Chapter 3
Patch Testing Methodology

Jean-Marie Lachapelle and Howard I. Maibach

3.1 Historical Background

Jozef Jadassohn is the father of patch testing [1]. At the time of his discovery in 1895, he was professor of dermatology at Breslau University (now Wroclaw in Poland). He initially reported a patient who had developed an eczematous reaction to mercury plasters. He recognized the potential for eczematous reactions to occur in some (sensitized) patients when chemicals were applied to their skin; he thereby introduced the world to the contact test, then referred to as "Funktionelle Hautprüfung" [2].

Bruno Bloch (professor at Basel and Zurich universities) is considered by the international community as an outstanding pioneer in the field of patch testing, continuing and expanding Jadassohn's clinical and experimental work. In some textbooks and papers, patch testing is sometimes quoted as the Jadassohn-Bloch technique.

In retrospect, it is difficult to assess the real place of the patch test procedure for the diagnosis of contact dermatitis between 1895 and the 1960s. Some points seem obvious:

- The technique was used extensively in some European clinics and ignored in others.
- No consensus was reached concerning material, concentrations of allergens, time of reading, reading scores, etc.
- Differential diagnosis between irritant and allergic contact dermatitis was often unclear.

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J.-M. Lachapelle, H. I. Maibach (eds.), *Patch Testing and Prick Testing*,
https://doi.org/10.1007/978-3-030-27099-5_3

It is no exaggeration to say that patch testers were acting like skilled craftsmen. Nevertheless, they provided, step-by-step, new information on contact dermatitis.

During that long period, clinicians often equated a positive patch test with the fulfillment of Koch's postulate [3]. They inferred that because a patient with dermatitis was shown to develop a positive reaction to compound X, the same compound must therefore be the cause of the dermatitis. In other words, there was little attempt to interpret correctly patch test results. Relevance was a neglected concept.

Credit must be paid to the former members of the International Contact Dermatitis Research Group (ICDRG) for their invaluable contribution to the standardization and interpretation of patch test procedures. Their efforts have encouraged many dermatologists, immunologists, chemists, and pharmacists.

Patch testing is now a well-recognized diagnostic tool, constantly being refined.

3.2 Definition and Aims

General considerations need to be pointed out about patch testing methodology.

First of all, patch testing aims to reproduce "in miniature" an eczematous reaction by applying allergens under occlusion on intact skin of patients suspected to be allergic. It is the in vivo visualization of the elicitation phase of a delayed-type hypersensitivity (type IV) reaction. Therefore, it is not intended to reflect an irritant reaction, considering its occurrence an untoward event, to be avoided by any means.

It is primarily aimed to detect "culprit" allergens in ACD, but its field of interest has been extended to some cutaneous systemic drug eruptions (see Chap. 12). It is submitted to general rules of evidence-based medicine applied to investigative procedures [4].

3.2.1 Requirements for an Ideal Patch Testing Procedure

Several requirements are advocated to reach an ideal patch testing procedure [5]:

- A perfect patch test should give neither false-positive nor false-negative reactions.
- It should cause as few adverse reactions as possible, particularly no patch test sensitization. False-positive, false-negative, and adverse reactions are all dose dependent.
- Simplicity, safety, and low cost of patch testing methodology are highly recommended.
- Patch testing must have a good positive predictive value, defined as the percentage of true cases in those with a positive test, when this test is used in a given population.
- Patch testing must also have a good negative predictive value, defined as the percentage of disease-free individuals in those with a negative test, when this test is used in a given population.

- Positive and negative predictive values depend on several parameters, which cannot be dissociated:
  - Sensitivity defined as the probability of a positive test in an individual with the disease
  - Specificity defined as the probability of a negative test in an individual without the disease
  - The prevalence of the disease in the given population
- A good screening test has also to be reliable, which means that it has to be precise and must have good intraobserver and interobserver reproducibility.

3.2.2 Is Patch Testing the "Gold Standard" to Investigate Patients with Allergic Contact Dermatitis?

"Tests reactions properly performed and interpreted are acceptable as scientific proof of a state of allergic sensitization."

The question is: can Rietschel's statement [5] be fulfilled by patch testing? At present the answer is as follows: patch testing even with optimum concentration and vehicle for a given allergen is, like most diagnostic tests, neither 100% sensitive nor 100% specific [6].

Despite its limitations, patch testing is by no means the cornerstone of the diagnostic procedure.

Its reliability is increased if it is sustained by additional tools of investigation, such as the following:

- Use of complementary testing approaches, that is, semi-open tests, ROATs, etc. (see Chap. 7)
- Other methods for assessment of clinical relevance of patch test reactions (see Chap. 8)

Conventional patch testing, as described in this chapter, is used worldwide. Allergens are produced and purchased separately from patch test units plus tapes.

TRUE TEST is an alternative way of patch testing described in Chap. 6.

3.3 Patch Test Units

3.3.1 Nonchamber Patch Tests

Various types of nonchamber patch test material units have been long available. Their characteristics were quite different from one brand to another.

Today, there are two nonchamber tests marketed:

- The Curatest F® Lohmann and Raucher, Rengsdorf, Germany, used mainly by some German dermatologists, due to its inexpensiveness
- The Torii Patch Test®, Torii Pharmaceutical Co, Chuo-Ku, Tokyo, Japan, routinely used by Japanese colleagues

Most dermatoallergologists are currently using chamber tests (see Sects. 3.3.2 and 3.3.3).

3.3.2 Chamber Patch Tests

3.3.2.1 Finn Chambers

Finn Chambers were developed by Professor Veikko Pirilä, a founding member of the ICDRG. Professor Pirilä's family ran the business successfully for more than 30 years under the company name Epitest Ltd Oy. (Tuusula, Finland). Keys to its success were a focus on high quality and customer service. Because of Epitest's strong specialization and technology in the diagnosis of skin allergy, its reach internationally was at the core of its business. In 2008, SmartPractice purchased Epitest and moved the facility to Phoenix, Arizona, where it is manufactured today in an ISO 13485:2003 certified facility for the design, manufacture, storage, and distribution of patch test allergen delivery systems.

Finn Chambers® is a patch test device (Fig. 3.1) which provides good occlusion because of the chamber design. Finn Chambers are available as loose chambers (8, 12, and 18 mm) which allows the clinician to select their preferred tape or pre-mounted on Scanpor tape (Actavis Norway AS/Norgesplaster, Vennesla, Norway) (Fig. 3.2). The chambers are made of aluminum but are also available with a polypropylene coating. The 12 and 18 mm (inner diameter) are not intended to be used for the practice of patch testing but for specific research projects. The 8 mm (inner diameter) Finn Chambers is therefore the one to be used. The 8 mm inner diameter provides a 50 mm^2 area. Finn Chambers on Scanpor are available in strips of 10 (2 × 5), 5 (1 × 5), and single chambers. The strips of ten chambers are practical when testing with a large number of substances, for example, with routine tests. Smaller strips are suitable for small test series and individual tests.

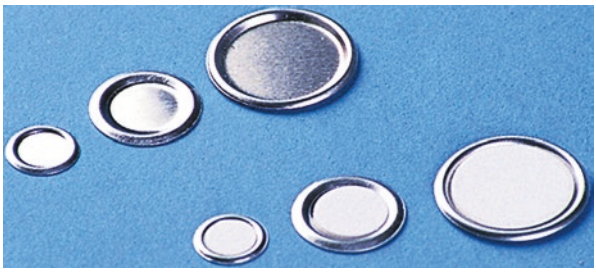

Fig. 3.1 Finn Chambers® of different sizes without and with filter paper

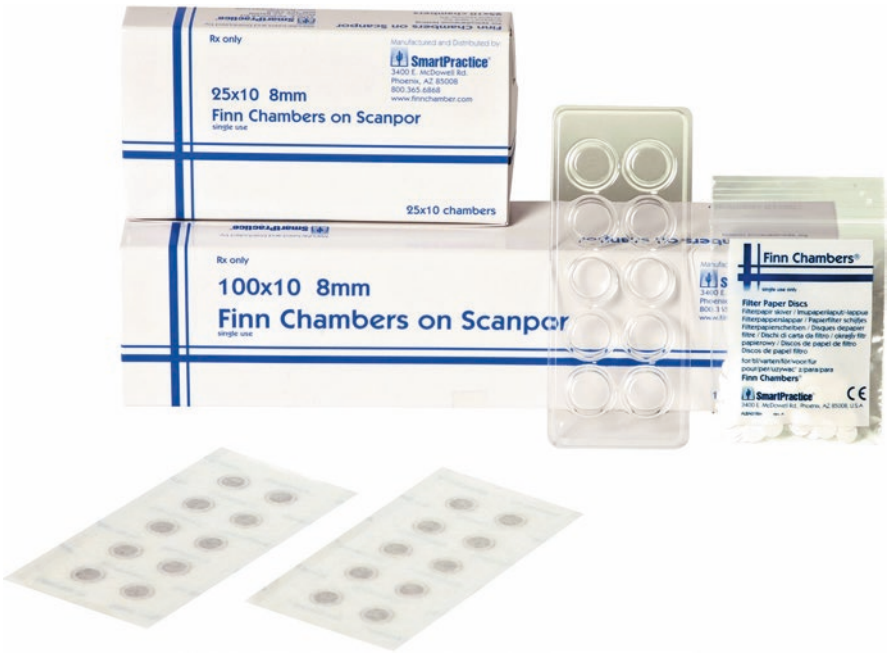

Fig. 3.2 Detailed presentation of the Finn Chambers® on Scanpor®

Test substances are usually applied in petrolatum. The concentrations of allergens in most standard series are suitable for Finn Chambers. When using uncommon test substances, the administering physician should choose carefully the substances and concentrations. It is advisable to use low concentrations with irritating test substances due to tight occlusion provided by the chamber. Most commercial test substances are suitable for Finn Chambers. The substances incorporated in a semisolid base are applied directly into the chamber (Fig. 3.3). For liquids, a filter paper disk is placed in the chamber and saturated with the liquid. For locating the test sites, a special reading plate is recommended.

Allergic reactions to aluminum and Scanpor tape are rare [6, 7]. However, occasional cases of contact sensitivity to aluminum, for example, due to vaccination or hyposensitization of allergic rhinitis patients with aluminum precipitated antigens, have been reported. Many vaccines can be incriminated, for example, those against diphtheria, tetanus, and poliomyelitis (but also many others). In particular, they can provoke (mainly in children) the formation of dermal nodules of allergic nature, which are sometimes persistent for long periods of time. In those cases, allergic contact dermatitis to all Finn Chambers applied on the skin may occur, being stronger at their periphery (edge effect). Polypropylene-coated chambers should be used in these cases. It is the same when testing with mercuric compounds and particularly with mercuric solutions which dissolve the aluminum [8]. As with all patch testing, the skin may react to the removal of the tests with a slight mechanical irritation, indicated by erythema on the area covered by the tape.

Fig. 3.3 Filling a Finn Chamber® with thiuram mix dispersed in petrolatum

Application of the Test Substances Mark identification on the top of each tape to show the order of the test substances throughout the testing procedure. Remove the protective paper and place the tape on the desk or tray with the chambers up (Fig. 3.4). Keep a narrow strip of the protective paper on the tape until the tape has been attached onto the skin.

Semisolids (e.g., petrolatum as the vehicle) are applied directly into the chamber, filling more than half the chamber volume (a bar of about 5–6 mm if the diameter is 2 mm). Do not use filter paper disks with semisolids. For liquids place a filter paper disk in the chamber. Moisten the disk thoroughly without surplus. Excess liquid should be removed, for example, with porous paper. Place the test onto the skin within a few minutes. Do not let the filter paper disk dry because this may result in

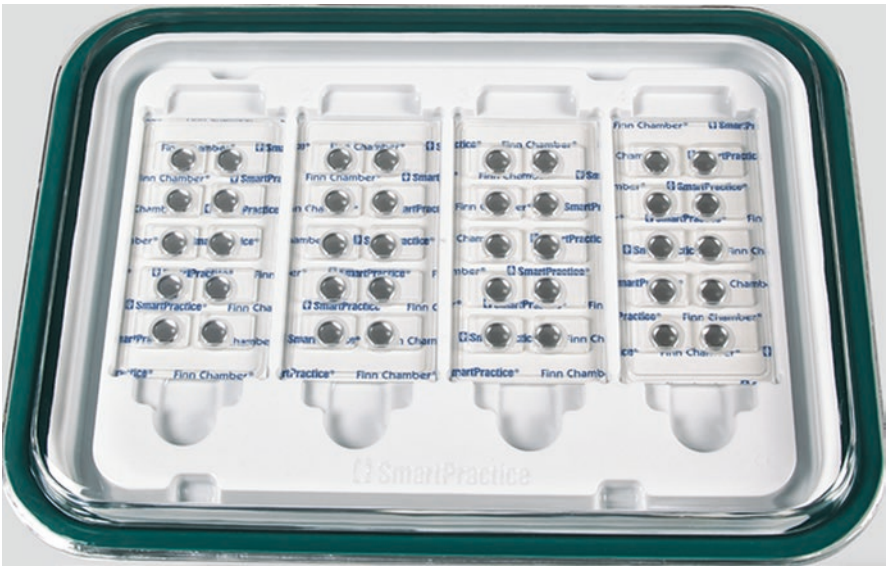

Fig. 3.4 Strips of Finn Chambers can be stored in a tray kept in a refrigerator

weak or false-negative reactions. A small dab of petrolatum under the filter paper may help keep the filter paper in place during application.

Finn Chambers should be applied to the back starting with the lower part and pressing the chambers from below to let the air escape. After having applied the tape this way, press each chamber containing a semisolid gently with the finger to get an even distribution of the test substance. Rub the tape gently but firmly with the palm against the skin, especially on the corners, to ensure good adherence. The tests are removed after 48 h. Immediately after removal, the clinician should check for the ring-shaped depression around each test to verify occlusion and validate the test, especially in the case of negative reactions.

Finn Chambers® on *Scanpor*® *tape* is manufactured and distributed worldwide through SmartPractice® 3400 E. McDowell Road, Phoenix, Arizona 85008 (phone: 1-800-365-6868; fax: 1-800-926-4568; e-mail: info@allerderm.com; website, http://www.finnchamber.com).

3.3.3 Plastic Square Chambers

Two companies (Chemotechnique and SmartPractice) have models of square plastic chambers as an alternative. The square shape of the chambers is intended theoretically to differentiate allergic and irritant reactions. Both plastic square chambers have similar characteristics (with minimal differences) and the choice is dictated by geographical availability.

3.3.3.1 IQ Square Chambers Chemotechnique

There are three IQ chambers available: the original IQ chamber, the IQ Ultra chamber, and the IQ Ultimate chamber (Fig. 3.5).

Features/Benefits of IQ Chamber, the Original Chamber
Each IQ chamber unit is composed of ten pieces of injection-molded polyethylene chambers mounted on hypoallergenic surgical tape attached to a stiff plastic cover

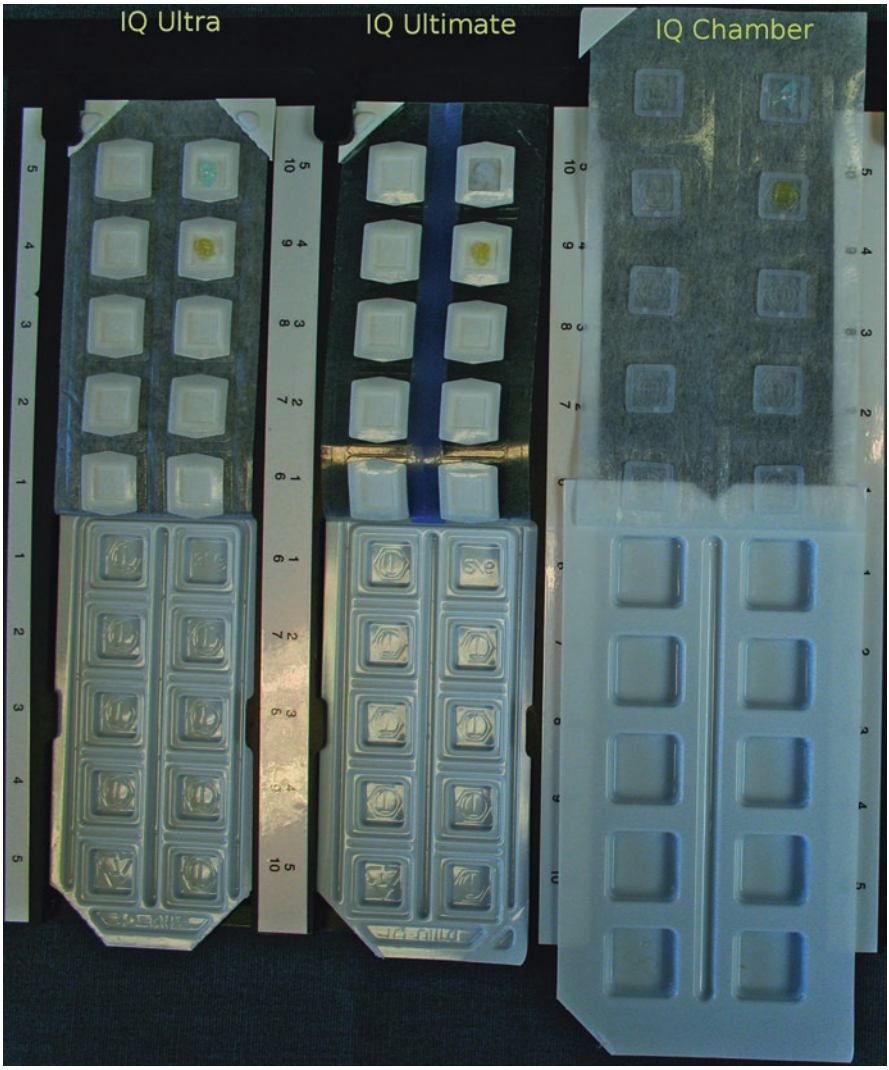

Fig. 3.5 The three IQ chambers® Chemotechnique. (From left to right: IQ Ultra®, IQ Ultimate®, and IQ chambers® [9])

with ten compartments corresponding to each of the ten chambers on the tape (Fig. 3.6). The cover makes it possible to reattach the tape to the cover after filling the chambers with hapten preparations. The volume of the chamber is 65 μL, and the inside area of the chamber is 81 mm^2. The width of the tape is 68 mm, and the length is 142 mm.

The chamber unit contains no aluminum and is therefore environmentally safe and recyclable, and due to its inert feature, it has the same advantages as the IQ Ultra® and IQ Ultimate® patch test units. The IQ chamber application device makes advance filling of test substances easy and makes the routines of patch test preparations efficient, thus saving valuable time.

Features/Benefits of IQ Ultra® and IQ Ultimate® Patch Test Units
The patch test units have important advances and are based on laminated tapes, and the products have valid patents in numerous countries, contain no aluminum, and are therefore environmentally safe and recyclable. Undesired side effects in the form of allergic reactions to the test unit itself are avoided due to the chemical stability of the polyethylene plastic. The effect of reactive test substances on the test chamber which may result in secondary toxic reactions during the patch test is also avoided due to polyethylene's chemical resistance to these types of substances. By using inert plastic material such as polyethylene, the risk of inactivation, modification, and absorption of the hapten during contact with the surface of the test chamber is avoided.

Each unit contains two rows of five chambers/row, and each chamber has a filter paper incorporated which eliminates adding loose filter papers.

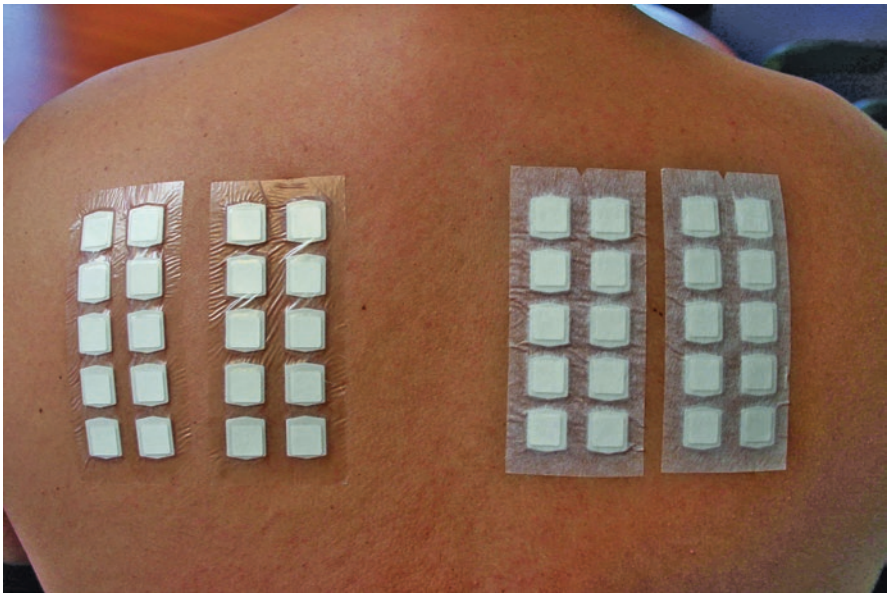

Fig. 3.6 Application of IQ chambers on the skin

The rim of each chamber has an adhesive layer to optimize adhesion to the skin and to eliminate leakage. This makes IQ Ultra® and IQ Ultimate® a closed-cell system enhancing occlusion and confining the test reaction within the chamber parameter. The opening of the chamber is square to make it easier to differentiate between allergic and irritant reactions.

The size of the IQ Ultra® and IQ Ultimate® is small to allow the application of multiple test units to patients' backs. The chambers are made of thin and soft polyethylene foam material thus making them even more comfortable for the patients. The width of the tape is 52 mm, and the length is 118 mm. The volume of the chamber is 32 μL, and the inside area is 64 mm^2.

The highest quality hypoallergenic surgical tape is used for the IQ Ultra® and corresponding thin elastic polyurethane film for the IQ Ultimate®. Each strip of ten chambers of the test units is attached to a protective plastic cover with corresponding compartments which makes it possible to reattach the tape after advance filling of the chambers with the haptens.

IQ Ultimate® uses a water-resistant, transparent, and elastic thin carrier tape made of polyurethane film, making it possible for the patient to avoid the restrictions that normally are a drawback during patch testing. This is the only difference between IQ Ultra® and IQ Ultimate® patch test units.

The IQ Ultra®/IQ Ultimate® application device makes advance filling of test substances easy and makes the routines of patch test preparations efficient thus saving valuable time.

The IQ chambers, the IQ Ultra chamber, and the IQ Ultimate chamber are marketed by Chemotechnique.

3.3.3.2 allergEAZE Chambers SmartPractice

There are now two allergEAZE chambers available: the allergEAZE patch test chamber and the allergEAZE clear patch test chamber.

3.3.3.3 allergEAZE Patch Test Chamber

The allergEAZE patch test chambers are designed as an allergen delivery system and provide a means to place allergens or allergen mixes in contact with the surface of the skin:

- The allergEAZE patch test chambers are designed for excellent occlusion with a small chamber area, ideal depth, increased spacing, and raised edges.
- Chambers are easy to preload due to the removable protective paper covering the adhesive.
- Panels are user-friendly with precut registration holes and prefixed filter paper.
- Patients can be comfortable wearing allergEAZE patch test chambers due to rounded corners (panel and chambers) and flexible material. Panel adhesive and material are also nonirritating and nonsensitizing.

- The allergEAZE patch test chambers meet the regulatory requirements of the US Food and Drug Administration for a medical device and bear the CE mark.

Its specifications are as follows:

- The allergEAZE patch test chambers are available in boxes of 100 panels, with 10 chambers per panel.
- Each panel contains two rows of five square (8 mm × 8 mm^2) chambers, which are made of pharmaceutical polyethylene terephthalate.
- The square chambers are mounted on a rectangular patch (120 mm × 60 mm) of nonwoven polyester.
- The panel adhesive is an acrylic copolymer emulsion, similar to many surgical tape adhesives.
- Chamber volume is 40 μL.
- Spacing between rows is 16 mm.
- Spacing between chambers is 9 mm.

3.3.3.4 allergEAZE Clear Patch Test Chamber

allergEAZE clear polyurethane panel allows freedom of movement and provides excellent adhesion, even when damp. It is designed to provide optimum occlusion and enhanced patient comfort. The allergEAZE clear open chamber design allows for easy panel setup:

- The allergEAZE clear patch test chambers are designed to provide excellent occlusion with increased comfort.
- The small chamber area, depth and enhanced spacing between chambers, and raised chamber edge facilitate allergen contact with the skin.
- Each patch test panel consists of two rows of five square (8 mm × 8 mm) pharmaceutical polyethylene terephthalate (PET).
- The chambers are mounted on a rectangular patch (120 mm × 60 mm) made of polyurethane film.
- Pharmaceutical polyethylene terephthalate (PET) chambers eliminate adverse reactions that may be caused by metal chambers.
- The panel and its adhesive are nonirritating, nonsensitizing, and covered with a protective paper liner that is easily removed.
- The transparent film panel material flexes to allow freedom of movement.

Its specifications are as follows (Fig. 3.7):

- Chamber volume is 40 μL.
- Spacing between chambers is 9 mm.
- Spacing between rows is 16 mm.
- The panel adhesive is an acrylic copolymer emulsion, consistent with most state-of-the-art surgical tapes.
- Removable adhesive strip and blue-colored finger lifts on both ends for improved patch placement control.

- Precut registration holes.
- Prefixed filter paper.
- Raised, rounded panel and chamber corners.

allergEAZE© patch test chambers (Fig. 3.8) are available worldwide through SmartPractice®.

Fig. 3.7 AllergEAZE clear patch test chambers

Fig. 3.8 Prefilled patch test panel

3.3.4 Reinforcement of Patch Test Units

The patch test units may be reinforced by extra tape applied at the margins or covering the total surface of the original tape and extending over its margins. The procedure is particularly recommended in hot climate to avoid detachment of the strips. Its use is also advisable but facultative in temperate climates.

Various tapes are convenient for this purpose: Fixomull® Beiersdorf, Scanpor® Alpharma, and Micropore® 3 M. Liquid adhesives such as Matisol® are also used to help secure patch test panels.

3.4 A General Overview of Allergens

3.4.1 Allergens

The first standardized allergens (in the 1970s) were manufactured and marketed by Trolab in Denmark. At that time, the company worked in close cooperation with former ICDRG members.

Today, the standard and/or additional series of patch test allergens are sold by two companies, following the advice of the ICDRG or other international and/or national groups.

The coordinates of companies involved in the field are detailed in Appendix C.

The vast majority of allergens of the baseline and/or additional series are dispersed in white petrolatum (Fig. 3.9). The petrolatum used as a vehicle is considered to be the purest marketed [10].

Fig. 3.9 Plastic clinical trays provide convenient temporary storage of allergens and patch test panels

White petrolatum can be considered inert when applied onto the skin but may be responsible in exceptional cases for an irritant reaction. The semisolid allergens may be prepared in advance. The plastic trays are refrigerated. There is a limitation to these practices: fragrances, acrylates, methacrylates, and isocyanates, due to their volatility. Therefore, they need to be prepared extemporaneously, i.e., immediately before application on the skin.

A few allergens cannot be dispersed in petrolatum due to their chemical instability. This is the reason why they are supplied in aqueous solutions. Some examples include formaldehyde, Cl + Me-isothiazolinone, phenylmercuric acetate, cocamidopropyl betaine, ammonium thioglycolate, chlorhexidine digluconate, benzalkonium chloride, etc. Hydrocortisone-17-butyrate is dissolved in ethanol 70%. An extensive list of chemicals not available in marketed lists of allergens has been gathered in de Groot's textbook [11], which provides useful and accurate information about test concentrations and vehicles. Vehicles that are referred are water, acetone, ethanol 70%, methyl ethyl ketone, olive oil, and petrolatum. Liquid vehicles are recommended for some allergens, since they facilitate penetration into the skin, but they have also 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 and in research projects.

In textbooks on contact dermatitis and patch testing and in suppliers' catalogs, the concentration of an allergen is given as a percentage. In one catalog [9], molality (*m*) is given together with percentage (weight/weight). The traditional method of presenting concentrations as a percentage is simple and probably practical but has been questioned [12], 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 number of moles applied that is of interest.

Finding the ideal test concentration is complicated; the currently recommended concentrations have been determined taking many important factors into account.

The general principle has been to use the highest concentration that does not provoke any irritation when testing in groups of patients enrolled in prospective joint studies. Doing so, false-positive (irritant) and false-negative (due to a too low concentration) reactions are avoided. Therefore, the choice of the concentration tends to reach an ideal (but sometimes unattainable) compromise.

The substances with petrolatum vehicle are supplied in 5-mL polypropylene syringes or in 5-mL color-coded polypropylene tubes, while those in a liquid solution are supplied in 10-mL polypropylene dropper bottles.

The allergens should be kept to minimize degradation. In accordance with their stability, it is recommended that all substances should be renewed according to the expiry stated on their labels. Nonmarketed allergens are prepared freshly; allergens diluted in liquids should be kept in dark bottles.

3.4.2 Bioavailability of Allergens

To obtain optimal bioavailability of an allergen, one can influence the following five parameters:

- Intrinsic penetration capacity
- Concentration
- 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.

3.4.3 Quality Control of Allergens

The dermatologist is recommended to obtain protocols of chemical analyses and data on purity from suppliers of test preparations. We encourage allergen suppliers to make the information readily available.

3.4.4 Appropriate Amounts of Petrolatum to Be Applied at Patch Testing

The prerequisite for a patch test is the requirement that the whole test area is covered with the allergen.

The ideal test situation is (a) the test area completely covered with the test preparations and (b) without any spreading outside the test area, to avoid overlapping at reading.

Fischer and Maibach anticipated this practical issue [13, 14] when elaborating TRUE TEST®.

There were no recommendations related to the amounts of petrolatum to be applied. Bruze et al. [15] and Isaksson et al. [16] have conducted studies on behalf of the ESCD to answer this important question. After several trials, they concluded that, when using the Finn Chambers, the optimal dose for pet preparation was 20 mg (Fig. 3.10). Similar studies were conducted with the van der Bend Chamber. The authors could not draw a definite conclusion, but a minimal dose of 35 mg seems advisable. Similar studies do not exist for the other plastic square chambers.

This dose has been illustrated by Elsner and Schliemann [17].

3.4.4.1 TruVol®: A Recent Ancillary Item

SmartPractice has recently developed the TruVol® precision allergen dispenser (Fig. 3.11). It provides a standardized dose of allergen every time! Simply attach Truvol to allergEAZE petrolatum-based allergen syringes and get more consistent patch test results without the guesswork!

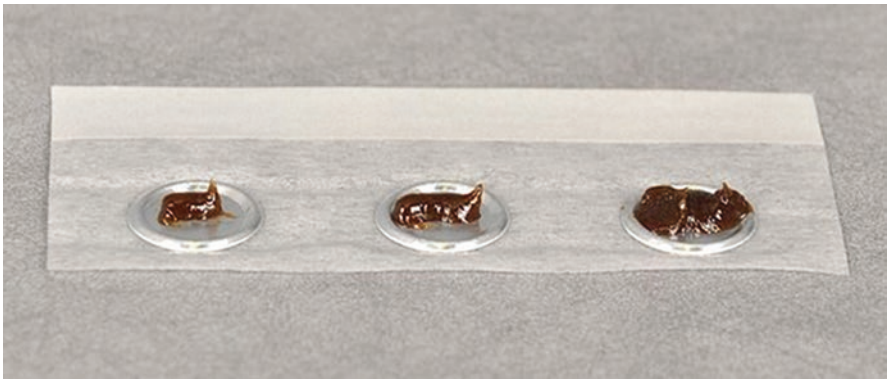

Fig. 3.10 Doses of petrolatum allergen preparations in Finn Chambers with 10 mg (left), 20 mg (center), and 40 mg (right) of a petrolatum allergen preparation; 20 mg is the correct dose. Doses that are too low may lead to unreliable or false-negative readings and doses that are too high, to spreading of the allergen

Fig. 3.11 TruVol® with syringe (lateral side)

- Accurately delivers a standardized dose (20 µl) per use
- Helps save money by eliminating overfilled chambers
- Works with most petrolatum allergen syringes

(SmartPractice Canada: Allergen EAZE catalog 2018/2019)

3.4.5 Appropriate Amounts of Liquids to Be Applied at Patch Testing

The prerequisites are similar to those described in Sect. 3.4.4.

The conclusions are clear-cut:

- For water solutions, the Finn Chambers is highly recommended. The amount of liquid, delivered by a calibrated pipette, is 20 µL.
- For ethanol and acetone solutions, the amount of liquid that fulfills requirements is 20 µL.

The chambers are immediately applied onto the skin to avoid evaporation of liquids. No irritation from ethanol or acetone is noted.

3.5 Specific Recommendations When Considering Patch Testing Patients

Some general rules as well as recommendations have to be taken into consideration when patch testing patients. This seems useful in practice.

3.5.1 Patch Testing on Intact Skin Is Critical

The general rule is to avoid by any means patch testing at skin sites presenting currently or recently any type of dermatitis, to avoid false-positive reactions and/or the angry back syndrome (see Sect. 3.14.2). This includes not only contact dermatitis (either primary or "id" reaction) but also atopic dermatitis, nummular eczema, and seborrheic dermatitis. Similar considerations are applied to various skin diseases, such as pityriasis versicolor, psoriasis, lichen ruber planus, pityriasis rubra pilaris, pityriasis lichenoides, pityriasis rosea, Darier's disease, and others. Complete healing or remission is needed before patch testing.

Atopic dermatitis is of special concern: it is up to the clinician to decide when patch testing can be performed. A good criterion is perhaps to consider that the patient is free of any inflammatory phase of the disease, does not require any "active" topical drugs (tacrolimus, pimecrolimus, corticosteroids), and is exclusively treated by emollients, useful for treating xerosis.

3.5.2 Medicaments and Patch Testing

3.5.2.1 Corticosteroids

Treatment of test sites with topical corticosteroids [18] can give rise to false-negative reactions.

Testing a patient on oral corticosteroids creates uncertainty. The problem was studied 25–30 years ago [19] by comparing the intensity of test reactions before and during treatment with corticosteroids (20–40 mg prednisone). Diminution and disappearance of test reactions were irregularly noted in several cases. 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 questionable reactions were not evaluated. Another study called this dogma in question [20]. When patch

testing with serial dilution tests with nickel, it was found that the total number of nickel patch tests decreased significantly when the patients were on 20 mg of prednisone compared to those on placebo. The threshold concentration to elicit a patch test reaction increased, and the overall degree of reactivity to nickel shifted toward weaker reactions. The last study referring to this problem was published in 2008 [21]; the authors concluded that successful testing during concomitant low dose of prednisolone was achieved. Nevertheless, we conclude that interpretation of patch test results in patients treated with corticosteroids needs great caution; repeating patch testing after treatment discontinuation can be useful when in doubt.

3.5.2.2 Antihistamines

The interference of antihistamines on patch test results is a subject of controversy.

Few studies refer to this specific question. In one study, oral loratadine reduced patch test reactions, evaluated clinically and echographically [22]. These results also give the dermatologist a feeling of uncertainty. Therefore, in most clinics, antihistamine treatment is discontinued during testing, which is deferred. However, this option is not universally accepted [23].

3.5.2.3 Immunomodulators

So far, there is little data available on the reliability of patch testing in patients taking immunosuppressive agents other than corticosteroids. A recent study [24] concluded that patch test reactions can be elicited in patients taking azathioprine, cyclosporine, infliximab, adalimumab, etanercept, methotrexate, mycophenolate mofetil, and tacrolimus.

However, it remains unclear what effect these immunosuppressive drugs may have on suppressing allergic patch test reactions, and further studies should be carried out to determine the reliability of testing in these circumstances.

Analyzing the results of this publication, our viewpoint is that false-negatives can occur and that only positive reactions are meaningful.

Topical immunomodulators (tacrolimus, pimecrolimus) are almost exclusively used in the treatment of atopic dermatitis.

3.5.2.4 Irradiation

Irradiation with UVB [25] and Grenz rays [26] 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 [25].

From a practical viewpoint, avoid patch testing on markedly tanned persons, and a minimum of 4 weeks after heavy sun exposure should be allowed before testing.

3.5.3 Pregnancy and Patch Testing

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. Therefore, the general rule adopted by the members of the ICDRG is: do not test pregnant women, taking into account medicolegal considerations, not scientific ones. In some clinics, this view is also adopted for lactating women.

3.5.4 Patch Testing in Children

In children, patch testing has the same indications as in adults. Most authors agree that patch testing in children is safe, and the only problem being mainly technical because of the small patch test surface [27]. It is usually advised to use the 8-mm Finn Chambers. Reinforcement of patch test units is suitable due to hypermobility of children, which may result in loss of patch test materials.

Instructions should be given to parents about the test procedure and the measures that may be taken to optimize the patch test conditions [27].

There has been much debate about the concentrations of allergens to be used in children. Some authors have recommended lower concentrations, but nowadays, there is a general consensus of using the same concentrations as in adults. Nevertheless, it is well known that irritant reactions from patch testing are more frequent in children. When in doubt, the clinician is advised to retest with a lower test concentration. The problem is raised mainly in children under the age of 5. Similarly, most authors agree upon the fact of applying in children the classical standard series, as well as additional series, if needed. Some authors have advocated the use of a limited series of patch tests [28] adapted for the usually more restricted environment of children, but there is no general agreement about this opinion.

Several recent papers, worthwhile to be consulted, make the point about this difficult and controversial issue [29–32].

3.6 Application of Patch Tests on the Skin: Some Practical Suggestions

The accurate application of patch test units onto the skin is a prerequisite to ensure optimal reading and interpretation of patch test results.

Some suggestions to optimize the technique of application are listed below.

3.6.1 Test Sites

The preferred site is the upper back (Fig. 3.6). For a small number of allergens, for example, at retesting, the outer aspect of the upper arm is also acceptable. False-negative results can be obtained when testing on the lower back or on the volar forearms.

The avoidance of applying patch tests on nevus or seborrheic keratoses is self-evident, but not always respected. When lesions are numerous and do not allow proper application of tests, the choice of another patch testing site is encouraged.

3.6.2 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. It is advisable to clip hair 1 or 2 days before patch testing, whenever possible. This procedure does not offer absolute guarantee in terms of skin irritation.

3.6.3 Degreasing of Test Site

In cases of oily skin, gentle treatment with ethanol or other mild solvents could be recommended. The solvent must evaporate before the test strips are applied. Practically, no degreasing is performed in European clinics.

3.6.4 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.

3.6.5 Instructions to Patients

Patients should be informed 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 and discomfort. Occasional loosening of patches can occur; frequent check

by the patient is advisable during the application period. Reinforcement of test strips is recommended (material delivered to the patient when patch tests are applied). Such written instructions and guidelines for patients are highly recommendable.

3.7 Reading Time

Reading is the most important step in the patch test procedure. It should be done by the clinician himself or herself and interpreted carefully. There is a need for constructive dialogue between clinician and patient. This requires time, skill, and perseverance to achieve the specific aim of tracing the source of allergy. The reading allows the clinician to complete past and current history in each individual patient. It cannot be dissociated from the search for relevance or nonrelevance (see Chap. 8). A decision must be made about whether to continue the investigations by additional patch tests and/or other tests such as repeated open application test (ROAT), for instance (see Sect. 7.4). Therefore, it may be considered that in many cases the reading is only an intermediate step in the investigatory process.

There are controversies in the literature regarding the optimal reading time, as discussed in the following sections. Therefore, the "best" reading time is always a matter of compromise.

3.7.1 Standard Patch Test Occlusion and Reading Time

The standard patch test technique involves application of the test allergen strips onto the skin under occlusion for 2 days (48 h). Conventionally, patch test reading is performed 15–30 min after the removal of the occlusive strips to allow the transient erythema caused by the occlusive effects of allergens and plasters to subside. This will eliminate false-positive reactions. The 2-day occlusion ensures that adequate allergen penetration has occurred to provoke an allergic contact reaction on the test site.

Reading is further performed at day 3, 4, and 7 after occlusion (i.e., 1, 2, and 5 days after the removal of the patch test strips) thereafter.

3.7.2 Conventional Patch Test Reading Time

Conventionally, patch test reading is performed in most patch test clinics at day 2 when the patch test strips are removed and again at day 4. Allergic reactions are then identified and checked for relevance. Patients are then instructed to report back to

the dermatologist if any additional positive reaction appears at day 5 or beyond to detect any late reactors or sensitization that may have occurred.

3.7.3 Reading at Day 2, Day 3, and Day 4

Positive reactions at day 2 after the removal of the test strips should not be considered positive unless the reactions persist into day 3 and beyond [33]. True allergic reactions should persist or may appear at days 3 and 4.

3.7.4 Reading at Day 7

Reactions occurring at day 7 or later are regarded as late reactions. Some allergens are "late reactors," and delayed positive reactions may appear at day 5 or later. Examples of such late reactors include neomycin, corticosteroids, nickel sulfate, p-t-butylphenol formaldehyde resin, Cl + Me isothiazolinone, and gold thiosulfate. This is particularly true for corticosteroids: in many instances, when readings are made only on day 2 and day 4, some positive reactions are missed, since they appear later [34]. In some cases, late reactions reflect active sensitization (see Sect. 3.14.1), but this latter interpretation requires cautious appreciation. To corroborate this point, a late reaction to paraphenylenediamine is often considered an active sensitization. It is certainly not always the case [35].

3.7.5 Single Reading Versus Multiple Reading

Single reading carried out at day 2 may result in false-negative reactions. Reading of diagnostic patch test should not cease at day 2, as numerous allergic reactions need more time to evolve to become positive. Further recommended reading times include day 3, day 4, and day 7. In most patch test clinics around the world, patch test reading is carried out at day 4.

3.7.6 Day 3 Versus Day 4 Reading

Day 4 reading yields better results (fewer false-negative results) than day 3 reading alone because some positive results appear only after day 3 [36].

At this stage, it must be recalled that several exogenous factors, for example, surface concentration of the allergen, total amount applied, penetration properties of

the allergens and the vehicle, patch test technique, and allergen exposure time, are major determinants in the elicitation of positive patch test reactions [37].

3.7.7 One-Day Occlusion Versus Two-Day Occlusion

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). A 1-day exposure would reduce the number of questionable reactions. No definite conclusion can be drawn from the studies published to date [38].

In tropical climates where the environmental temperature and humidity are high, 1-day occlusion may be adequate to elicit positive patch test reactions. The shorter occlusion will be more tolerable to the patients and is more likely to improve compliance and cooperation from patients to accept the patch test procedure [38, 39].

3.7.8 Marking the Skin

When several readings are performed, it is useful to "mark" the patch test sites.

The Chemotechnique Skin Marker is a suitable marking pen designed for marking efficiently the patch test sites. Its content is methylrosanilin (gentian violet), 1%; silver nitrate, 10%; and denatured ethanol/aqua in equal parts at 100%. Duration of the marking is approximately 5–7 days. Marking may be repeated to ensure durable staining.

For dark skin types or when a nonstaining ink is required, the Chemotechnique UV Skin Marker (yellow fluorescent ink) provides a good alternative. Its content is disulfonic acid derivate of stilbene, 2%, and dimethyl sulfoxide (DMSO)/denatured ethanol in equal parts at 100%. DMSO increases fixation of the ink to the outer layer of the skin. The tip has tapered edges, which facilitate precise markings. The duration of the marking is approximately 5–7 days. The UV Skin Marker requires the use of a Wood's light at each reading session (Fig. 3.12). A similar marker has been developed by SmartPractice.

Some authors do not use skin markers but a reading plate (i.e., reading plate for Finn Chambers on Scanpor Epitest), which is a real template for the patch skin sites.

A practical, clean, durable, and inexpensive alternative method of marking was reported [40]. It requires A4 (21 × 29.7 cm^2) transparencies used for transparent photocopies and two or three colors dry erasable pens. Contours of patch test areas are carefully marked with a pen. The transparency is used for further readings.

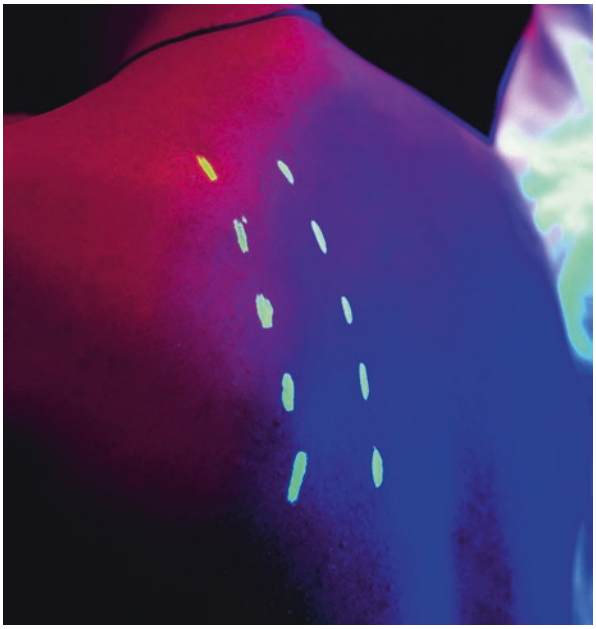

Fig. 3.12 Marking the skin with the Chemotechnique UV Skin Marker: examination under Wood's light

3.7.9 Positive Control

To exclude hyporeactivity, an impaired inflammatory response, and the possibility that the test patches do not adhere properly, sodium lauryl sulfate and nonanionic acid have been suggested as positive controls [41].

3.7.10 Immediate Urticarial Reactions to Some Allergens

Seldom, some allergens (e.g., balsams of Peru, cinnamic aldehyde, cobalt) are responsible for an immediate urticarial reaction about 20–30 min after applying patch tests. It is the reason why some authors remove the tests for a short while at 30 min and reapply them immediately at the same site. This practice, that is in essencewise, is not usually performed by dermatologists. The reaction can be reproduced when applying the allergen in an open test. Meticulous investigators apply systematically in each patient balsams of Peru on the volar aspect of the left forearm and cinnamic aldehyde on the volar aspect of the right forearm, as an open test (see Sect. 7.2). Readings occur at 20 and 30 min. In some cases, this observation has no clinical meaning, but in some others, it reflects the existence of a contact urticaria syndrome (see Sect. 10.1), coexisting eventually with ACD.

Rarely, some other allergens such as parabens provoke an immediate urticarial reaction.

3.8 Reading and Scoring Patch Test Results

3.8.1 Scoring Codes According to the ICDRG

It is important for patch tests to be scored according to the reaction seen and not only according to the interpretation placed on the reaction by the reader. Irritant reactions should be recorded as positive irritant and not as negative. In our view, the best scoring system remains as that recommended by Wilkinson et al. [42] and reproduced in Table 3.1. Some variants of scoring exist in textbooks of contact dermatitis; they include the occasional occurrence of papules, as an additional clinical sign of + and ++ reactions. Papules are purposely omitted in our scoring system for two reasons: they do not provide any complementary useful information and histopathologic examination of papules observed in some positive patch test reactions reveals that they are, in fact, tiny vesicles (Fig. 3.13).

3.8.2 Proposal for Modified Scoring Codes of Positive Patch Test Reactions, According to ESCD and EECDRG

Menné and White suggested a modification of the scoring codes to be submitted to the ESCD [43]. Their concern was based upon discrepancies in the reading of the + reaction encountered in the current literature.

Two schools have developed: one which defines the "+" reaction as homogeneous redness in the test area with scattered papules and the other requires homogeneous redness and homogeneous infiltration in the whole test area. The conflict is

Table 3.1 Scoring of patch test reactions according to Wilkinson et al. [42], on behalf of the ICDRG

Score	Interpretation
−	Negative reaction
?+	Doubtful reaction[a]; faint erythema only
+	Weak (nonvesicular) reaction[b]; erythema, slight infiltration
++	Strong (edematous or vesicular) reaction; erythema, infiltration, vesicles
+++	Extreme (bullous or ulcerative)[c]
IR	Irritant reactions of different types
NT	Not tested

Note that photopatch tests (see Chap. 5, Sect. 5.5) are graded similarly with a prefix Ph: Ph−, Ph?+, Ph+, Ph++, Ph+++, Ph IR, Ph NT

Reading and scoring have to be repeated at each individual visit to check the progression or regression of the reaction (day 2, day 4, day 6, or day 7)

[a]?+ is a questionable faint or macular (nonpalpable) erythema and is not interpreted as a proven allergic reaction

[b]+ is a palpable erythema, suggestive of a slight edematous reaction

[c]From coalescing vesicles

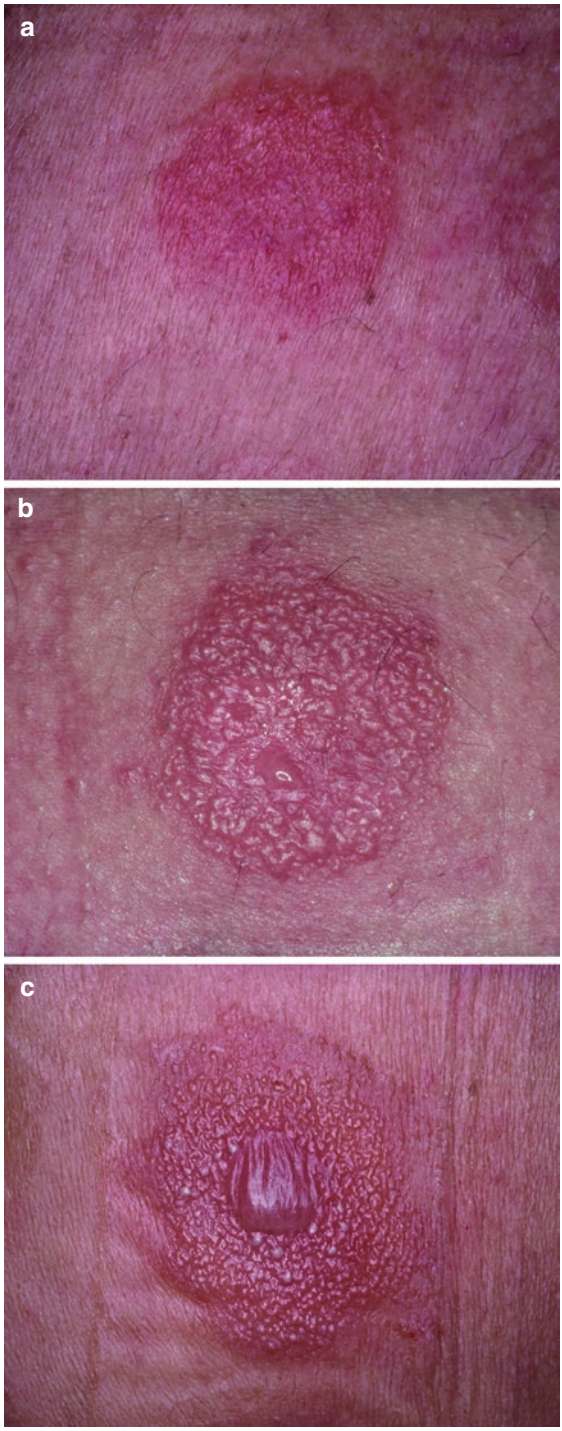

Fig. 3.13 Scoring positive allergic patch test reactions. (**a**) + reaction; (**b**) ++ reaction; (**c**) +++reaction (see explanation in text)

Table 3.2 Scoring of patch test reactions [43], on behalf of ECDS and EECDRG

+	Homogeneous redness in the test area with scattered papules
++	Homogeneous redness and homogeneous infiltration in the test area
+++	Homogeneous redness and infiltration with vesicles
++++	Homogeneous redness and infiltration with coalescing vesicles

well known. The stronger the patch test reaction, the higher the degree of relevance and reproducibility. Yet a weak positive reaction may be relevant and reproducible as well. The classification of patch test reactions depends exclusively on descriptive morphology. A pragmatic way, which will allow comparison between the different databases, is to introduce an extra grade of patch test reaction. To encompass the two main schools of current practice, the following scale is therefore suggested for debate. This alternative scoring system is presented in Table 3.2.

To date, no consensus has been reached in the matter.

3.8.3 Rating Patch Test Reactions Based on Digital Images

A study in Germany [44] assessed the diagnostic validity of readings of 20 digital images of various patch test reactions graded by congress attendants. One hundred and 22 volunteers took a patch test quiz offered during the eighth ESCD meeting, September 2006, Berlin. The "gold standard" grading determined by an EECDRG expert panel was disclosed while the quiz was open. The distinction between? + and + reactions proved rather difficult, but most images prompted a fair proportion of correct classifications.

Results were largely valid. Thus, the method could be used for continuing medical education and standardization in multicenter networks.

3.8.4 Bioengineering Methods for Evaluating Skin Irritation and Allergic Reactions: A Comparison with Visual Scoring

Farage et al. have analyzed the current views in the matter [45].

Visual assessment of skin reactions has long been used to evaluate the safety of chemicals and preparations that contact the skin and to meet regulatory requirements.

Furthermore, as bioengineering methods were developed that can quantitate certain aspects of skin irritant and/or allergic reactions, it is important to consider whether such measures should supplement or replace visual assessment. Examples of investigations comparing the outcomes of studies that use visual scoring and those that use bioengineering measures are discussed. These examples provide little

evidence that bioengineering measures provide an improvement in overall quality in comparison with current testing methods that rely on visual assessment. In addition, such measuring techniques can add considerably to the complexity of testing protocols. When benefits and costs are weighed in the balance, the visual assessment scales remain an effective, practical method of evaluation.

3.8.5 Remarks About Reading and Scoring Patch Test Results

3.8.5.1 Size of the Reaction

The size of the reaction differs from case to case. The use of current patch test units (i.e., chambers) has limited the size of the reaction to the patch area in most cases; nevertheless, the reaction may sometimes spread all around the patch area, outside the chamber's margins (see Sects. 3.4.4 and 3.4.5). It can be concluded that the reactions are more limited nowadays (thus more comfortable for the patient) than previously, when older patch tests (i.e., nonchamber) units were used. Readings are therefore easier because of the absence of overlap between neighboring positive reactions.

3.8.5.2 Edge Effect

The occurrence of "ring-shaped" allergic positive patch test reactions to allergens dissolved in a liquid vehicle (i.e., formaldehyde) is not uncommon [46]. Such reactions can be explained by the accumulation of the chemicals at the periphery of the patch test site. We previously coined the term "edge effect" because some patch test units are square in shape. When using such units, the liquids accumulate at the "edges" of the squares. The occurrence of the "edge" or "ring" effect could be due to pressure [47]. Besides this pressure mechanism, capillary migration could be responsible for an enhanced edge effect. Exceptionally, "ring-shaped" reactions can occur with allergens dispersed in petrolatum, the explanation of which could also be the effect of pressure (Fig. 3.14). Exceptionally, an edge effect has also been observed when using TRUE TEST® (for similar reasons, see Chap. 6).

A particular type of edge effect can be seen when patch testing with corticosteroids. The margins of the positive test are red, while the central area is whitish. This could be related to the vasoconstrictive effect of the corticosteroid, due to an enhanced penetration of the chemicals in the central area. Vasoconstriction and reduction of the inflammatory process most probably counteract the expression of the allergic response.

3.8.5.3 What Must Be Done in Case of "?+" (Doubtful/Questionable) Reactions?

"?+" reactions are labeled "doubtful" in the files. There is no real problem when allergens of the standard and/or additional series are concerned, since that type of reaction reflects in a few cases the true allergic nature of the reaction.

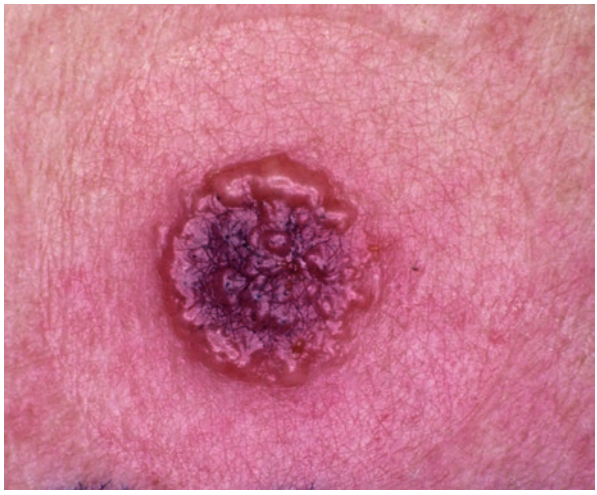

Fig. 3.14 Edge effect. Allergic positive ++ patch test reaction to paraphenylenediamine. Such a reaction can be explained by the accumulation of the chemicals at the periphery of the patch test chamber

More attention must be paid if the reading occurs in a hot climate, due to the potentially increased irritancy of some allergens, such as the fragrance mix.

A caveat does exist: "?+" reactions cannot be easily interpreted as irritant or allergic when patch testing with less common allergens, and even more so with products of unknown content, the irritancy of which is to a large extent unknown.

To circumvent these difficulties, the following strategy can be adopted by the clinician:

(a) Repeat the patch test in the patient to verify its reproducibility. This may include serial dilutions of the suspected allergen (dose/concentration relationship).
(b) Apply the same test in control subjects.
(c) Conduct additional investigations in the patient, such as open tests, semi-open tests and ROATs, and eventually use tests.
(d) Consider performing serial dilution testing. Allergic responses often reproduce at lower concentrations marginal irritation reactions (chromates, parabens, fragrance mix, and formaldehyde do so frequently).

To strengthen the validity of such investigations, note that when applying patch tests in the same patients (left vs. right sides of the back), most discrepancies in patch test readings do occur with "?+" and/or "+" reactions [48].

3.8.5.4 What Must Be Done in Cases of Pustular Reactions?

The occurrence of pustules in positive allergic patch test reactions is common. This is particularly true with metallic salts (chromates, nickel, cobalt, etc.) mainly, but not exclusively, in atopics. If some doubt exists in relation with its allergic meaning, repeating the tests would be wise, including a serial dilution test. This step-by-step procedure can avoid false-positive reactions and permits an unequivocal positive or negative reassessment of the allergic nature of the test.

3.9 Irritant Patch Test Reactions

In older days, when patch testing did not respond to definite rules (due to the lack of international standardization), irritant reactions were not uncommon. This was due to (a) the nature of substances and/or mixtures applied to the skin and (b) a too high concentration of some allergens, above the threshold of irritation.

Such irritant reactions may still occur nowadays when inappropriate methodology is used (Fig. 3.15).

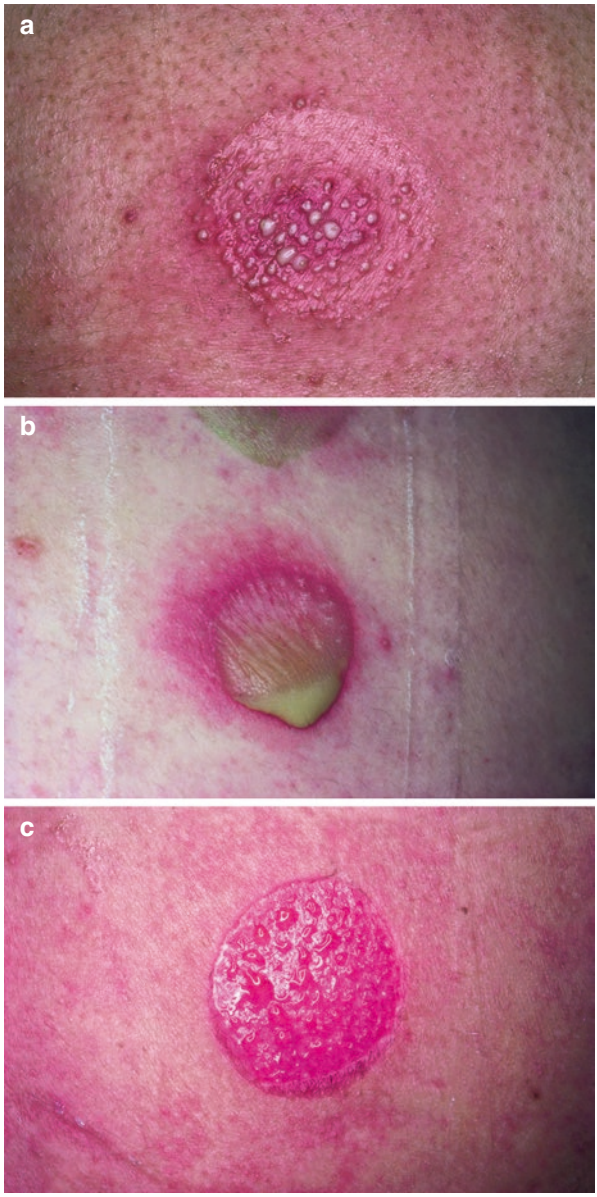

Fig. 3.15 Examples of irritant reactions: (**a**) pustular follicular, (**b**) pustular diffuse, (**c**) necrotic

The clinical signs of irritant patch test reactions vary in relation with the nature and/or concentration of irritants [52].

They are classically described as follows:

(a) Erythematous Reactions

Erythema is strictly limited to the site of application of substances, with sharp well-delineated margins. This means that when a square patch test unit is used, erythema has a square shape. The reaction is sometimes discretely scaly, but usually not edematous.

Allergens from the standard and/or additional series may provoke in some patients mild erythematous irritant reactions; they occur "at random" and are probably related to skin hypersensitivity in these patients.

Among allergens of the standard series, fragrance mix, thiuram mix, and paraben mix and formaldehyde in water are usually quoted as candidates for such marginal irritant reactions. In those cases, strategies to be applied for further patch testing are explained in detail (see Chap. 7).

(b) Purpuric Reactions

Purpuric patch test reactions are common with some allergens, in particular, cobalt chloride. About 5% of patients tested with 1% cobalt chloride in petrolatum show this petechial hemorrhage (Fig. 3.16). Histopathologic examination reveals slight perivascular lymphocytic infiltration, swollen endothelium, and extravasation of erythrocytes, mainly localized to the epidermis and acrosyringium. Purpuric reactions can also be observed when patch testing with paraphenylenediamine, IPPD, and some drugs.

(c) "Soap or Shampoo Effect" Reactions

These are so named because they are typically produced by patch tests with soaps and detergents. The skin is red or slightly shiny and wrinkled; there are usually no vesicles; pruritus is uncommon. It is therefore recommended not to test with soaps or detergents, unless appropriately diluted. In recent years, the careful evaluation of the various components of detergents leads to the detection of contact allergens and allows a better interpretation of results obtained from this investigation. Such reactions may still occur with soluble oils (which do contain detergents), when the test concentration is inappropriate.

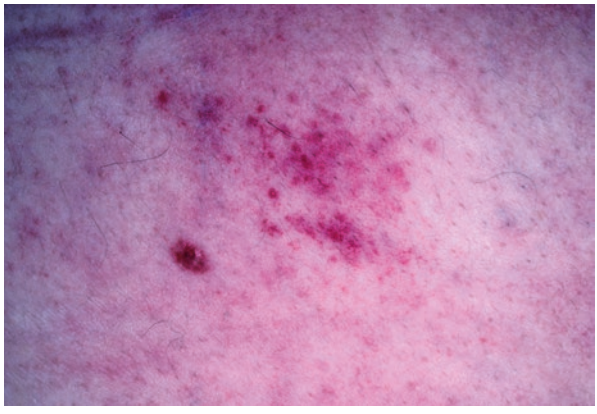

Fig. 3.16 Purpuric patch test reaction. Purpuric macules are scattered at random on the patch test application site (mainly observed with cobalt chloride (see explanations in text))

(d) Blistering (or Bullous) Reactions
Blistering occurs after testing with nondiluted or overly concentrated caustic products, such as acetone, gasoline, kerosene, and turpentine. Patch tests with quaternary ammonium salts may blister even when low concentrations are used.

(e) Pustular Reactions
These are sometimes consecutive to bullous reactions. Pustules are the result of an influx of polymorphonuclear neutrophils (sterile pustules) or are less often due to superinfection (mainly *Staphylococcus aureus*). In those circumstances, a unique large pustule is observed at the site of application.
Another type of pustular reaction may occur. The application area, uniformly erythematous, is dotted with small follicular pustules. This type of reaction mainly occurs with metallic salts (such as chromate, cobalt, nickel, copper, mercury) in atopic patients. The reaction can be exclusively irritant in nature or be superimposed onto a true allergic reaction. Formerly, a similar pattern of reaction (purely irritant, nonallergic) was observed when croton oil was applied to the skin ("croton oil effect").

(f) Necrotic or Escharotic Reactions
These are the most violent irritant reactions. For example, caustic soda or kerosene provokes such reactions.

3.10 False-Positive Patch Test Reactions

False-positive reactions can be defined as positive patch test reactions occurring in the absence of contact allergy. These are manifold; nevertheless, the following list (Table 3.3) is mainly related to technical errors (which can be avoided) or to a misinterpretation of the test results, in particular, when using inadequate concentrations of allergens.

Table 3.3 False-positive patch test reactions

1. Too high a test concentration for that particular patient (some allergens are more concerned)
2. Impure or contaminated test substance
3. Vehicle is irritant (especially solvents and very rarely petrolatum)
4. Excess of test preparation applied
5. Test substance, usually as crystals, is unevenly dispersed in the vehicle. This can occur when prepared at the hospital (i.e., not by manufacturers)
6. Influence from adjacent test reactions
7. Current or recent dermatitis at test site (excited skin syndrome) [49]
8. Current dermatitis at distant skin sites (excited skin syndrome) [49]
9. Pressure effects of tapes, mechanical irritation of solid test materials, furniture, and garments (see Sect. 3.14)
10. Adhesive tape reactions
11. The patch itself has caused reactions

Table 3.4 False-negative patch test reactions

1. Insufficient penetration of the allergen
(a) Too low a test concentration for a defined allergen
(b) The test substance is not released from the vehicle or retained by the filter paper
(c) Insufficient amount of test preparation applied
(d) Insufficient occlusion
(e) Duration of contact too brief; the test strip has fallen off or slipped
(f) Test not applied to the recommended site: the upper back
2. Reading is made too early, for example, neomycin and corticosteroids are known to give delayed reactions
3. Test site has been treated with topical corticosteroids or irradiated with UV (see Sect. 3.5.2)
4. Systemic treatment with corticosteroids or immunomodulators has to be taken into consideration (see Sect. 3.5.2)
5. Allergen is not in active form, insufficiently oxidized (oil of turpentine, rosin compounds, d-limonene) or degraded
6. Compound allergy (see Sect. 3.12)

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

3.11 False-Negative Patch Test Reactions

False-negative reactions can be defined as negative patch test reactions occurring in the presence of contact allergy [49]. The most common causes have been summarized in Table 3.4.

Some are self-evident and can be predicted and monitored by the dermatologist, while others cannot. Examples of the latter category may arise when (a) testing has been performed in a refractory or "anergic" phase [49]; (b) the test does not reproduce the clinical exposure (multiple applications), where some adjuvant factors are present (sweating, friction, pressure, damaged skin), or penetration at the site is lower than that of clinical exposure (eyelids, axillae). A stripping skin technique is recommended in the last case, where the test sites are stripped with tape before application of test preparations (see Sect. 7.1).

The differential diagnoses: photoallergy (see Chap. 5) and contact urticaria (see Sect. 10.1) should also be considered.

3.12 Compound Allergy

The concept of "compound allergy," popular among dermatologists, cannot stricto sensu be considered a false-positive or false-negative patch test reaction. It is the reason why it is described in a separate section.

The term "compound allergy" describes the condition in patients who are patch test positive to formulated products, usually cosmetic creams or topical medicaments, but test negative to all the ingredients tested individually [50]. This phenomenon can sometimes be explained by irritancy of the original formulation, but in some cases it has been demonstrated that the reactivity was due to the combination of the ingredients to form reaction products. 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). Pseudocompound allergy, due to faulty patch testing technique, is likely to be more common than true compound allergy. Several proven or possible compound allergens were listed in some papers. The formation of allergenic reaction products can take place within the product ("chemical allergenic reactions") but also metabolically in the skin ("biological allergenic reactions") [50].

The "quenching phenomenon" is a consistent finding whereby cinnamic aldehyde alone induces sensitization but when mixed with other fragrance compounds such as eugenol or d-limonene induces no sensitization. Patients who are sensitive to cinnamic aldehyde can sometimes tolerate perfumes containing this allergen because of presumed chemical changes (quenching) that occur during the usual aging process of a "mature" perfume [51].

3.13 Cross-Sensitization, Concomitant Sensitization, and Polysensitization

This section deals with situations wherein patients present several (two or more) contact allergies.

3.13.1 Cross-Sensitization

Cross-sensitization (syn.: cross-sensitivity, cross-allergy) means that contact allergy caused by a primary allergen is combined with allergy to other chemically closely related substances. In other words, in those patients who have become sensitized to one chemical (primary allergen), an allergic contact dermatitis can be provoked or worsened by several other related chemicals (secondary allergens).

Examples follow:

- A patient positive to *p*-phenylenediamine not only reacts to the dye itself but also to immunochemically related chemicals that have an amino group in the *para* position, for example, azo compounds, some local anesthetics, and sulfonamides.

- Cross-sensitization occurs with some antibiotics: neomycin, framycetin, kanamycin, and gentamycin.
- Cross-sensitization is often mentioned with nonsteroidal anti-inflammatory drugs. This issue is controversial: in some cases, true cross-sensitization seems to occur (ketoprofen and tiaprofenic acid), whereas in some others, reactions are interpreted as examples of concomitant sensitization (see Sect. 3.13.2).
- In the realm of plant dermatitis, true examples of cross-sensitization occur (e.g., catechols from different species of Rhus), but some are misinterpreted, since they are representative of a concomitant sensitization (see Sect. 3.13.2).

When investigating cross-sensitization, it is essential to use pure test compounds.

3.13.2 Concomitant Sensitization

Concomitant sensitization (syn.: cosensitization, cosensitivity, simultaneous sensitization) should not be confused with cross-sensitization.

It refers to the circumstance that certain substances often occur together in some products and that sensitization to the different substances often takes place on the same occasion. Thus, often cosensitization occurs to nickel and cobalt on contact with nickel items where cobalt is present as an impurity and toward chromates and cobalt on contact with cement. Lisi et al. [52] have conducted an extensive study on concomitant sensitization between different metals. The same applies to sensitization to various rubber chemicals (e.g., thiurams and thioureas). Another example of concomitant sensitization refers to proparacaine and tetracaine ophthalmic formulations.

The synonym "simultaneous sensitization," preferentially used in some papers, only means that at reading positive patch test reactions to some noncross-reacting substances do occur at the same time, that is, during the same test session. This does not imply that the patient has been sensitized "simultaneously" (or not) to those substances; this cannot be assessed retrospectively.

3.13.3 Polysensitization

Polysensitization (syn: multiple sensitization) refers to a specific population of patients who are "polysensitized," that is, sensitized to different categories of chemically nonrelated allergens. It has been arbitrarily stated that this concerns patients who are allergic to three or more categories of allergens [53]. A lack of knowledge still persists, as regards the respective role played by environmental and genetic factors [53, 54].

3.14 Unwanted Adverse Reactions of Patch Testing

The greatest hazard is omission of patch testing procedures in the management of patients who have certain dermatoses. Such omission dooms these patients to repeated attacks of avoidable contact dermatitis [6].

Side effects of patch testing patients are listed in Table 3.5. Some are described in detail. Such unwanted effects are seldom encountered in daily practice. In this respect, it must be emphasized that the risk-benefit equation of patch testing is much in favor of the benefit.

Table 3.5 Unwanted adverse reactions of patch testing

Patch test sensitization	("Active sensitization") see Sect. 3.14.1
Excited skin syndrome	("Angry back") see Sect. 3.14.2
"Ectopic" flare of dermatitis	On rare occasions, a positive patch test reaction may be accompanied by a specific flare of an existing or preexisting dermatitis that was caused by the test allergen. This side effect can be minimized by testing patients free of any current active dermatitis
Persistence of a positive patch	
test reaction	A notorious patch test reaction for persisting for more than 1 month is that due, for example, to a 0.5% aqueous solution of gold chloride in a gold-sensitive patient. Its meaning is partly understood (see Sect. 2.1.3)
Pressure effect	This consists of a red, usually depressed mark "imprinted" into the skin. It is a transient effect due to the application of solid materials. In practice, it can be due to (a) the pressure of chamber's rings or squares. This is a physically induced edge effect, distinct from the chemically induced edge effect (see Sect. 3.8.2); (b) the use of allergens in a solid form
Koebner phenomenon	A positive patch test reaction in a patient who has active psoriasis or lichen planus may reproduce these dermatoses at the patch test site during the weeks following patch test application [55]. The use of a topical corticosteroid usually quickly clears the lesion. Rarely, a similar Koebner phenomenon is observed in patients with lupus erythematosus [56] and lymphocytic infiltration of the skin (Jessner-Kanof) [57]
Hyperpigmentation	Hyperpigmentation from patch tests occurs infrequently and is most likely in darkly pigmented persons. It fades progressively after applying repeatedly topical corticosteroids. Sunlight or artificial UV exposure, immediately following removal of patch tests especially to fragrance materials, leads to hyperpigmentation of patch test sites in relation with photosensitivity, as in berloque dermatitis. This side effect is more common in Oriental populations (see Sect. 3.15.2)
Hypopigmentation	Postinflammatory hypopigmentation may occur at the sites of positive patch test reactions. It is usually a transient event (e.g., phenol)
Bacterial and viral infections	These adverse reactions have been occasionally described but are exceedingly rare
Necrosis, scarring, and keloids	Foolhardy testing with strong irritants (acids, alkalis, or chemicals of unknown composition) may produce such adverse reactions. Good practice of patch testing has entirely suppressed the occurrence of these complications, which are only of historical interest
Anaphylactoid reactions	Anaphylactoid reactions, or shock from, for example, neomycin and bacitracin, have been reported and are exceptional

3.14.1 Patch Test Sensitization ("Active Sensitization")

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 risk of active sensitization from the baseline and/or additional series. Common examples are *p*-phenylenediamine, thiuram mix, epoxy resin, sesquiterpene lactone mix, primula extracts, and, in recent years, isothiazolinones [58] or acrylates [59]. The risk, however, is uncommon when the testing is performed according to internationally accepted guidelines. Sensitization by a patch test rarely causes the patient any subsequent dermatitis or affects the course of a previous dermatitis.

In recent years, there has been concern about active sensitization from *p*-phenylenediamine. Gawkrodger and English [60] have made an extensive review of the literature and, when analyzing the different studies, they conclude:

- The overall percentage of active sensitization is low (1–1.5%).
- Even in case of active sensitization, the risk of developing allergic contact dermatitis from hair dying is small.

Moreover, late reactions to *p*-phenylenediamine are not always an indication of active sensitization [35].

In conclusion, it must be emphasized that the overall risk-benefit equation of patch testing patients is much in favor of the benefit. On the other hand, we advise against "prophetic" patch testing of non-dermatitic potential employees because in that case, the risk-benefit equation is much in favor of the risk of active sensitization.

3.14.2 Excited Skin Syndrome ("Angry Back")

This represents an important issue. Mitchell [61] used the term "angry back" to describe a regional phenomenon caused by the presence of a strongly positive reaction, a state of skin hyperreactivity in which other patch test sites become reactive, especially to marginal irritants, such as formaldehyde or potassium dichromate. He believed that these concomitant "positive" reactions cannot be relied on. Indeed, when retesting, these reactions were negative. He suggested that the true index of sensitivity was falsely exaggerated by concomitant patch testing. Nickel sulfate and potassium dichromate were considered best examples of such false-positive reactions. To confirm or deny the significance of individual reactions found on the "angry back," he recommended sequential testing later with each substance alone.

Because patch test may be performed elsewhere besides the back, Maibach [62] and Mitchell [63] broadened the term "angry back" to the "excited skin syndrome" (ESS), which was extensively reviewed later [64]. The pathogenesis of ESS has not yet been clearly elucidated.

When in doubt about the occurrence of ESS in a patient, the strategy to be conducted is individual *sequential retesting*, with each incriminated allergen, prefera-

bly on a different skin site. This procedure can be completed by additional tests, such as ROAT tests (see Sect. 7.4). It is a matter of the utmost importance in medicolegal situations.

ESS is now less frequent, possibly for two main reasons: (a) patch testing only on intact skin in patients free of any current dermatitis and (b) using smaller amounts of allergens, in relation with new patch test units (chambers).

The ESS is distinct from the "status eczematicus," contrary to what is written in most textbooks on contact dermatitis. Status eczematicus means that, at many patch test sites, there are positive nonspecific reactions, due to a state of skin hypersensitivity. This does occur when general rules of patch testing are not respected, such as patch testing patients with active atopic dermatitis or other types of dermatitis. Status eczematicus makes reading impossible; it can be avoided by using correct procedures.

3.15 Patch Test Readings in Different Ethnic Populations

Most publications dealing with patch test readings refer to Caucasian populations. It seems important to know whether differences may occur when reading patch test results in different ethnic populations.

Ethnicity may play an important role in reading and/or interpreting patch test reactions. In many publications, this problem has been discussed at length, without definite answer.

In a detailed review of all parameters involved, some authors have analyzed the different aspects, which could be of help when reading allergic and/or irritant patch test reactions [65, 66].

3.15.1 Patch Test Reading in Oriental Populations

3.15.1.1 Particular Aspects of Reading

The skin color in Oriental races (Japanese, Chinese, Korean, etc.) varies from white fair skin (equivalent to Fitzpatrick classification types II to IV) to dark complexion (equivalent to Fitzpatrick classification skin types V and VI).

For dark-skinned individuals (skin types V and VI), skin marking of patch test sites is important because by the second and fourth day, it is often difficult to identify the location of the patch test sites. Special markers incorporating silver nitrate (though it may cause irritant reactions) may be more effective than marking the skin test sites in a conventional way.

Goh in Singapore uses the following marker solution:

Gentian violet 1%
Methyl alcohol (95%) 50%
Silver nitrate 20%
Distilled water to 100%

However, the preparation may cause skin irritation. Freshly prepared ink may be preferred, as the constituents become too concentrated as the solvent evaporates over time.

For fair skin (type II to type IV), a patch test reaction is not difficult to interpret. Allergic patch test reactions are usually easily discernible. The erythema, papules, and mild edema of allergic patch test reactions are usually obvious in skin types II and III. In darker skin types (types V and VI), a mild positive allergic patch test reaction may be overlooked as the erythema may not be obvious. However, the edema and papules/vesicles are usually obvious and palpable.

In darker skin of Malays and Indians, allergic patch test reactions may be difficult to discern. Erythema is barely visible. Much will depend on the appearance of papules/vesicles and edema. Palpation of the patch test site may help to detect allergic reactions. Associated pruritus on papular eruptions on the patch test site helps to affirm the possibility of the presence of a positive allergic patch test reaction.

Finally, there is little evidence of statistically significant differences in the irritant response between Oriental and Caucasian groups [67]. Therefore, it can be anticipated that patch test irritant reactions are not more frequent in Asian than in Caucasian populations.

3.15.1.2 Pigmented Contact Dermatitis

Pigmented contact dermatitis is a particular entity characterized by a diffuse brown, slate-colored, grayish-brown, reddish-brown, or bluish-brown pigmentation. It occurs in the weeks following an acute episode of irritant or allergic contact dermatitis. Pigmented contact dermatitis is rare in Caucasians but common in Mongoloids. Most recent cases have been reported from Japan. Various allergens have been incriminated, namely, naphthol AS, 1-phenyl-azo-2-naphthol, parabens, trichlorocarban, jasmine oil, rose oil, benzyl salicylate, musk ambrette, and some others. Positive patch tests to these allergens become hyperpigmented in the days or weeks following patch test application and remain so for long periods of time.

Pigmented contact dermatitis has occurred in many individuals, following diphenylcyclopropenone treatment for alopecia areata [68]. Note that patients who showed hyperpigmentation were poorer responders to the treatment.

Histologically, skin specimens showed lichenoid or vacuolar interface dermatitis with necrotic keratinocytes and dermal melanophages. Taking into account these characteristics, it is very similar to lichen planus pigmentosus; pigmented contact dermatitis could be called pigmented lichenoid contact dermatitis.

3.15.2 Patch Test Reading in Black Populations

Most textbooks on contact dermatitis do not mention particular aspects of patch test reading in black populations. In practice, reading does not cause insurmountable difficulties.

Two specific points deserve special attention:

- Erythema is distinctly visible in some cases or may present itself as a darker black hue in some others. It is advisable to read the patch test site under oblique light.
- In black skin, vesicles of eczematous reactions (including positive patch test reactions) do not tend to burst readily (Fig. 3.17); since they exhibit a yellowish hue (Fig. 3.18), they can be confounded with tiny pustules. This particular aspect is certainly related to the fact that, in black skin, stratum corneum has more cell layers and requires more tape strips to remove it than that of Caucasoid stratum corneum [69].

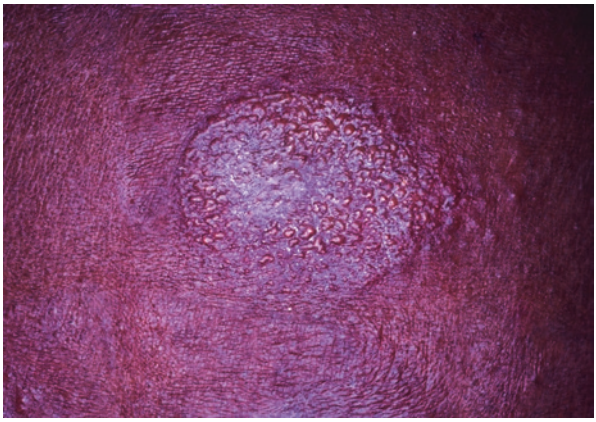

Fig. 3.17 Patch test scored ++ on a black skin. Darkening of the skin color replaces erythema. Infiltration and vesicles (read at 72 h)

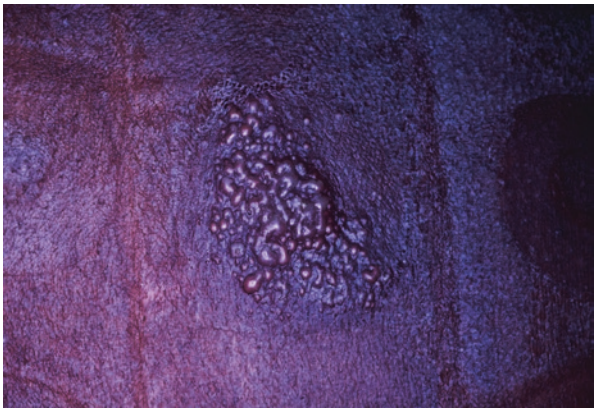

Fig. 3.18 Patch test scored ++; major infiltration of the central part, whitish tense vesicles mimicking minipustules. This particular image (yellowish hue) is due to the greater thickness of the stratum corneum in Blacks. The vesicles only burst as tension increases (read at 48 h)

The darker the skin, the more difficult it is to mark. For very dark skin, a fluorescent marking ink is probably best, the dots being located by a Wood's light in a dark room.

Once again, there is little evidence of statistically significant differences in the irritant response between Black and Caucasian groups. Therefore, it can be anticipated that patch test irritant reactions are not more frequent in Blacks than in Caucasoids.

Nevertheless, it is possible that intraindividual variations do exist, but further studies need to be conducted before a definitive statement can be made. Therefore, vigilance is requested at patch test reading to evaluate correctly potential irritant reactions.

3.16 Patch Testing Techniques in Different Climatic Environments

The patch testing procedures should be modified in different climatic conditions. This is because of the adherence of the tape and moisture of the skin surface under different climatic environments [70].

3.16.1 Temperate Climates

In some temperate countries, patch testing is performed only during the cooler seasons and discontinued during summer time because the hot humid climate in summer may cause the tape to be dislodged more readily and patients generally find it uncomfortable to have strips of tape left on their skin for 48 h.

In many places, there is no real need to interrupt patch testing activities during summer time. The only reason why this habit does occur is for practical convenience, in relation with personnel holidays.

Useful information is related to seasonal variations in patch test reading in temperate countries:

- Chapping of the skin during winter predisposes to irritant contact dermatitis and also increases the incidence of false-positive reactions to substances such as formaldehyde, mercurials, and propylene glycol.
- Some authors found many positive reactions in summer but far fewer during cooler weather. Thus, occlusion and sweating may increase the number of positive reactions to some substances, whereas propylene glycol, which is hygroscopic, and some other marginal irritants may often appear to be more of an irritant in winter.

3.16.2 Tropical Climates

Allergic contact dermatitis from whatever cause can be aggravated by environmental factors such as heat, high humidity, and dust.

In the tropics where there is little seasonable variation, there is no "ideal" season when patch testing can be done most comfortably. Patch testing is usually performed throughout the year. Because of the high ambient temperature and high humidity, the patch testing procedure may need some modification to ensure that the occlusive effects of the patch test chamber are maintained and that patients comply with the instructions carefully.

In addition, because of the higher ambient temperature, it is recommended that the patch test allergens be stored in a cool place when not in use. The test allergens should be kept in a refrigerator.

3.16.3 Patch Testing Procedures in the Tropics

The warm humid environment in the tropics makes patch testing an uncomfortable experience for the patients. Miliaria can occur at the sites of patch testing due to occlusion. Patients should be given clear instructions on the patch testing procedures.

3.16.3.1 Instructions for Patient

To ensure compliance, the following instructions may be given to the patients:

- Patients will be allowed to continue to take light showers or bathes to clean their face, chest, limbs, and lower torso. They should avoid washing the back (patch test sites) with water.
- The back where the patch test tapes are placed will be allowed to be cleaned daily with light moist towels, avoiding the test strips area.
- Patients should avoid outdoor activities and remain in a cool air-conditioned environment whenever possible.

3.16.3.2 Technical Adaptations

Patch testing can be performed with the various patch test chambers available commercially. The Finn Chambers are widely used for patch testing in the tropics. However, the hot, humid environment causes sweating and makes plaster adhesion to the skin poor. Patch test plasters tend to come off easily. Reinforcement of the patch test plaster is useful to ensure proper occlusion. An effective way is to reinforce strips of plasters on the edges of the patch test tapes.

The conventional skin marker does not remain on the skin due to perspiration. The silver nitrate skin marker is a useful marker for identifying patch test sites.

3.17 Is Self-assessment of Allergic Contact Dermatitis by Patients Recommendable?

3.17.1 Self-assessment by Questionnaires

Many studies have been conducted in the last decades. A recent review [71], focused on nickel allergy, has clearly shown that the validity of self-reported nickel allergy is low. The questions regarding nickel allergy overestimate the true prevalence of nickel allergy.

In conclusion, this approach is only indicative and can be considered of limited scientific value. Nevertheless, it remains an interesting preliminary step before starting controlled studies on cohorts of patients.

3.17.2 Self-readings of Patch Tests by Patients

We consider that the same restrictions can be applied to self-reading of patch test by patients.

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