) is lower than that of clinical exposure (eyelids, axillae). A stripped skin technique is recommended in the last case, where the test sites are stripped with tape before application of test preparations.

The differential diagnoses photoallergy and contact urticaria should also be considered. Skin hyporeactivity in relation to patch testing was recently reviewed [100] and it was pointed out that the failure to elicit a response might be due to a faulty immune response, a defective inflammatory response or both. The defective inflammatory response can be evaluated by using a positive control, such as the irritant sodium lauryl sulfate [41] or nonanoic acid [39].

22.5.6.2 Compound Allergy (Item 6)

The term "compound allergy" is used to describe the condition in patients who are patch test positive to formulated products, usually cosmetic creams or topical medicaments, but are test negative to all the ingredients tested individually [101]. This phenomenon can sometimes be explained by irritancy of the original formulation, but in some cases it has been demonstrated that reactivity was due to combination of the ingredients to form reaction products [102, 103]. Another reason might be that the ingredients were patch tested at the usage concentrations, which are too low for many allergens (e.g., MCI/MI, neomycin). Pseudo-compound allergy, due to faulty patch testing technique, is likely to be commoner than true compound allergy. In recent publications [104, 105], several proven or possible compound allergens were listed. The formation of allergenic reaction products can take place within the product ("chemical allergenic reactions") but probably also metabolically in the skin ("biological allergenic reactions") [104]. The topic remains the subject of continuing debate [106, 107]. False-positive and false-negative reactions have recently been reviewed [59].

22.6 Ethnic and Climatic Considerations

Problems and recommendations when patch testing at different climatic environments and in oriental and black populations were recently reviewed [62].

22.7 Effect of Medicaments and Irradiation on Patch Tests

22.7.1 Corticosteroids

Treatment of test sites with topical corticosteroids [108] can give rise to false-negative reactions (see Sect. 22.5.6.1, Common Causes).

Testing a patient on oral corticosteroids always creates uncertainty. The problem was studied 25–30 years ago [109–111] by comparing the intensity of test reactions before and during treatment with corticosteroids (20–40 mg prednisone). Diminution and disappearance of test reactions were noted in several cases, but not regularly. These findings have been interpreted as allowing us to test patients on oral doses equivalent to 20 mg of prednisone without missing any important allergies. However, the test reactions studied were strong (+++), and fairly weak (+) and questionable reactions were not evaluated. In a recent study [112] patch testing with serial dilution tests with nickel, it was found that the total number of positive nickel patch tests decreased significantly when the patients were on 20 mg prednisone compared to on placebo. The threshold concentration to elicit a patch test reaction increased and the overall degree of reactivity to nickel shifted toward weaker reactions. In clinical practice we prefer to defer testing until the patient's dermatitis has cleared. When testing a patient with labile skin there is also the risk of excited-skin syndrome [97]. In selected cases where one or two allergens are strongly suspected, we choose to test for these only, even if the patient is on oral corticosteroids. However, when the dermatitis has cleared, we repeat the test with the whole series to relieve our uncertainty.

22.7.2 Antihistamines

In one study [110], the antihistamine Incidal did not influence reactivity, while in another [113] a decrease in intensity was seen in 6 out of 17 patients after cinnarizine had been administered for 1 week. Oral loratadine was found to reduce patch test reactions; evaluated clinically and echographically [114]. These results also give the dermatologist a feeling of uncertainty, and we prefer either to discontinue antihistamine treatment during testing or to defer testing. However, this contraindication is not universally accepted [115].

22.7.3 Immunomodulators

Topical cyclosporine inhibits test reactions in humans and in animal models [116–118]. As yet there is no comparison of test reactions in allergic patients before and during treatment with orally or parenterally administered cytostatic agents.

22.7.4 Irradiation

It has been shown that irradiation with UVB [119] and Grenz rays [120, 121] reduced the number of Langerhans cells and the intensity of patch test reactions in humans. Repeated suberythema doses of UVB depressed reactivity even at sites shielded during the exposures. This indicates a systemic effect of UVB [119]. Experiments to clarify the mechanism behind these observations have been carried out on experimental animals, but their relevance to humans is not finally settled [122, 123].

22.7.5 Seasonal Variations

Seasonal variations in patch test reactivity is not fully explored. In Israel negative patch test reactivity was found among 55% in winter and 70% in summer among tested patients [124]. In a German study [125] formaldehyde exhibited a distinct increase in questionable or irritant as well as weak-positive reactions associated with dry, cold weather. In a more recent German study [126] it was concluded that ambient temperature and humidity and sodium lauryl sulfate reactivity independently contribute information on individual irritability at the time of patch testing. We recommend avoidance of patch testing on severely tanned persons and that a minimum of 4 weeks after heavy sun exposure should be allowed before testing. At our clinic we refrain from testing during July and August.

22.8 Complications

Reported complications of patch testing are listed below. However, most can be predicted and avoided:

1. Patch test sensitization
2. Irritant reactions from nonstandard allergens or products, brought by the patient
3. Flare of previous or existing dermatitis due to percutaneous absorption of the allergen
4. Subjective complaints
5. Depigmentation, e.g., phenols
6. Pigmentation, sometimes after sunlight exposure of test sites
7. Scars, keloids
8. Granulomas from beryllium, zirconium
9. Anaphylactoid reactions or shock from, e.g., neomycin, bacitracin (regarding penicillin, see below)
10. Infections (bacteria, virus)

22.8.1 Patch Test Sensitization (Item 1)

By definition, a negative patch test reaction followed by a flare-up after 10–20 days, and then a positive reaction after 3 days at retesting, means that sensitization was induced by the patch test procedure. There is a small risk of active sensitization from the standard series and common examples are *para*-phenylenediamine, primula extracts and, in recent years, isothiazolinone [63], acrylates [127], and a bleach accelerator (PBA-1) [128]. The risk, however, is an extremely low one when the testing is carried out according to internationally accepted guidelines.

It must be emphasized that the overall risk–benefit equation of patch testing patients is much in favor of the benefit.

22.8.2 Subjective Complaints (Item 4)

Subjective complaints, e.g., fever, fatigue, indisposition, vomiting, headache, dizziness, were more often reported on the day of test application compared to the day of reading, however with one exception – itch on the back [129]. This itch can mainly be related to positive patch test reactions and irritation from adhesive tapes. However, 10–15% of patients with positive test reactions, but without itch, reported complaints such as tiredness, feeling unwell, headache, shakiness, and light-headedness [130]. Of patients without complaints on the day of application 36% later reported complaints other than itch [131].

22.8.3 Penicillin (Item 9)

Penicillin can give rise to anaphylactoid reactions or shock and is therefore not recommended for routine patch testing (see also Chap. 40). To minimize the risk, which is essential also from a medicolegal point of view, we recommend radioallergosorbent tests, an oral provocation test with half or one tablet of penicillin, and an open test prior to the closed patch test.

22.9 Open Tests

22.9.1 Open Test

"Open test" and "Use test" (see Sect. 22.10, Use Tests) are sometimes used as synonyms and no clear-cut definitions seem to exist. Open testing usually means that a product, as is or dissolved in water or some solvent (e.g., ethanol, acetone, ether), is dropped onto the skin and allowed to spread freely. No occlusion is used.

An open test is recommended as the first step when testing poorly defined or unknown substances or products, such as those brought by the patient (paints, glues, oils, detergents, cleansing agents based on solvents, etc.). The test site should be checked at regular intervals during the first 30–60 min after application, especially when the history indicates immediate reactions or contact urticaria (see Chap. 26). A second reading should be done at 3–4 days.

The usual test site is the volar forearm, but this is less reactive than the back or the upper arms. A negative open test can be explained by insufficient penetration, but indicates that one dares to go on with an occlusive patch test.

22.9.2 Semi-open Test

This method was introduced by Goossens [132] and is mainly used for products – brought by the patients – with suspected irritant properties due to solvents or emulsifiers, e.g., detergents, shampoos, paints, resins, varnishes, glues, waxes, cooling fluids, pharmaceuticals, and cosmetics. The product (solution or suspension) is applied with a cotton swab as is in a small amount (about 15 µL) to an area of 2 × 2 cm. After complete drying it is covered with acrylate tape for 2 days. The site is checked for contact urticaria and at days 2 and 4 for signs of contact eczema.

22.10 Use Tests

22.10.1 Purpose

The original (provocative) use (or usage) tests (PUT) were intended to mimic the actual use situation (repeated open applications) of a formulated product such as a cosmetic, a shampoo, an oil or a topical medicament. A positive result supported the suspicion that the product had caused the patient's dermatitis. The primary goal was not to clarify the nature (allergic or irritant) of the dermatitis – just to reproduce it!

Nowadays these tests are increasingly used to evaluate the clinical significance of ingredient(s) of a formulated product previously found reactive by ordinary patch testing. The concentration of the particular ingredient can be so low that one may wonder whether the positive patch test reaction can explain the patient's dermatitis.

22.10.2 Repeated Open Application Test

The repeated open application test (ROAT) in a standardized form was introduced by Hannuksela and Salo [133]. Test substances, either commercial products, as is, or special test substances (e.g., patch test allergen) are applied twice daily for 7 days to the outer aspect of the upper arm, antecubital fossa or back skin (scapular area). The size of the test area is not crucial: a positive result may appear on a 1 cm × 1 cm area 1–2 days later than on a larger area. The amount of test substance should be approx. 0.1 ml to a 5 cm × 5 cm area and 0.5 ml to a 10 cm × 10 cm area [134, 135].

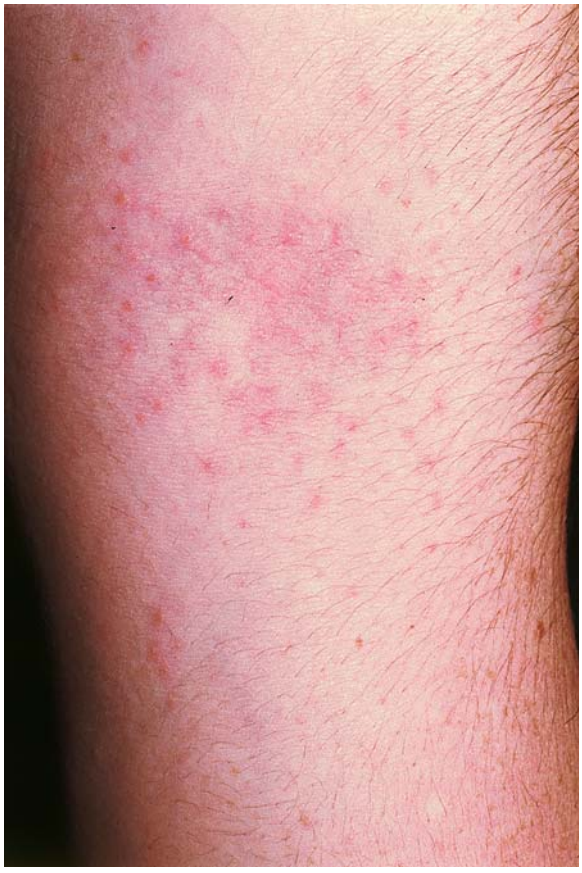


Fig. 7. A positive ROAT on the third day in a patient allergic to iso-eugenol. (Courtesy of P.J. Frosch)

A positive response – eczematous dermatitis – usually appears on days 2–4 (Fig. 7), but it is recommended to extend the applications beyond 7 days in order not to miss late-appearing reactions [136–138]. A refined scheme for scoring of ROAT reactions has recently been presented [139]. The patient is told to stop the application of the test substance(s) when he or she notices a reaction [133].

If a ROAT is carried out with a formulated product, the observed reaction may be due to allergy to an ingredient, but irritancy from other ingredients cannot be excluded. At our clinic we therefore use two coded samples – one containing the allergen and one without it. We instruct the patient to apply one product to the left arm and the other to the right arm, according to a special protocol where the treatments and any observed reaction can be noted. If there is a reaction only at the test site where the allergen-containing product has been applied, we consider the initial patch test reaction relevant. On the other hand, we interpret reactions of the same intensity on both arms as an expression of irritancy.

The value of ROAT has been verified in cases with positive, negative or questionable reactions at initial patch testing [136, 137, 140–142] and in animal studies [138], and it was pointed out that Use testing has significant potential in refinement of the evidence-based diagnosis of clinical relevance [143].

22.11 Noninvasive Techniques

To reduce the well-known interindividual variation when scoring patch test reactions, several attempts have been made to introduce objective bioengineering techniques for assessment. Erythema and skin color can be assessed by laser Doppler flowmetry (LDF), skin reflectance and colorimeters, and edema with calipers, ultrasound and electrical impedance. The advantages and limitations of these methods have been reviewed [144]. These sophisticated techniques cannot replace visual assessment and palpation of test sites by the dermatologist, but are valuable in research work [145]. The topic is further reviewed in Chap. 28.

A significant correlation between visual scoring of patch test reactions and LDF values was claimed by Staberg et al. [146]. The method discriminated between negative and positive reactions, but failed to quantify strong positive reactions. However, in a recent guideline from the standardization group of the European Society of Contact Dermatitis it was stated that laser Doppler perfusion imaging does not directly distinguish between allergic and irritant patch test reactions [147].

It has also been shown that the combination of allergen, vehicle, patch, and tape will cause a transient increase in skin blood flow, even in healthy subjects [80]. An increase was noticed for 1–2 days after removal of the patches, without causing any visual changes. Skin blood flow must be increased three to four times before the naked eye can detect an erythema [148].

22.12 Quality Control of Test Materials

22.12.1 Identification and Purity

As pointed out above (see Sect. 22.2.1.2, Allergens), the dermatologist is recommended to obtain protocols of chemical analyses and data on purity from suppliers of test preparations. Some dermatologists have the laboratory facilities to check the information presented, but most just have to accept it. Especially when "new" allergens are detected, in cases of unexpected multiple reactivity or suspected cross-reactivity, detailed information on purity, chemical identification, and stability of the allergen is indispensable [24]. Some mixes, such as fragrance mix, contain emulsifiers (sorbitan sesquioleate) and a correct retest with ingredients of a mix should thus include the individual fragrances as well as the emulsifier.

22.12.2 Test Preparations Under the Microscope

Light microscope examination (magnification ×100–400) of commercial test preparations with petrolatum as vehicle is usually disappointing. Crystals [149–151] or globules [152] of different size are seen and one wonders how this influences the bioavailability of the allergen. However, in one comparative study no difference in reactivity was found [153].

In the TRUE Test, the allergens are incorporated in hydrophilic gels and are evenly distributed [27].

22.12.3 Fresh Samples

In cases of unexpected negative test reactions, the items listed in Sect. 22.5.6.1, Common Causes should be considered. If the case remains unsolved, it is suggested that a fresh sample of the allergen be purchased from a different supplier.

22.12.4 Adhesive Tapes

A significant development in tape quality has taken place [33, 154] (see also above Sect. 22.2.1.5, Tapes).

22.13 Tests with Unknown Substances

22.13.1 Warning!

A word of warning: totally unknown substances or products should never be applied to human skin! Scarring, necrosis, keloids, pigmentation, depigmentation, systemic effects following percutaneous absorption, and any other complications listed earlier can appear and the dermatologist may be accused of malpractice.

22.13.2 Strategy

When patients bring suspected products or materials from their (work) environment we recommend that adequate product safety data sheets, lists of ingredients, etc. are requested from the manufacturer so that a general impression of the product, ingredients, concentrations, intended use, etc. can be formed. There are usually one or two ingredients that are of interest as suspected allergens, while the rest are well-known substances of proven innocuousness for which detailed information is available. For substances or products where skin contact is unintentional and the dermatitis is a result of misuse or accident, detailed information from the manufacturer is required before any tests are initiated.

22.13.3 Test or Not?

The next step is to look for the suspected allergens. If they are available from suppliers of patch test allergens [21, 23], one can rely on the choice of vehicle and concentration. If one suspects that impurities or contaminants have caused the dermatitis, this can only be discovered via samples of the ingredient from the manufacturer.

If it is an entirely new substance, where no data on toxicity, etc. are available, the patient and dermatologist have to decide how to find an optimal test concentration and vehicle, and to discuss the risk of complications. To minimize the risk, one can start with an open test and, if this is negative, continue with occlusive patch testing. Most allergens are tested in the concentration range 0.01–10% and we usually start with the lowest and raise the concentration when the preceding test is negative. A very practical method is to apply 0.01% and 0.1% for 1 day in a region where the patient can easily remove the patch her- or himself (upper back or upper arm). If severe stinging or burning occurs, he or she should be instructed to remove it immediately. If the test is negative, the concentration can be raised to 1%. Occasionally, the likely irritant or sensitization potential of a chemical may be such that starting with concentrations of 0.001% and 0.01% is advisable, increasing to 0.1% if negative. An alternative is to start with a higher concentration, but with reduced exposure time (5 h) [76]; but this procedure is not sufficiently standardized.

If the test is positive in the patient, one has to demonstrate in unexposed controls that the actual test preparation is nonirritant [66]. Otherwise the observed reaction in the particular patient does not prove allergenicity. It is important to check the pH of products before testing.

When testing products brought by the patient, it is essential to use samples from the actual batch to which the patient has been exposed, but also when testing, for example, cutting fluids, unused products must be tested for comparison. When testing with dilutions, one runs the risk of overlooking true allergens by using over-diluted materials. See also Chap. 50.

22.13.4 Solid Products and Extracts

When a solid product is suspected (textiles, rubber, plants, wood, paper etc.), these can usually be applied as is. Rycroft [94] recommends that the material be tested as wafer-thin, regular-sided, smooth sheets (e.g., rubber) or as finely divided particulates (e.g., woods). A transient so-called pressure effect is sometimes seen when testing with solids. Plants and woods and their extracts constitute special problems, due to variations in the quantity of allergens produced and their availability on the surface. Extracts for testing can be obtained by placing the product or sample in water, synthetic sweat, ethanol, acetone or ether, and heating to 40–50°C. False reactions to non-standardized patch tests have been reviewed by Rycroft [94]. Patch testing with thin-layer chromatograms has been found valuable for products such as textiles, plastics, food, plants, perfumes, drugs, and grease [155].

22.13.5 Cosmetics and Similar Products

For most products with intended use on normal or damaged skin (cosmetics, skin care products, soap, shampoos, detergents, topical medicaments, etc.), detailed predictive testing and clinical and consumer trials have been performed. The results can usually be obtained from the manufacturer. For this category of products, open tests and Use tests probably give more information than an occlusive patch test on the pathogenesis of the patient's dermatitis. Suggestions on concentrations and vehicles can be found in textbooks [38, 52].

22.14 The Future

This chapter concludes with the following list of hopes and needs for the future:

- Diversified vehicles to obtain optimal bioavailability of allergens
- Statements in suppliers' catalogs on the purity and stability of individual allergens
- Decrease of test exposure times (24 h or less) with retained accuracy
- Consensus on the reading, scoring, interpretation, and relevance of weak test reactions
- Objective assessment of test reactions
- Further standardization of Use tests
- Irritancy from test preparations – refinement of predictive methods
- Systemic treatment with immunomodulators and antihistamines– influence on patch test reactivity
- Influence on patch test reactivity due to seasonal variation, latitude, temperature, and humidity.

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