

INTRODUCTION

This position paper is restricted to skin tests used for diagnosis of type I allergy (atopic, IgE-mediated allergy) and does not take into account skin tests used for the study of Type III and IV allergy.

In 1873, Charles Blackley published the result of the first scratch-test. He scratched an area of his skin, approximately 1 cm², applied pollen to the lesion and noted a huge swelling and erythema, that persisted for several days. Since then, the skin test method has been refined and used for many different purposes. Methods for performing skin tests vary between regions and also between medical specialities. During recent years, a rapid development of techniques for skin prick testing has taken place. This method is presently the dominating skin test method especially amongst pediatricians. Despite its wide use, there has not been any international agreement, how skin tests should be performed, evaluated and used for diagnostic, standardization and epidemiological purposes. In contrast to results obtained by laboratory methods, skin test results are often presented without giving any details on potency of allergen, method, precision and reproducibility in the hands of the investigator. This fact makes most published results from studies using skin tests, e.g. data from prospective epidemiological studies, less valuable.

During the annual meeting of the European

Academy of Allergology and Clinical Immunology, that took place in Copenhagen 1988, the Executive Committee of EAACI decided to form a "Sub-Committee on Skin Tests", and asked me to chair that Sub-Committee. At the first meeting of the subcommittee in Copenhagen, those present decided to prepare a position paper and publish it prior to the congress in Berlin, September 1989. The committee members agreed upon preparing this report by means of a collaborative work. As chairman, I was asked to outline a preliminary table of content. Then members of the Steering Committee of the Sub-Committee, each worked out a first draft of one section. The content was edited and partly rewritten by me and then sent out for comments to a number of allergologists, from all European countries, interested in the subject. Therefore, the content of this report reflects the present knowledge within the field and I am convinced that it will be of value for the quality of daily diagnostic work, and form the basis for epidemiological investigations, biological standardization and estimation of the relative potency of allergens and estimation of changes in skin reactivity during different kinds of therapeutic measures. Furthermore I hope it will stimulate scientific work within the field, because this position paper is just a report of what is known today.

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1. Pathophysiology of skin tests

In recent years our understanding of the mechanisms by which allergens and non-specific agents can induce skin reactions has improved considerably although studies on the mechanisms of skin tests began more than 50 years ago when Lewis and Grant (43) described the "triple response". The IgE-mediated allergic reaction of the skin consists of an immediate weal and flare reaction. It is inconsistently followed by a late phase reaction, i.e. a diffuse infiltrate covered and surrounded by erythema, developing some 2 to 3 hr later and resolving in approximately 24 hr.

1.1. THE TYPE I ALLERGIC REACTION

1.1.1. *Antibodies involved*

In humans, anaphylactic antibodies belong mostly to the IgE isotype. The ability to mount an IgE response to allergens is a prerequisite for the development of positive allergen skin tests. British investigators have shown that the skin reactions may be mediated by IgG short-term sensitizing antibodies (50) (S-TS). These skin reactions have the classical features of weal and flare reactions, but are less intense than those induced by IgE antibodies and reach their maximum about 5 min later than the IgE-induced weal and flare reaction. Moreover, it has been suggested that some IgG S-TS-mediated skin reactions were only demonstrable by intradermal tests (ICT) but not skin prick tests (SPT) which might be explained by the low concentration of antibodies (See Chapter 2).

1.1.2. *Factors determining the skin reactivity*

There are other factors than the antigen-antibody reaction that determine the skin reac-

tivity to an allergen, including the amount (potency) of allergen introduced into the skin and the composition of that allergen, the number of mast cells (MC) and the degree of sensitization of cutaneous mast cells, whether the sensitizing IgE is active (IgE+) (44) or not, presence of non-immunologic triggering factors, and the reactivity of the dermal tissue to mediators, particularly histamine released from mast cells.

1.1.3. *Non-allergenic substances causing skin reactions of similar appearance as those induced by allergens*

Skin tests can also be performed with vaso-active agents, mast cell secretagogues such as histamine, PAF-acether, sulfidopeptide leukotrienes, neurotransmitters such as cholines and substance P. Many diverse chemicals can trigger mast cells in vitro, causing the release of vaso-active mediators but only a few of them have been found to give a typical weal and flare reaction. Among them, compound 48/80, polymyxin B, ACTH and opiate derivatives such as codeine phosphate and morphine are the most widely used. Histamine induces only a weal and flare reaction whereas kinins, PAF-acether or mast cell secretagogues are able to elicit both an immediate and a late phase reaction (for review see (14)).

1.2. HISTOLOGY OF THE NORMAL SKIN

The skin is a complex organ with an intricate and rich vascular and nerve supply. Resident cells are capable of developing an inflammatory response. Epidermis is the superficial, non-vascular layer of the skin covering the dermis

which has a rich blood supply. T-lymphocytes are scattered in the skin whereas there are no B cells. They are present mainly around dermal superficial blood vessels, and occasionally also found in epidermis and interstitial dermis. Antigen presenting cells (AGPC) are mainly Langerhans cells in the epidermis, whereas macrophages have this function in the dermis. There are 5,000 to 12,000 mast cells per mm³ distributed mainly round the vessels. Most of them are connective tissue mast cells, i.e. MC sensitive to treatment with formalin which are also characterized by their content of both tryptase and chymase. There are very few granulocytes in normal skin. These are mainly found around superficial venules (13).

1.3. HISTOPATHOLOGY OF TYPE I ALLERGIC REACTION

Within 10 - 20 minutes after introduction of allergen into the skin of an allergic patient, there consistently appears an immediate reaction, which is characterized by a weal and flare, inconsistently followed by a late reaction, i.e. a diffuse swelling and redness, starting 2 to 3 hours after the allergen injection, peaking at 6-12 hours and disappearing within 12-24 hours.

1.3.1. *Immediate or early phase reaction (EPR)*

According to systematic histopathologic studies of human allergic responses in the skin an influx of fluid occurs within 5 min after allergen injection, followed after 10 minutes by the appearance of inflammatory cells, predominantly neutrophils. The inflammatory cell infiltrate persists for up to 24 hr (3,4,57). Skin mast cells degranulate at allergen challenge (33) suggesting a role for the mast cell in the early phase reaction and a possible involvement in the generation of the cell inflammatory response. Furthermore, mast cell secretagogues, e.g. morphine (16) are capable of inducing release of mast cell mediators and thereby a weal and flare reaction.

Anti-histamines such as hydroxyzine failed to alter the cell response after antigen in-

jection but decreased the weal response (4). Thus the immediate weal response is mainly induced by histamine, whereas the cellular infiltration occurring after mast cell degranulation depends on other factors.

1.3.2. *Late phase reaction (LPR)*

Dolovich et al (21) showed that 5 to 6 hours after injection into the skin of ragweed allergic individuals of anti-IgE antibodies or ragweed antigen, a late reaction occurred, peaking between 6 and 12 hours after the injection. Biopsies revealed a mixed cellular infiltrate of predominantly mononuclear cells but also of eosinophils, basophils and neutrophils. These authors did not find any evidence of a type III immune reaction. Solley et al (58) and De Shazo et al (19) highlighted the importance of IgE antibodies in the LPR and found that LPR was distinguished from the EPR by more pronounced infiltration of mononuclear cells, basophils, neutrophils and eosinophils and oedema and deposition of fibrin. However, in principle the same pattern of cellular infiltration was found after immediate weal and flare reactions that did not lead to a macroscopic LPR (19,53).

1.4. STUDY OF SKIN BLISTER FLUIDS

Dunsky and Zweiman (29) proposed to induce a skin blister to study the skin response to allergen. The epidermis was denuded and the dermis covered with a skin chamber. After stimulation with allergen or mast cell secretagogues, most often for four hours, blister fluid containing mediators and cells were recovered. This technique was adapted by several groups (24,28). It has been of great importance for the study of the allergic skin reaction, especially the LPR. However, the extended exposure to allergen (most often 4 h) makes firm conclusions impossible, regarding the time course of events after normal skin testing.

1.4.1. *Immediate reaction*

Electron microscopic studies of skin blister fluids and cells showed evidence of mast cell

degranulation occurring within 15 seconds after antigen injection (63). Histamine release was observed in the same experiment series, beginning about 5 minutes after allergen injection and peaking at 30 minutes (29).

1.4.2. Late phase reaction

Several major events have been studied with respect to LPR: The cellular infiltration, mediator release, and the effect on skin of injected mast cell mediators.

The biochemical and cellular events produced during the development of a LPR have been evaluated using skin blister fluid analyses. Chamber fluid histamine and tryptase increased for up to 4 hr following allergen challenge (62,63). The total amount of histamine released correlated with the magnitude of the LPR (62), indicating a possible relationship between mast cell degranulation and the occurrence of the LPR. More recently it was also shown that the magnitude of LPR was significantly correlated with the levels of PGD₂ level 5.5 hrs after allergen challenge (51). Skin blister fluid collected during LPR contains a factor releasing histamine from human basophils but not from human mast cells (70). Accumulation of eosinophils and neutrophils (10) was directly related to the mast cell activation (29). The exact role of basophils and mast cells in LPR still remains to be elucidated. The mediator involved in eosinophil accumulation was not histamine but rather a low molecular weight eosinophil chemotactic factor (65,66). Neutrophils appear early in skin blister fluids, their number correlates with the intensity of the mast cell response (30). Polymorphonuclear cells are activated and can cause severe skin damage (55,72).

Several mediators have been recovered during LPR from skin blister fluids after allergen challenge, mostly for four hours. They include histamine (5 min to 4 hr), kallikrein (26), thromboxane B₂ (26), PGD₂ (50) (start 30 min and peak at 6 hr after challenge), LTC₄

(peak 2-4 hr after challenge) and minimal concentrations of LTB₄ (12,27,61), and PAF-acether (peak 5-6 hr after challenge) (45,56).

1.5. INJECTION INTO THE SKIN OF INFLAMMATORY MEDIATORS

Inflammatory mediators released by various cell types have been studied. Some mimic the immediate effects of allergen by their vasoactive properties. On the other hand, the LPR is related to a cellular influx. Chemotactic mediators are likely to be involved in this reaction and their intradermal injection can reproduce the LPR.

1.5.1. Histamine

Histamine is released not only during the immediate type reaction but for 4 hours after allergen challenge (51,62,63). When injected into the skin by SPT or ICT histamine perfectly mimics the allergen-induced weal and flare reaction. However, histamine never produces LPR, in contrast to allergens and mast cell secretagogues.

1.5.2. Sulfidopeptide leucotrienes (LT)

Leucotrienes include LTC₄, LTD₄/LTE₄. They are released in the skin during allergen challenge with a time course compatible with a LPR (12,27,61). The intradermal injection of LTD₄ and LTE₄ induces a burning, erythematous weal and flare reaction persisting for up to 4 hours, with a characteristic central pallor supposed to be caused by arteriolar constriction. LTC₄ elicits a response similar to that induced by LTD₄/LTE₄. Histologically, the weal and flare is associated with dermal oedema and marked dilation of venules and capillaries (59).

LTB₄ is a potent leukocyte chemotactic substance released by many cell types including mast cells. It is inconsistently released in skin blister fluids during the LPR (61). Intradermal injection of LTB₄ in human skin has been shown to induce a transient weal and

flare reaction followed by a LPR which histopathologically demonstrated a polymorphonuclear leukocyte influx and some fibrin deposition (15,60). Using an improved technique, Michel et al (49) observed that injection of LTB_4 resulted in an intense dose and time dependent neutrophil migration. These cells were found to be activated.

1.5.3. Prostaglandin D_2 (PGD_2)

Prostaglandin D_2 is the major prostaglandin released by mast cells but not by basophils. It is released during the weal and flare reaction as well as during the LPR (51). When injected, it induces an asymptomatic weal and flare reaction resolving within 2 hr, but its role appears to be minor by comparison to that of histamine (9). It caused dermal oedema and vessel dilation, with a subsequent neutrophil infiltration beginning 2 hr after challenge and lasting for a further 4 hr.(51)

1.5.4. Platelet activating factor (PAF)

Platelet activating factor was found to be released in skin blister fluids after allergen challenge 5 to 7 hours after the beginning of the challenge (45,56). This autacoid released by a wide array of cells participating to the allergic inflammation including some MC, and human skin fibroblasts (46), modulate in vitro the actions of a variety of target cells including platelets, neutrophils, macrophages, eosinophils and neurons (49). Intradermal PAF administration results in both acute and delayed effects, i.e. a marked EPR resolving within 30 to 60 minutes followed by a LPR persisting for hours (2,7). The weal and flare reaction is due to the vaso-active properties of the autacoid. However, since PAF-acether is rapidly metabolized in vivo by fluid-phase and cell-associated phospholipases, its delayed effects may only be explained by its potent chemotactic activity, especially on eosinophils, or its effects on the release of IL-1 and tumor necrosis factor (13). These factors also stimulate

PAF-acether synthesis by several cell types including human monocytes (67). The late release of PAF-acether in skin blister fluids may be due to this positive feed-back loop. IL-1 is a potent pro-inflammatory agent that may prolong the biological effects of PAF-acether. Moreover, PAF-acether is the most potent eosinotactic factor known as yet and a very potent chemotactic factor for other cells too. Its release induces an influx of various cells known to be part of LPR. Prolonged influence of PAF-acether induced migration of neutrophils and basophils (34). It was also able to attract eosinophils but only in allergic individuals (38).

1.5.5. Other preformed mediators

A number of other preformed mediators capable of activating the coagulation, fibrinolytic and bradykinin pathways have been described. The intradermal injection of kallikrein leads to a LPR characterized by a cell infiltrate similar to that induced by allergen (20), whereas bradykinin only causes vasodilation and vasopermeability without inflammation.

1.6. PHARMACOLOGIC STUDIES

Studies of the modulation of the early and late phase reactions by pharmacologic agents have given indirect evidence for the role of different mediators for the development of allergen induced skin reactions (34,52).

1.6.1. Immediate reaction (EPR)

High dose topical corticosteroid treatment and anti-histamines inhibit the weal and flare reaction (for review see (14)), whereas cromolyn does not inhibit the codeine-induced immediate skin reaction (64).

1.6.2. Late phase reaction (LPR)

The LPR is blocked by prior administration of topical or systemic corticosteroids (35)(for review see (14)). Eicosatetraynoic acid (ETYA) applied topically blocked both the cy-

clo-oxygenase and the lipoxygenase pathways of arachidonic acid metabolism and induced a reduction of the LPR (22). Cyclooxygenase inhibitors incompletely altered oedema formation (23) and dazoxiben, a selective inhibitor of thromboxane synthesis, increased the weal and flare reaction and partly decreased the LPR (25). Topical indomethacin, another cyclooxygenase inhibitor, decreased the flare without any effect on the weal and reduced the LPR (60). PAF-antagonists decreased skin reactions to PAF-acether and to a lesser extent those induced by allergen (7,36).

Interestingly, the antihistamine cetirizine has an inhibitory effect not only on the histamine release and the EPR, but also inhibits the release of PAF acether and recruitment of eosinophils (47).

These studies confirm the importance of histamine for induction of the EPR, whereas eicosanoids, prostaglandins, thromboxanes and PAF appear to be more important for the generation of the LPR.

1.7. NEUROMEDIATORS

The skin receives a rich supply of nerve fibers, many of which are peripheral ramifications of primary sensory neurons. There is a close relationship between mast cells and nerve endings (71), which might be of significance. Several neuropeptides have been found to be associated with these neurons: substance P, somatostatin, vasoactive intestinal peptide (VIP), neuropeptide Y, calcitonin gene related peptide (CGRP) (69). The physiologic significance of the antidromic stimulation of small diameter afferent nerve fibres localized to the skin is still incompletely known but it was found to cause both cutaneous vasodilation (8) and increased vascular permeability (42). The importance of neuromediators in the flare response is exemplified by the lack of flare in patients with spinal cord injury and after topical dermal anesthesia (52). Substance P is one of the neuromediators involved in the axon reflex causing vasodilation and plasma extravasation (32,37,40).

Our understanding of the relationship between the neurogenic and cellular inflamma-

tions, respectively, in the generation of EPR and LPR is limited.

1.7.1. Immediate reaction

Intradermal injection of substance P in humans produced a weal and flare reaction similar to that elicited by histamine (37). The mechanisms of production of plasma extravasation (weal) differ from those of vasodilation (flare) (32). The flare induced by intradermal injection of substance P is blocked by H_1 -histamine receptor antagonists whereas the weal is only minimally affected (32,37). These findings suggest that substance P can release histamine from skin mast cells and histamine may in fact be the direct mediator of flare. This hypothesis is substantiated by (i) the close contacts observed between skin mast cells and neurons, (ii) the ability of substance P to cause histamine release from human skin mast cells in vitro, (iii) the in vivo histamine release by mast cells after antidromic stimulation of sensory nerves, (iv) the decrease in the flare response due to anti-dromic stimulation when histamine has been depleted from mast cells and (v) the reduction of antidromic vasodilation by H_1 -histamine receptor antagonists. Because of the time course of the erythema induced by CGRP, it cannot be a mediator of the erythema of EPR but might be associated with vasodilation of later phases of skin inflammation (62).

1.7.2. Late phase reaction

Nothing is known regarding the interplay between cells and neuromediators during the late phase allergic reaction.

1.8. SUMMARY OF THE PATHOPHYSIOLOGY OF THE IMMEDIATE AND LATE PHASE ALLERGIC REACTIONS

1.8.1. Immediate reaction

The weal and flare reaction induced by an IgE-mediated immune response is mainly due

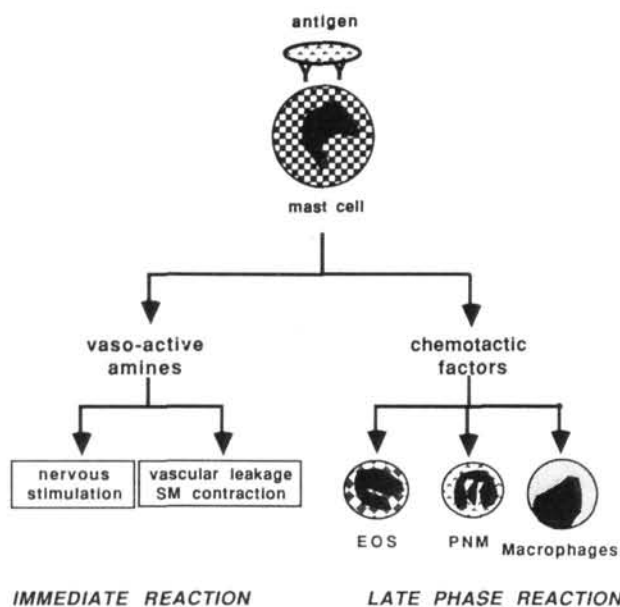


Figure 1. Mast cell release of mediators inducing the immediate or early phase reaction (EPR), and of chemotactic factors attracting cells participating in the late phase reaction (LPR).

to the activation of mast cells (6) releasing vaso-active agents causing both plasma extravasation and vasodilation. Skin MC were shown to release histamine, PGD_2 and to a lesser extent LTC_4 and LTB_4 (11). It is likely that cellular and neurogenic inflammations are linked. Substance P is released by the axon reflex that can be triggered by histamine released by mast cells located close to afferent nerve endings. Furthermore, substance P enhances the release of histamine from mast cells (positive feedback loop)(Fig. 1 and 2).

1.8.2. Late phase reaction

Although many basic facts are known about the LPR, the interplay between cells, their preformed and synthesized mediators, chemotactic factors, and neuromediators has to be further elucidated.

1.8.2.1. MAST CELLS (MC)

Mast cells appear to be the trigger cell of the reaction since the LPR almost never appears without an immediate reaction. These cells release LTB_4 and possibly PAF-acether and other vaso-active mediators, which produce

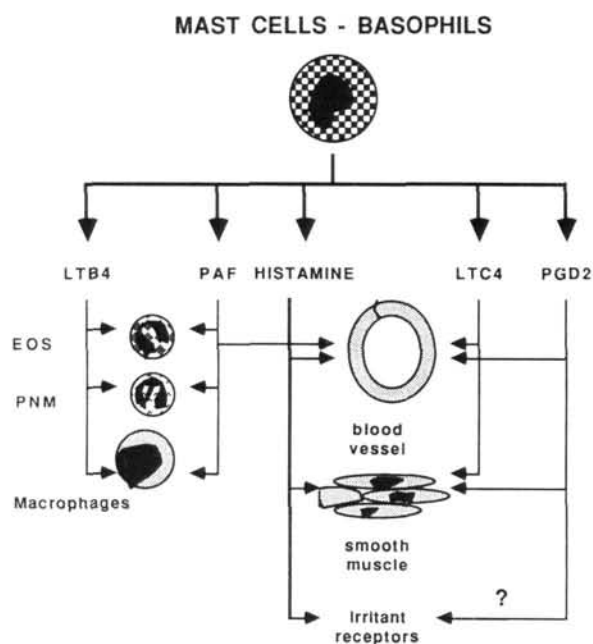


Figure 2. Some mediators released by MC and basophils, and their principal action on tissues.

vasodilatation and increase vascular permeability. Furthermore, chemotactic mediators enhance migration of inflammatory cells.

1.8.2.2. CHEMOTACTIC FACTORS

The most potent chemotactic factors such as PAF-acether, LTB_4 and HETES are released by various cells, primarily mast cells but also neutrophils and macrophages. The release of these factors explains the cell infiltrate observed before and during the LPR including neutrophils, macrophages, lymphocytes, eosinophils, mast cells and basophils (73).

1.8.2.3. EOSINOPHILS

Eosinophils are known to produce cytotoxic proteins such as the major basic protein (MBP) and eosinophil cationic protein (ECP) that were shown to be involved in the LPR of the nose and/or the bronchi (42,49) but their role in the cutaneous LPR has to be demonstrated (Fig. 3).

1.8.2.4. LYMPHOCYTES

Lymphocytes dominate the cell infiltrate of the LPR and may be involved in the inflammation

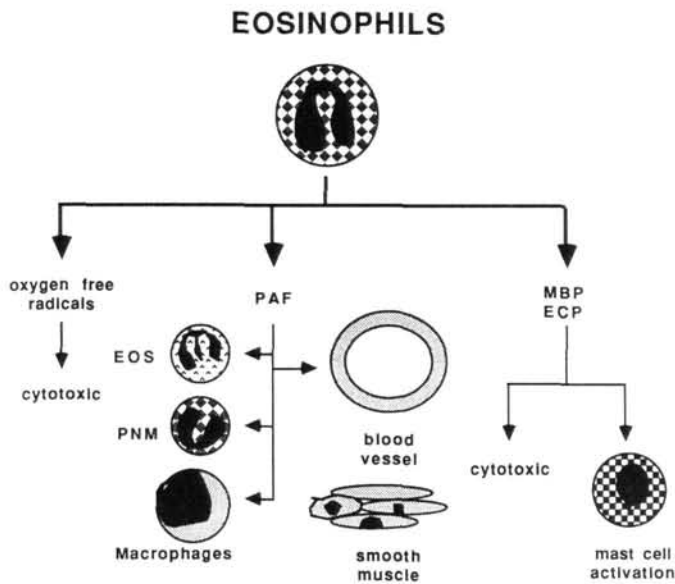


Figure 3. Some mediators released by eosinophils, and their principal action on tissues.

since they release IL-1 which has strong pro-inflammatory effects and histamine releasing factors (HRF) enhancing the activation of mast cells present in the infiltrate.

1.8.2.5. MACROPHAGES AND NEUTROPHILS

Macrophages and neutrophils release chemotactic factors but also oxygen free radicals and enzymes involved in the inflammation. Neutrophils represent the first cell type present in the inflammatory infiltrate following the EPR. They are in an activated state, i.e. they can initiate the cutaneous damage and attract other cells, particularly eosinophils (15).

1.8.2.6. HYPERREACTIVITY

Allergen challenge of the nasal or bronchial mucosa leads to non-specific hyperreactivity characterized by the occurrence of symptoms of rhinitis or asthma after the inhalation of non-specific triggering factors such as tobacco smoke, histamine, acetylcholine or other irritants. This phenomenon plays a major role in the severity of rhinitis or asthma (18). In the skin, no such non-specific hyperreactivity was observed (1). Among the several possible explanations for the lack of allergen-induced

hyperreactivity following the skin reaction are the differences between skin and mucosal mast cells (39).

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2. Methods for skin testing

2.1. GENERAL ASPECTS OF SKIN TESTING (ST)

The weal size of the immediate or early phase reaction (EPR) is most often used as a measure of the skin reactivity to allergens and mediators/mediator releasing substances used as references. Although the late phase reaction (LPR) is probably of great importance for our understanding of allergic diseases, it is less often used as a measure of skin reactivity in routine diagnostic work, for standardization purposes and in epidemiological studies. Therefore, emphasis is put on the EPR in this chapter.

2.1.1. *Skin diseases and skin testing*

Skin tests should not be performed on anatomical locations with eczema or obvious dermatographism (2,3,8).

2.1.2. *Skin testing and drug treatment*

Antiallergic drug treatment may significantly suppress EPR to ST (51). A general rule is that treatment with conventional, relatively short-acting antihistamines should be discontinued at least 3 days (51) prior to ST. Long-acting, non-sedating antihistamines, e.g. astemizole suppress the EPR for up to 2 months (51,8). Bronchodilators including epinephrine (adrenaline), theophylline and oral β_2 -adrenergic agonists, have not been shown to significantly suppress the EPR to neither allergen nor histamine (51). Corticosteroids in doses equivalent to 30 mg of prednisone/prednisolone per day for one week do not reduce the response to skin test (56), whereas local application of potent steroid ointments suppress the ST response markedly (52). There is no need to discontinue low dose oral glucocorticoids, i.e. doses less than 10 mg of prednisolone per day (51).

2.1.3. *Anatomical location of skin testing*

Skin tests can be performed on the volar surface of the forearm or on the back (8,46). If only a limited

number of tests are performed, the volar aspect of the forearm is the most convenient area. The skin can, but need not, be cleaned with either isopropyl alcohol or 70 % ethyl alcohol. It should be totally dry before ST's are performed (46). The test sites should be marked with an appropriate code, and allocated at least 5 cm and 3 cm apart for ICT and SPT, respectively, to avoid overlapping of reactions and influence of strong reactions on slightly positive or negative tests (8,34,46). Tests may not be placed within 5 cm from the wrist and 3 cm from the elbow (8,46).

Reactivity to both allergen and histamine varies between different parts of the skin surface. Using ICT (42) and SPT (27), the back was found more reactive than the forearm. However, this was not true for all test substances (27). The antecubital fossa is the most and the wrist the least reactive part of the volar side of the forearm (46).

There is a circadian rhythm of the size of skin reactions to allergen and histamine, with maximum weal size during the night (32). When the total population is regarded, the reaction to histamine is less pronounced in infants, increases in young adults and then declines gradually (53). However, in children with manifest atopic allergy, the skin has similar reactivity from 1 year of age until puberty (14).

2.1.4. *Allergen extracts*

A prerequisite for scientific and routine diagnostic ST is the availability of standardized, stable, allergen preparations, i.e. with known composition, potency and stability (2,8,15,22,44,47). The extracts should satisfy the following requirements:

- They must contain active allergens with relative amounts largely resembling that of naturally occurring allergen source materials.
- The concentration should be known and given in defined biological units, e.g. the Nordic Biological Units (BU/ml) (44), the US Allergy Units (AU/ml) (4,57,58) or the index of Reactivity (IR)

(63). Furthermore, the potency should be given in International Units (IU/ml)(45) when International Standard Preparations are available and the content of major allergen(s) by weight should be given when appropriate methods are available for determination of these antigens.

- They must not be contaminated with other allergens (to the extent possible). This is crucial as a 0.01 % contamination with one allergen may induce a positive reaction in highly sensitive individuals (16)(See Chapter 3).
- There may not be any irritating or toxic components in the final product (except naturally occurring enzymes such as phospholipase and hyaluronidase in Hymenoptera venoms).
- They should have documented stability within accepted limits, i.e. according to the Nordic Guidelines 50 to 200 % of labelled value (44).
- The batch to batch variation must be limited and the variation included in the limits given for stability in relation to a given reference (Company or laboratory freeze-dried In-House Reference preparation - IHR). Fig. 1 (25) outlines a suitable procedure.

Human serum albumin 0.03 % should be added to extracts used for ICT or SPT to increase stability. Addition of glycerol 50 % increases the stability still more. Due to its irritating properties, 50 % glycerol may only be added to extracts used for SPT, but may not be added to allergen solutions intended for ICT. This is due to the fact that larger amounts of test solutions are introduced into the skin by the ICT method, i.e. 0.01-0.05 ml, to be compared with approximately 3×10^6 ml by SPT (8). Because of the lower volume introduced by SPT in relation to ICT, about 10 000 - 30 000 times stronger extracts should be used for SPT than for ICT, to obtain the same skin response (38,59). The potency of allergen extracts used for routine diagnosis should be suitable for detection of all patients with clinical allergy (15,20), i.e. have a high sensitivity (27)(See Chapter 3).

Some allergens, e.g. food allergens, are not available as well characterized, freeze-dried allergen preparations with known allergenic composition. Then fresh fruit, meat, nut etc., can be used for testing according to the prick-prick method (22), i.e. first pricking the food, then the skin with the same needle. Furthermore, foods which need

extraction before testing, e.g. flour, can be extracted in 0.1 molar ammonium bicarbonate or 0.01 phosphate buffer with saline at + 4°C, (filtered) and frozen in small aliquots, which then are thawed when needed for testing (14).

2.1.5. Negative and positive controls

To evaluate unspecific reactions caused by the trauma of the skin induced by test devices (dermographism), tests should be performed with a negative control solution in parallel with the allergen tests (2,8,36,61), in routine, at least in cases with positive tests to all tested allergens. To document normal reactivity of the skin, i.e. no major change in skin reactivity due to intake of anti-allergic drugs, skin tests should be performed with a positive control, e.g. histamine or mast cell secretagogues such as codeine or compound 48/80 (2,8,23,36,44). Histamine dihydrochloride 10 mg/ml (54,3 mmol/l), equivalent to 6.14 mg/ml of histamine base, is recommended for use as positive control (8,15,17,36,38,44). In ICT, histamine dihydrochloride 0.1 mg/ml should be used (8,42).

2.1.6. Recording of skin reactions

Test sites should be inspected and reactions recorded after 15 minutes (8,41,46,60,61). Since in some patients reactions take longer to develop, test sites should be reinspected at 25 - 30 minutes, and the largest reaction recorded (45). This is true especially when ST's are used for scientific purpose. To obtain a permanent record, the contours of the weal and erythema should be outlined with a fine filter tip or ball-point pen and the contours transferred by means of a translucent tape to a record sheet. The size of the skin reaction should be recorded as the mean of the longest and the midpoint orthogonal diameters, the sum or the product of these diameters (8) which all correlate to the more precise area obtained by planimetry (48). In SPT the weal size is normally used, whereas both the weal and erythema sizes are measured to quantify the skin reaction in ICT (46,60). Other methods such as the laser doppler technique (55) determining the blood flow in the weal and erythe-

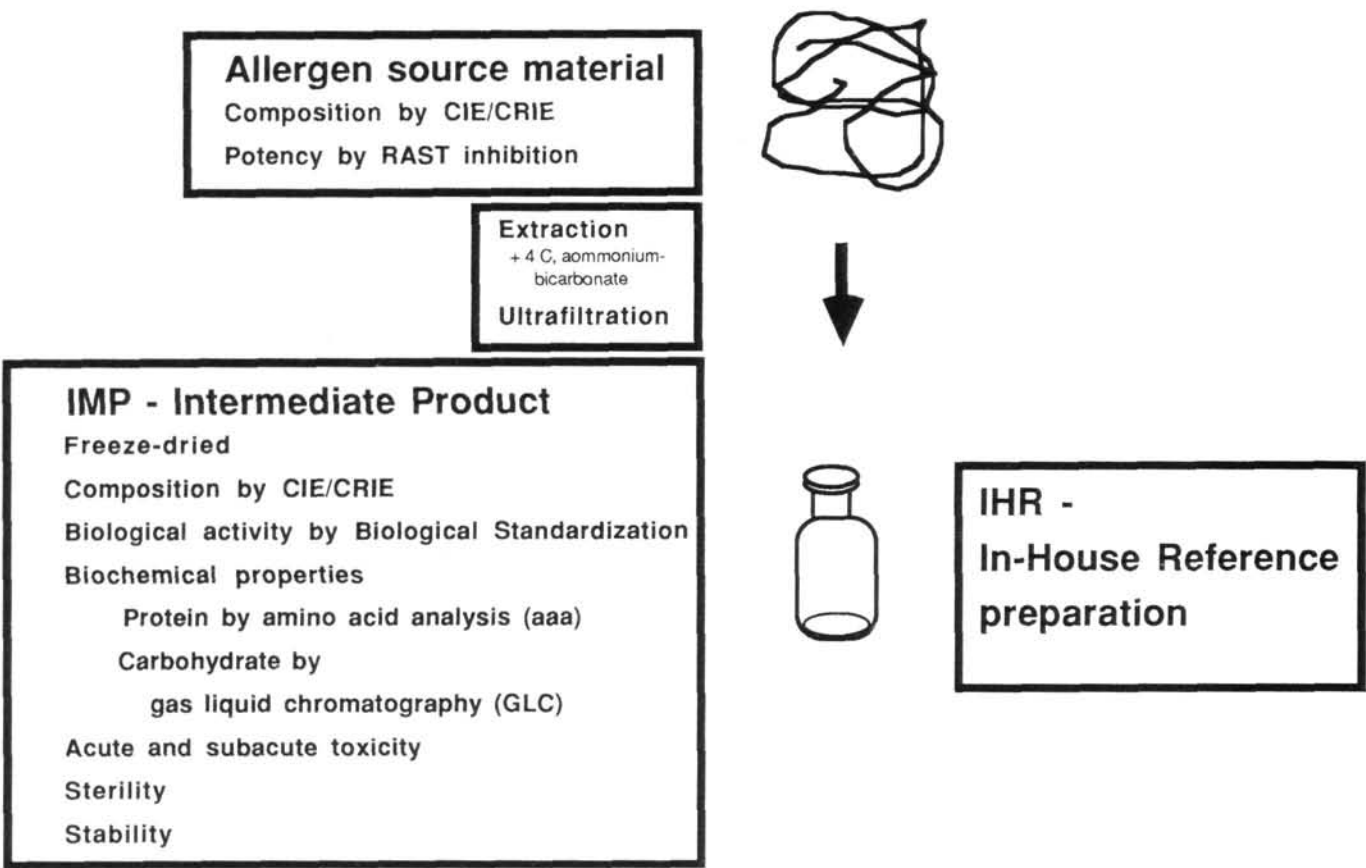


Figure 1. The principle for production of standardized, freeze-dried allergenic preparations by manufacturers and research laboratories. Modified after (25).

ma, respectively, and ultrasound (54) estimating the area, thickness and volume of the weal, can be used for scientific purposes.

To assure reproducibility single ICT's are sufficient, provided no leakage occurs from the primary weal (59). Skin prick tests should always be performed in duplicate, as single negative tests (5 %) will be obtained in clearly sensitive patients even by skilled technicians (19) and the risk for false negative tests due to technical problems is high in patients with low skin sensitivity (16). For scientific purposes quadruplicate tests with each test solution are recommended (15,34,44). The reproducibility of the ST should be estimated either based on duplicate tests performed in every patient or based on 20 tests performed at intervals in the same patient with the same allergen solution or histamine. The latter method is probably less reliable, since it does not supervise the variation in technique on a daily basis. For SPT, a coefficient of variation (cv) less than 40 % based on area, or 20 % based on mean diameter) is recommended (15,35,34,37,38,44), but cv's about 15 - 20 %

based on area, 7-10 % based on mean diameter, can be reached by skilled technicians (11,19,20).

2.2 INTRACUTANEOUS (INTRADERMAL)(ICT) SKIN TEST METHOD

2.2.1. Practical performance

For ICT, a sterile unitized, disposable 0.5 ml syringe with a built-in 27-gauge needle is recommended (8). The syringe should be filled with 0.1 ml of test solution and all bubbles expelled (8,46). The ICT is performed by stretching the skin, then the needle is introduced at 45 degrees angle to the skin surface with the bevel facing down, the point of the needle is gently inserted in a forward lifting motion so as to pick up the skin with the tip of the needle. As the tip enters the skin, the pick up motion is gradually converted into a forward, downward pressure, while the barrel is lowered to eliminate the angle to the skin surface. The bevel should enter the skin entirely and lie between the

layers of the skin, with the remainder of the needle outside but in contact with the skin. According to different procedures, 0.01 - 0.05 ml of test solution is injected (8,46), giving a bleb with a diameter of 3 to 5 mm, respectively. The size of this “primary weal” increases during 15 minutes to approximately 5 to 7 mm in diameter. If no bleb is formed, air is splashed into the skin or test solution leaks out of the bleb, a new test should be performed at suitable distance from the first test.

2.2.2. Evaluation of test results

The sensitivity and the specificity (26) of both the SPT and the ICT depend on the concentration of allergen and technical performance of the test. The ICT method is 10 000 - 30 000 times more sensitive than the SPT method and should therefore be preferred to SPT for detection of patients with low grade sensitization and for evaluation of allergenic extracts of low potency (provided higher concentrations are not available)(7,8,59). The diagnostic capacity is discussed in Chapter 3.

2.2.3. Cut off limit

The cut off of a method is for skin tests the size of the reaction that best discriminates between negative control and positive test result. For ICT, the lowest concentration producing a weal reaction larger than that of the negative control, but no erythema, is considered the end point. In the US a 2+ reaction according to Norman (46) is most often used as a criterion for positivity (mean of the longest and the midpoint orthogonal diameters):

Grade	Erythema	Weal
0	< 5 mm	< 5 mm
±	5-10 mm	5-10 mm
1 +	11-20 mm	5-10 mm
2 +	21-30 mm	5-10 mm
3 +	31-40 mm	10-15 mm or with pseudopods
4 +	> 40 mm	> 15 mm or with many pseudopods

Another method to determine the potency of an extract or the sensitivity of a patient is to estimate the concentration of test solution inducing an erythema with a sum of the longest and the mid-

point orthogonal diameters = 50 mm, i.e. the ID₅₀EAL method given by FDA (4,57,58)(See Chapter 4 and 5).

2.2.4. Dose response relationship

Using the least square regression method on skin reactions as a model of the dose response relationship between allergen concentration and weal size can be estimated. By logarithmic transformation ($\text{Log (size of skin reaction)} = a + b \text{ Log (concentration of allergen)}$) a linear dose response relationship with the same standard deviation of e.g. weal size at different concentrations is obtained. A high value of the coefficient of correlation (r) between allergen concentration and weal size indicates a strong linear relationship. In some practical applications a value of $r \geq 0.85$ has been set up as a criterion for approval of data. (32, 36, 57, 59). The erythema shows a steeper slope (b) than the weal using a linear (size of reaction) / log (concentration of test solution) model (59), since the size of the erythema increases rapidly at allergen concentrations just a little higher than that eliciting the end point reaction (see table above). This phenomenon has been utilized in the US to outline methods for determination of relative potencies between allergenic extracts and estimation of patient’s sensitivity (See Chapter 4 and 5). At higher concentrations the weal and erythema reactions are largely parallel (38,59). There is also a dose response relationship of the LPR (64).

2.2.5. Comparison between ICT methods

Detailed recommendations have been given for ICT (8,46). These should be followed. In many scientific studies micrometers have been used for determination of the injected volume of test solution. This seems to be an unnecessary precaution, since injection of approximately 0.05 ml by plastic disposable syringes until the blebs were of similar size, was at least as precise as using a micrometer syringe (cv % 9,5 and 10, respectively)(24).

2.2.6. Recommendations

The risk of eliciting adverse reactions is higher with ICT (33), provided titration, starting with

very low concentrations, is not performed (41), but negligible with SPT. Therefore, SPT can be recommended only to clinicians with long experience of ICT performed with strict safety precautions (41). With the availability of standardized allergen extracts with predetermined potency, relevant for diagnosing clinically sensitive patients (See Chapter 3), ICT has no advantage over SPT (8,15,38,42,43). Intracutaneous tests should only be performed (with precautions (34)) provided SPT is negative and higher concentrations of allergen extract are not available. Regarding concentration vs diagnostic sensitivity and specificity, see Chapter 3.

2.3 SCRATCH SKIN TEST METHOD

2.3.1. Practical performance

The first skin test performed by Charles Blackley, published in 1873 (10) was a scratch test. Blackley abraded an area of the skin, approximately 1 cm² in size, but later investigators have used a scarifier to perform a 1 cm² scratch of the skin, without causing any bleeding. One drop of test solution is then applied (29). The scratch test method has also been used in combination with application of fresh food with the aid of the so called Finn Chamber (31).

2.3.2. Evaluation of test results

The scratch test method is 500 times less sensitive than the ICT method (29), i.e. only about 20-60 times more sensitive than the SPT method, that is much less traumatic. Therefore, more potent extracts should be used, than with ICT. False positive reactions are more easily elicited by the scratch method than with the SPT or ICT methods. This is due to the more pronounced trauma of the skin in scratch testing, resulting in large reactions even to negative control solutions (29).

2.3.3. Cut off limit

The large unspecific weal reactions (triple response) induced by the trauma, makes it difficult

to define the cut off limit between negative and positive tests.

2.3.4. Dose response relationship

Using a + grading system of the skin reaction and a log/log model, a parallel dose response relationship has been documented for the scratch test method (31).

2.3.5. Comparison between scratch test methods

There are no valid comparisons between different methods for scratch testing.

2.3.6. Recommendations

Scratch tests are not recommended for routine diagnostic work nor for scientific use, due to the high background of the test (triple response to trauma).

2.4. SKIN PRICK (PUNCTURE) TEST METHOD (SPT)

2.4.1. Practical performance

Until 10 years ago, SPT was mainly performed by the method of Pepys (50) using a fine disposable needle or an ordinary blood lancet. The tip of the device is inserted at an angle of 60 - 70° to the skin surface through a drop of test solution placed on the skin. The tip is introduced into the superficial layer of the skin, which is caught and gently lifted. Thereby, a minute canal is formed, through which the test solution penetrates. The test device is pulled out or further lifted. The extract may be wiped off immediately, but this is not an absolute demand (46).

In 1979 a new method was published, using a lancet with 1 mm tip and shoulders preventing further penetration (48). This lancet should be pressed (15), not jabbed (40,47), at 90° to the skin surface through a drop of test solution (5, 11,15,27). The same pressure should be applied

every time (15). The same technique is used with other devices such as the plastic Morrow-Brown needle (Stallerpoint®, Stallargenes)(12,42), and the steel lancets, the Allergy Pricker (DHS Prick Lancet)(5,11,35) and the Phazet® (Pharmacia) (6,15,27,30,62). Lancets pre-loaded with allergen should remain in the skin for 1 second (15,62). The bifurcated needle, recommended by FDA in USA but not available in Europe, has two several mm long points and is used in a similar but not identical manner to European steel lancets. For allergens not available standardized, the prick-prick method, i.e. first pricking the food, then the skin with the same needle, should be employed (22).

2.4.2. Evaluation of test results

Until recently, the value of SPT has been limited by the low potency of allergenic extracts (15, 38,46), especially non-pollen allergen preparations (21,38). The use of low potency extracts results in many false negative results, i.e. low sensitivity. Even when high potency extracts are used, SPT doesn't elicit too many false positive reactions, i.e. they are still indicating the presence of IgE sensitization. Regarding the clinical relevance, i.e. sensitivity and specificity in relation to clinical disease, see further chapter 3.

2.4.3. Cut off limit

Most studies on SPT have used only the weal not the erythema reaction for evaluation of the response (5,6,9,11-13,15,19,20,23,27,30,35-40, 43,48,49,53,62). The weal induced by the trauma of the needle and the negative control solution is often 0 but may amount to 2,5 mm in diameter (5 mm²) in single tests in patients without otherwise demonstrable dermographism. Therefore, weals < 3 mm in mean diameter should be regarded as negative. However, when repeated negative controls do not show a lower response, then the cut off limit may be lowered. Thus, without special precautions, only weals ≥ 3 in diameter should be regarded as positive (15,35,62).

The cut off point is also determined by the technique and the potency of the allergen prep-

aration. Its implication will be further discussed in chapter 3.

A grading system system for SPT based on the relation between the reactions induced by allergen and the histamine reference has been used in Scandinavia (3):

Grade	Percent of the area of the weal induced by histamine reference
-	Same size as negative reference
+	25
++	50
+++	100
++++	200

This original grading system was based on histamine HCl 1 mg/ml and has not been re-evaluated after the recommendation by the Nordic Committee on Standardization of Allergen Extracts of using histamine HCl 10 mg/ml. This recommendation was originally meant for biological standardization (44), but has led to the use of this concentration of histamine even in the routine. This method does not take into account the potency of the extract used.

2.4.4. Dose response relationship

Careful studies of the dose response relationship of SPT have shown the slope of the allergen induced weal reaction, using a log/log model, to vary between patients. However, the mean slope in a sample is about 0.4 using the weal area as a measure of allergen response (9,15,19,20,21, 23,37,38), i.e. the weal area increases 2.5-fold by a 10-fold increase in concentration (16)(slope 0.2 and increase 1.5-fold using the diameter as a measure of response). The weal area induced by SPT increases slightly more than that induced by ICT (38,43), i.e. the slope is steeper.

Several studies have shown that the slope of the histamine weal increase is significantly less steep than that of allergen (19,23,37,38). However, this is only true for the mean slope in a sample of patients. In individual patients, there might be both some with steeper and some with flatter allergen than histamine dose response relationship. The slope of the allergen dose response relationship varies more than that of histamine. However,

in most patients the allergen and histamine dose response relationships are parallel according to statistical criteria (15,19,23).

Endpoint titration, i.e. determination of the concentration of allergen eliciting a just measurable weal response, has been used to determine the cut off. This method is controversial (38). Probably as good is therefore an estimation of the endpoint, using the median slope of the allergen dose response relationship, 0.4 (16), by simply dividing the weal diameter with 1.5 for each ten-fold dilution (or the area by 2,5). A simple yardstick which can be used is 2, 3, 4.5, 6.8, 10.1, 15.2, 22.8 mm in diameter for 10-fold increases in skin sensitivity. Thus, it can be estimated that a patient who reacts with a weal 22.8 mm in diameter to 100 000 International Units/ml (IU/ml)(or any BU), should have reacted with a 3 mm weal (cut off limit) to 1 IU/ml (BU/ml). This type of estimations are well correlated to results obtained with the parallel line assay method when applied to samples of patients. However, it can not be used for exact estimations of skin sensitivity in individual patients.

2.4.5. Comparison between SPT methods

Several comparisons have been made between different methods for SPT (5,11,22,30). Only results obtained by the same trained investigator can be used for comparison, as the precision varies considerably between centres. Several authors have found devices with 1 mm tip to have a better precision than the Pepys method (5,11) and Bousquet et al (11) who compared a number of devices found the original allergy pricker and the similar uncoated Phazet needle to give results which were more reproducible than those of other common devices on the market in Europe. The precision of the American bifurcated smallpox needle was similar to that of the uncoated Phazet (1 mm tip) tested with the same wet extract and the Phazet needle (27).

2.4.6. Recommendations

Skin prick tests are recommended as the method of choice (1,2,3,8,15,50) because of its safety and the SPT method of choice is the puncture method with

a standardized device with 1 mm tip. In the routine, SPT should if possible be performed in duplicate with one concentration (See chapter 3) and in scientific work in quadruplicate with each concentration. Standardized extracts with documented potency, composition and stability, either wet or dry on the tip of the needle, should be used. When a standardized technique and a defined concentration of allergen is used, then the size of the weal can be used as a measure of the degree of skin sensitivity, or used for comparison of an extract with unknown potency to that of a standard. The weal contours should be recorded. The potency of the allergen, the method of testing and the mean size of the histamine weal in the hands of the investigator (10 mg/ml ~ 6 mm in diameter) should always be given in scientific papers. The precision of the method should be documented in the hands of the scientist as well as the assistant using SPT in routine diagnosis. The c.v. % may not exceed 40 % (ideally 20 %) using the area and not 20 % (ideally 10 %) using the mean diameter as a measure of weal size. The 3 mm cut off limit is recommended, provided these recommendations are followed.

2.5. RECOMMENDATIONS

Skin tests should only be used by physicians trained to perform and interpret such tests.

For routine diagnostic work it is recommended to use SPT with standardized allergenic extracts, with a potency that has been documented regarding its diagnostic capacity, a standardized needle with 1 mm tip, duplicate tests, when possible, to be able to check the precision of the assistant, and to register on a record sheet, weal sizes induced by allergen and control solutions.

Intracutaneous skin tests are used in the routine in the Netherlands. It should be used only by experienced allergologists.

Recommendations how to use skin tests for scientific purposes will be further discussed in each separate chapter.

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3. Skin tests for diagnosis of IgE-mediated allergy

The most important part of clinical diagnosis of specific atopic allergy (to a certain allergen) is the history of the patient, taken by an experienced allergist. Skin tests are used to confirm IgE-sensitization of the skin which is one possibility to support specific allergy diagnosis. Recently several position statements on skin testing have been published (3, 31).

3.1 DIAGNOSTIC CRITERIA

Skin testing means introduction into the superficial layer of (application on) the skin of allergenic material. Provided IgE-antibodies, with an idiotype corresponding to the allergen introduced, are attached to skin mast cells, mast cell mediators are released, causing an early (immediate)(EPR) and under certain circumstances also a late phase reaction (LPR)(1)(See Chapter 1). The latter is more often found when high concentrations of allergen are used for testing in highly sensitive individuals (3). In this chapter, emphasis will be put on EPR, since LPR is seen in only 8% of adults using a concentration suitable for routine diagnosis by SPT (16). However, LPR is more easily induced by ICT than by SPT, since ICT is $\approx 30\,000$ times more sensitive than SPT (3). This fact should be kept in mind in scientific studies, evaluating the influence of therapeutic measures on LPR.

3.1.1. Definitions

3.1.1.1. SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUE AND EFFICIENCY OF SPT

As for all other diagnostic tests, the presence of a positive test does not always implicate clinical disease, and a negative test does not always de-

note absence of clinical allergy. The clinical meaning of results obtained by allergy skin testing highly depends on a number of factors, as mentioned under 3.2. Definitions of sensitivity, specificity, positive and negative predictive diagnostic value and efficiency are given in Table 1 (19). These terms can also be illustrated as in Fig. 1 (13). Results obtained under certain circumstances in a clinical trial, can only be applied to routine diagnostic work provided the same potency of allergen is used, the prevalence of disease is the same, the sample of patients subject to diagnostic work has the same characteristics as that studied in the trial etc.

3.1.1.2. SENSITIZATION VERSUS CLINICAL ALLERGY

Probably most individuals who get into contact with an appropriate protein antigen, capable of inducing an IgE response (an allergen) produce low amounts of allergen-specific IgE antibodies, i.e. they get sensitized. Atopic individuals are characterized by developing higher levels of idiotypic IgE antibodies, when exposed to very low doses of allergen (21), persisting for a longer period of time than in normals. However, all the children responding with IgE antibody production at early exposure and all adults exposed to high concentrations of allergen in their occupation, who also respond with IgE antibody production, do not show clinical allergy at contact with the allergen in question. Some develop clinical allergy later and in some the test may remain positive for years after clinical symptoms have disappeared. This phenomenon was described already in the thirties and forties by Carl Juhlin-Dannfeldt (24)(Fig. 2) who named the state of sensitization, without demonstrable symptoms, latent allergy. It has been studied in "modern time" e.g. by Horak (22).

Table 1

DEFINITIONS	
TP - <u>True positive</u> :	test positive and diseased, skin test and provocation test positive.
FP - <u>False positive</u> :	test positive but not diseased, skin test positive but provocation test negative.
TN - <u>True negative</u> :	test negative and not diseased, skin test and provocation test negative.
FN - <u>False negative</u> :	test negative but diseased, skin test negative but provocation test positive.

SENSITIVITY

True positive in percent of patients with disease

$$= \frac{TP}{TP + FN}$$

		Test		
		+	-	
Allergic	+	TP	FN	TP + FN
Non allergic	-	FP	TN	FP + TN
		TP + FP	FN + TN	TP + FP + TN + FN

SPECIFICITY

True negative in percent of patients without disease

$$= \frac{TN}{TN + FP}$$

		Test		
		+	-	
Allergic	+	TP	FN	TP + FN
Non allergic	-	FP	<u>TN</u>	<u>FP + TN</u>
		TP + FP	FN + TN	TP + FP + TN + FN

+ PREDICTIVE VALUE

Patients with disease in percent of test positive individuals

$$= \frac{TP}{TP + FP}$$

		Test		
		+	-	
Allergic	+	<u>TP</u>	FN	TP + FN
Non allergic	-	FP	TN	FP + TN
		<u>TP + FP</u>	FN + TN	TP + FP + TN + FN

- PREDICTIVE VALUE

Patients without disease in percent of test negative individuals

$$= \frac{TN}{FN + TN}$$

		Test		
		+	-	
Allergic	+	TP	FN	TP + FN
Non allergic	-	FP	TN	FP + TN
		TP + FP	FN + TN	TP + FP + TN + FN

EFFICIENCY

Tested individuals correctly classified by the test in percent of all tested

$$= \frac{TP + TN}{TP + FP + TN + FN}$$

		Test		
		+	-	
Allergic	+	<u>TP</u>	FN	TP + FN
Non allergic	-	FP	<u>TN</u>	FP + TN
		TP + FP	FN + TN	<u>TP + FP + TN + FN</u>

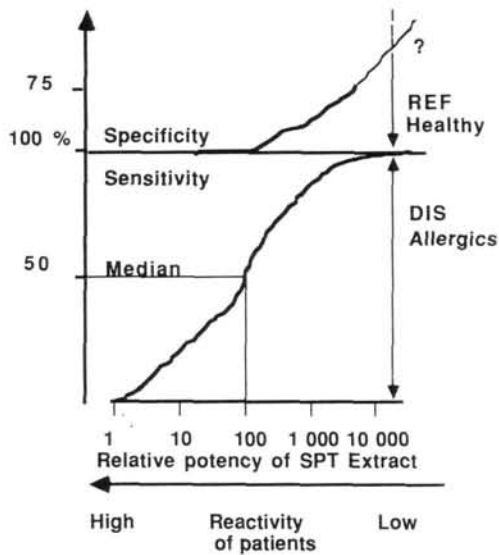


Figure 1. The higher the concentration of allergen used for skin testing (high to the right on the x-axis), the more patients will respond with a weal and flare reaction. 100 % sensitivity and specificity are illustrated by the horizontal line. To the left, specificity is high, the more to the right the lower the specificity and higher the sensitivity. At the concentration inducing a positive weal (≥ 3 mm in diameter) in all patients sensitive to the allergen investigated, sensitivity is 100 %. There is no need to further increase the allergen concentration. The two most important concentrations are those giving 100 % specificity and 100 % sensitivity (or close to it).

3.1.2. Suitable methods for illustration of diagnostic results

The sensitivity and specificity etc are most often given as a percentage based on cross tables as illustrated in Table 1 (19). Then only one concentration of allergen or weal size is used. However, by using the S-curve shown in Fig. 1, it is possible to illustrate the sensitivity and specificity using different concentrations, when end point titration is used, or weal sizes, when only one concentration of allergen is used. The most informative system for presentation of results of diagnostic test results in relation to disease, is that proposed by Gerhardt and Keller (20), which is illustrated by a principle drawing in Fig. 3a and an example in figure 3b (16). That system allows not only illustration of sensitivity, specificity and the other diagnostic parameters at one concentration but at several different concentrations, i.e. original data and results from several cross-tables, each one

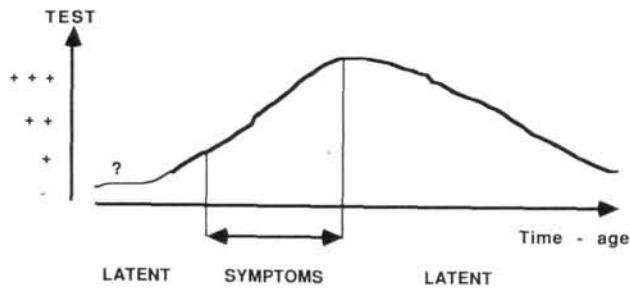


Figure 2. Patients are said to be latent allergic when they have a positive test (skin test) without any symptoms at contact. Latent allergy may precede, but also follow, clinical allergy. It probably also appears without clinical allergy, i.e. sensitization that disappears without clinical symptoms.

originating from one of several investigated concentrations (classes of weal sizes, when one concentration of allergen is used). This system makes it possible for readers of papers (later users of diagnostic tests like SPT or ICT) to understand the difference in diagnostic value when changing the cut off, as measured by allergen concentration or weal size.

3.2. FACTORS INFLUENCING THE RESULT

As mentioned above, a number of factors are of importance for the diagnostic information obtained by skin tests. These are: Definition of disease (allergy to the allergen under investigation); Potency of allergenic extract; Skin prick test technique and prevalence of disease (allergy) in the sample studied. When test techniques used by investigators and how they defined disease was well described, then their results can be applied to routine diagnosis, provided the same technique and diagnostic criteria are used by practising doctors. The degree of exposure to the allergen in question is of importance for the prevalence of allergy and in the case of continuous exposure with ongoing allergic inflammation also for the outcome of diagnostic tests, especially provocation tests.

3.2.1. Definition of disease

Definition of criteria for positive diagnosis.

3.2.1.1. HISTORY

The diagnosis of allergy to a certain allergen is mainly based on the clinical history. The history is

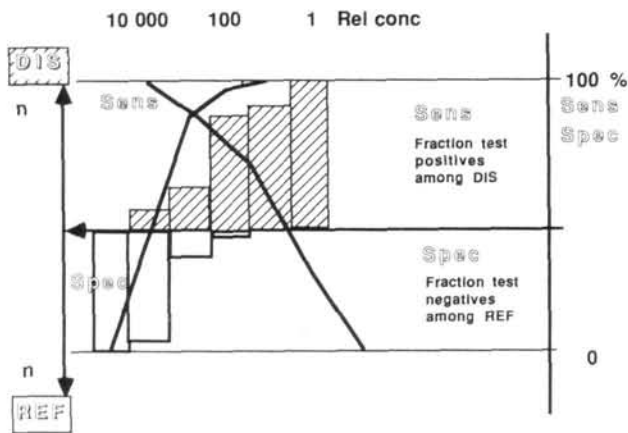


Figure 3 a. The system of Gerhardt and Keller (20). The number of individuals (left scale) with disease (allergy) are given by an histogram above the horizontal line, those without allergy, i.e. reference persons, below the line. For each class (positive ST at a given concentration) or weal size (log transformed), sensitivity and specificity can be calculated and plotted (right scale). In most situations a high sensitivity or a high specificity is preferred.

obvious, when patients get symptoms at direct contact with the allergen, e.g. animals, or when symptoms are only present during a short and well defined pollen season, e.g. the birch or plane pollen seasons. Sensitive patients exposed to low doses of allergen or patients who are only slightly sensitive and continuously exposed to high doses of allergen, do not give a clear history. Therefore, diagnostic tests must be used to support the history.

3.2.1.2. PROVOCATION TESTS

Since long, these tests have been regarded as the ultimate answer as to whether a patient is allergic or not. However, provocation tests are not physiologic, as high doses of allergen are administered during a short period of time (extract, pollens/spores or freeze-dried powdered allergen), whereas patients often are exposed to much lower doses over a long period of time. As yet, there has not been any agreement as to which doses of allergen should be used. The higher the dose, the more patients react (16). Probably, the most relevant tests are performed with pollen grains in naturally occurring doses (1 - 10 000 pollens per test) (4,8,10,30) or with powdered allergenic material (30). When conjunctival and bronchial provoca-

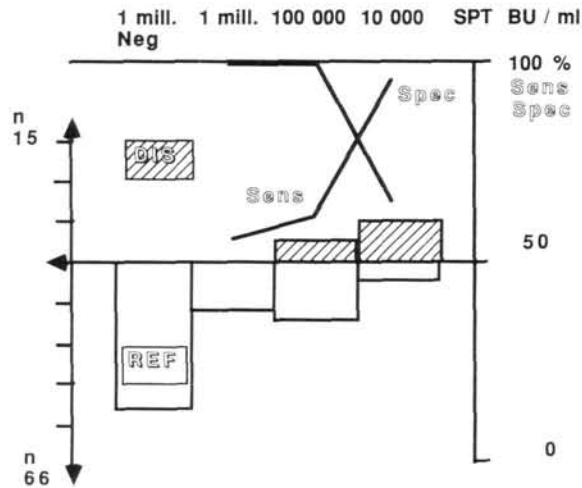


Figure 3 b. An example taken from reference (16). With dog allergy defined as symptoms at direct contact, SPT showed a high sensitivity at 100 000 BU/ml but still higher at 1 mill. BU/ml and the specificity was high at 10 000 BU/ml.

tion tests are performed in samples of patients with clinical sensitivity, then the distribution of threshold concentrations (concentration giving a positive test result at titration) has a similar S-shaped distribution as found for skin sensitivity (10). The difference in sensitivity between the most and the least sensitive patient in a sample is about the same as for SPT, i.e. 4 powers of ten (13-15,18).

It is well known that e.g. during the pollen season, patients have more symptoms during the last part of the season than in the beginning of the season, although pollen counts are similar. This has been explained by priming (10) of the shock organ by continuous pollen load for several weeks, that makes the shock organ more sensitive. This phenomenon is probably due to cumulation of late responses, which persist for several days. Thus, one pollen peak influences symptoms in patients with pollinosis for about one week (9,35). Many patients are continuously exposed to allergens, e.g. mite or animal dander allergens, and are therefore primed. Such patients will probably react to lower concentrations of allergen than non-primed individuals. It has also been shown that the hyperreactivity and sensitivity to allergen increases during the pollen season (5,6). Therefore, no definite concentration can be given that can be generally recommended as the highest relevant for clinical allergy, i.e. results from provocation tests can not be used as the golden standard for discrimination between clinical and latent allergy. This also applies to repeated low dose

provocation tests (23) as the way allergen is applied never can mimic the natural often continuous exposure to environmental allergenic particles.

3.2.1.3. OTHER CRITERIA

Patient's symptom scores correlating to exposure, e.g. pollen or mould spore counts, have been used to define patients with a true association of symptoms to the allergen in question (27,34). However, such complicated procedures are not possible to use in routine diagnosis and therefore only relevant for scientific studies aiming at increasing our knowledge about the clinical symptomatology of allergy to allergens without defined clinical picture or to define patient populations under study.

3.2.1.4 COMBINATION OF CRITERIA

The relevance of a certain less defined diagnostic criterion, does not increase because it is combined with another ill defined criterion. One possibility might be to define a sub-population of patients, obviously clinically allergic to the allergen, investigate which concentration that has the highest sensitivity in challenge testing, i.e. diagnoses most of these obviously sensitive patients by a certain challenge method, and then use this concentration for challenge testing of patients with the same specific allergy, but with less defined clinical history (16).

3.2.2. *Potency of allergenic preparations*

It has been calculated (36) that, to obtain the same response with ICT as with SPT, 30 000 times more diluted extracts can be used. Therefore, ICT should be used when extracts are available in low concentrations or the aim is to trace patients with low degrees of sensitization.

As illustrated in Fig. 1 and 3b, allergenic preparations with low potency does not diagnose all patients with clinical allergy (according to the definition of disease). In biological standardization trials, consecutive patients attending a University allergy department were included. The

sensitivity of these patients differed four powers of ten (13,16). To diagnose the least sensitive patient in such a sample, 100 000 BU/ml (See Chapter 4) should be used. To diagnose all patients in a community with clinical allergy to the same allergen but not with symptoms so severe that patients were referred to a University allergy clinic, a still higher concentration of allergen was needed (16). Actually, it was found that a 10 times higher concentration of allergen (1 mill. BU/ml) was needed to diagnose all patients (high sensitivity), whereas a ten times lower concentration (10 000 BU/ml) was sufficient to obtain a high specificity (16).

3.2.3. *Skin test technique*

When ICT is used, then the technique used is of little importance (3) provided recommended procedures are used. However, when the SPT method is used, it is of great importance (2) that a constant technique is used in the routine and that it is sufficiently documented when scientific trials are performed (13,28). Probably the pressure and the time during which this pressure is applied are the two factors which are most important. Therefore, no recommendations regarding allergen concentrations etc given by authors of scientific papers are possible to apply to routine diagnostic work, if not the same technique is used and the same precision is obtained in routine, as was achieved by the author of the scientific paper. This fact points on the need to document the technique, the precision and the size of the weal obtained with a well established reference substance such as histamine HCl 10 mg/ml (13,15,28, 37) or codein phosphate 9 mg/ml (7) in all scientific papers using diagnostic tests.

3.2.4. *Prevalence of allergy*

Diagnostic parameters such as sensitivity and specificity are most often investigated in patients attending hospitals, mainly University allergy outpatient clinics. Most often the prevalence in such studies is 40-60 %. In the population in general, there is a prevalence of different types of

allergies of a few - 20 % (See Chapter 6). If the same cut off limit (weal size or allergen concentration) is used when the test is applied to patients in the general populations with a low prevalence of that allergy, there will be more false positive, and less false negative results, provided the degree of sensitivity in individuals in the general population who are sensitive to the allergen in question, is similar to patients investigated in the trial. However, in practice it has been found that there are patients in the general population, who are clinically sensitive but to a lesser degree than patients referred to Universities (13,16). The problem is illustrated in Fig. 5. The sum of these effects was, that a ten times higher concentration should be used to obtain a high sensitivity with SPT in "the general population" (16).

3.2.5. Sensitization versus correlation to clinical allergy

From the discussion above it comes that positive skin tests indicate presence of specific IgE-antibodies on mast cells in the skin, i.e. the body has reacted with formation of allergen-specific homocytotropic antibodies attached to type 1 IgE Fc-receptors on MC, but that only part of the individuals with such sensitization complain of allergic symptoms at contact with the allergen in question. There exists no definite limit as measured by skin sensitivity between clinically sensitive and non-sensitive individuals with latent allergy (24), as illustrated in Fig. 4b.

3.3. RECOMMENDATIONS FOR SCIENTIFIC INVESTIGATIONS

Skin prick tests with a needle of the Østerballe type, standardized allergenic extracts, preferably with known potency in relation to International Standard Preparations (IU/ml)(33) and acknowledged Biological Units (Nordic BU/ml (32) or FDA AU/ml (36)) or any biological unit, provided the potency is also given in IU/ml, should be used (alternatively the same type of needle with dry standardized extract). At least duplicate, if possible quadruplicate, tests should be performed with each test solution, to avoid that patients fulfilling the diagnostic criteria used in the trial

are not diagnosed by the test because of accidental negative tests, due to the fact that the precision is bad in some centres (13). Weal (and erythema) contours should be encircled with a fine pen and the contours transferred to a record sheet for permanent storage. The mean of the longest and the midpoint orthogonal diameters (mean diameter) or the area should be used as a measure of weal (erythema) size, and ≥ 3 mm in diameter used as cut off limit for positivity (3,13,25)(See further Chapter 2).

The ICT method is preferred to SPT for diagnostic studies by some investigators (31). However, the clinical relevance of concentrations used should be documented.

3.4. RECOMMENDATIONS FOR ROUTINE USE

In routine the SPT method should be preferred to the ICT method, because it causes less pain and the lower risk of systemic reactions. Needles of the Østerballe type should be used in combination with standardized allergenic extracts (or coated with such extract) of known and clinically documented potency, i.e. the clinical value of a positive or negative diagnostic test is known. Furthermore, to allow continuous supervision of test technique/precision and assure patients with clinical allergy to be diagnosed, tests should be performed in duplicate. Results should be registered and evaluated according to guidelines given in Chapter 2.

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4. Skin tests used for standardization of allergenic preparations

Skin tests are mainly used for two different purposes in the standardization procedure of allergens. Biological standardization, i.e. estimation of the biological activity of a standard of allergens intended for diagnosis (and treatment) of atopic allergy. This procedure should not be repeated, but for scientific purposes. Furthermore, ST are used for calibration of the potency of a batch/substandard against a standard preparation (in-house standard preparations (IHS)(17,23), national (FDA Standards)(2,35) and International Standard Preparations(ISP)(30).

4.1. BIOLOGICAL STANDARDIZATION

Biological standardization (equilibration)(BS), is used for calibration of the potency of references of allergenic preparations intended for diagnostic use, against the population of patients sensitive to the allergen in question, with the aim to equilibrate the biological activity of allergenic preparations prepared from different allergen source materials (1,10,17,28,29,34). The Nordic method for BS (10,13,17,28,29) includes all patients, referred to a University Allergy Outpatient Clinic, clinically sensitive to the allergen in question. The principle, modified after Aas et al (1), is shown in Fig. 1. In the US, a method for biological standardization has been prescribed by FDA (2,34,35). This method uses ICT and only the most sensitive patients are included. The aim of this method is to find a concentration suitable for ICT and starting of immunotherapy (30,37).

4.1.1. *Biological standardization (BS) by SPT*

In principle, the skin reaction to an allergen preparation is estimated in a sample of patients sensitive to the allergen in question. The reaction is

related to the reaction of a reference substance (histamine, codeine) with the aim to minimize the influence of differences in test technique. However, some proposed methods do not include the use of a reference substance.

4.1.1.1 METHODS

In 1978, the Committee on Allergen Standardization, within the Nordic Association for Allergology, recommended a combination of methods for standardization of allergens (1). Immunoelectrophoretic methods were recommended for characterization of allergenic raw materials, intermediate and final products, RAST inhibition was proposed for estimation of allergenic potency of products in relation to standards, prepared from the same allergen source material, and biological standardization by SPT for equilibration of the biological activity between allergen preparations prepared from different allergen source materials. The idea was adopted by the Nordic Council on Medicines in their Guidelines for Registration of Allergen Preparations (28). No definite method was given. Thus several methods have been described. One of these (10,13,14,17) was chosen as reference method in the second edition of the Nordic Guidelines (28). The three important parts of methods for estimation of biological activity of allergenic preparations are: The SPT method used, the method for statistical evaluation and most important the patient selection criteria (10,12,13,15,18). The SPT method itself is probably of less importance, but most investigators have used the Østerballe - Weeke needle (32) or similar devices or especially in the beginning of the 80-ties, the technique described by Pepys (33). All but one method (5) prescribes a reference substance, either histamine HCl or codein phosphate, to minimize the influence of differences in technique between investigators.

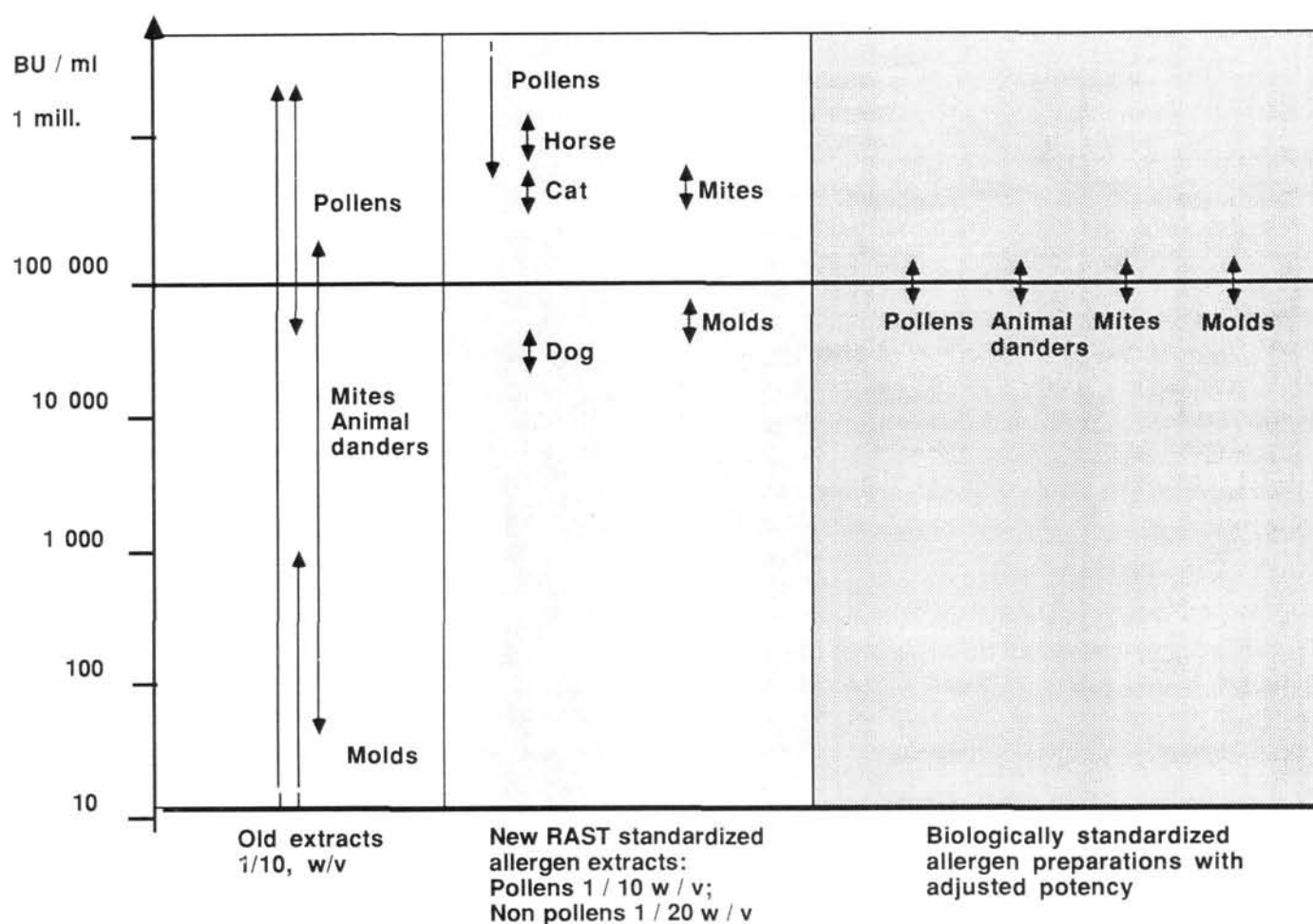


Figure 1. Old extracts varied at least 10 000 times in biological activity. In the centre is shown that it is possible to decrease the variation between batches of the same type of allergen source material, considerably by using RAST inhibition or similar methods, provided a stable reference is kept for calibration. However, this is not possible not between different allergen source materials, using in vitro methods. To the right is indicated that by BS, the difference in biological activity can be decreased also between non-related inhalant allergens (11).

All methods use the weal area or diameter as a measure of weal size. Most methods use a log/log model, since the dose response relationships of allergen, histamine and histamine releasing substances (20), e.g. codeine, are best fitted to a log/log model:

$$\log A (\text{area/diameter of weal response}) = a (\text{intercept}) + b (\text{slope}) \log C (\text{concentration of test solution})$$

The Nordic method and most other methods estimate the sensitivity of each patient in a sample. The slope of the dose response relationship is fairly flat, i.e. about 0.2 when the diameter of the weal is used (3,15,18-20) and 0.4 when the area is used (5,6,7,9,10,13,25,26). Only the method of Brighton (5) uses the mean of all weal areas obtained with a certain concentration of allergen and prescribes calculation of a regression line on the basis of six such points.

Selection of the sample of patients used for biological standardization of allergenic prepara-

tions, is the most critical point. Bjørkstén et al (3) and Dreborg et al (18) clearly showed that patient selection criteria are of great importance for the results obtained. However, by including consecutive patients referred to an University Allergy Outpatient Clinic, Dreborg et al (13,16,19) showed that results could be repeated in other regions, which is the best proof that patient selection criteria can be kept constant between investigators/samples.

Methods referred to below are illustrated in figure 2 a - d.

4.1.1.1.1 The Nordic Biological Unit (BU/ml)

In the second edition of the Nordic Guidelines, 1989 (28), the Nordic Council on Medicines, prescribes a method for BS that has been extensively documented (12,16,17). The principle is shown in Fig. 2 a. The concentration (C_h) of

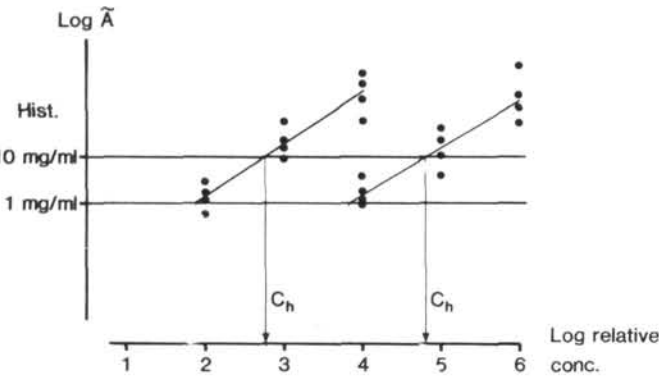


Figure 2 a. According to the Nordic method (11,15,27) three concentrations of allergen are chosen according to a preliminary test. The lowest of these allergen concentrations should induce a weal of similar size as that of histamine 1 mg/ml, and the two other should be 10 and 100 times stronger. These three allergen concentrations and histamine HCl 1 and 10 mg/ml are all tested in quadruplicate. The concentration of allergen inducing a weal response of the same size as that elicited by histamine HCl 10 mg/ml (C_h) is calculated by regression line analysis. The C_h of the median sensitive patient in a sample defines the unit 10 000 BU/ml.

allergen eliciting a weal of the same size as that of histamine HCl 10 mg/ml is estimated by regression line analysis using a log/log model, $\log \text{ weal area} = a + b \log \text{ conc.}$, to which the allergen dose response relationship is best fitted (See above). The Unit, 10 000 Biological Units/

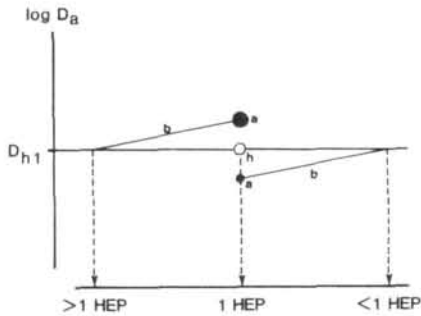


Figure 2 b. According to the simple method of Bjørkstén et al (3) and Dreborg (9,10) only one concentration of allergen and one of histamine HCl are tested. The concentration eliciting a weal of the same size as that of the histamine reference. There is a risk that negative results are obtained in clinically sensitive patients if low concentrations of allergen are chosen and that large weals are induced in sensitive patients if high concentrations are used. The normal range of skin reactivity in a sample of clinically sensitive patients is four powers or ten, i.e. weals 3–16 mm in diameter, provided a suitable concentration is chosen. If a ten times higher concentration is used, weals will range from 4.5 to 23 mm in diameter and if ten times too low, from 0 (20% negative) to 10 mm in diameter.

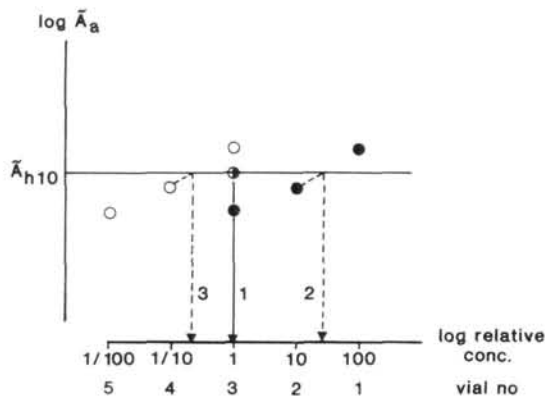


Figure 2 c. The simple method proposed by Dreborg (9,10). This method is described in the text and in an appendix to the original communication (9,10).

ml (BU/ml) is defined as the median C_h in a sample of patients clinically sensitive to the allergen in question. This method is regarded as the reference method.

4.1.1.1.2. Simple methods for estimation of BU/ml

Bjørkstén et al (3) described a simple method for Biological standardization, using only one allergen concentration and one concentration of histamine HCl (1 mg/ml) (Fig. 2b). However, the sensitivity of patients attending a University Allergy Outpatient Clinic varied four powers of ten (10,13,14,16,19). If the allergen concentration used is too low, many patients will get negative results and if it is too strong, many patients will get huge weals, i.e. > 20 mm in

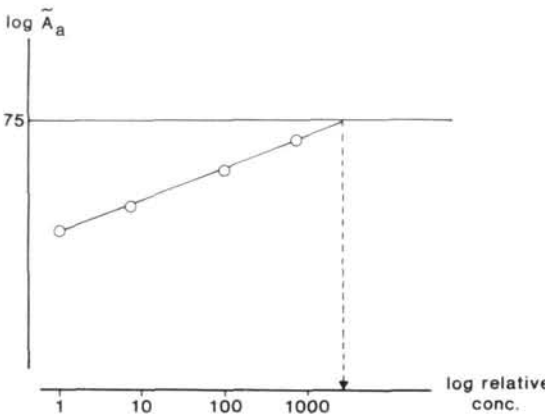


Fig. 2 d

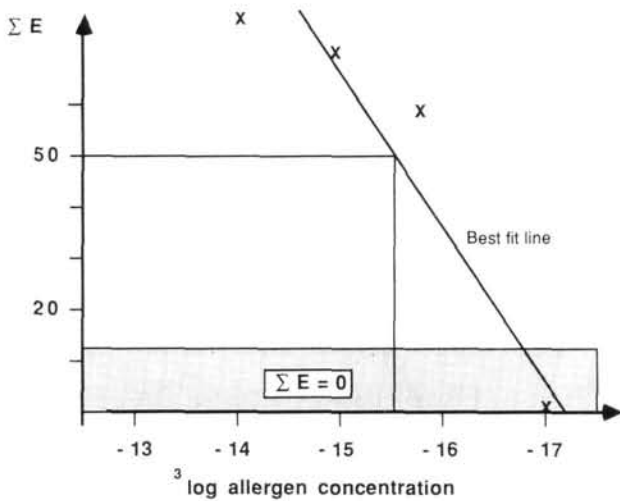


Figure 3. The sum of erythema diameters ΣE_{50} are given on the y-axis and the $3\log$ allergen dilutions on the x-axis. Erythemas less than the weal are given the value 0. Therefore the initial increase in ΣE is rapid at low concentrations, i.e. the slope is steep. The difference in potency is calculated by parallel line assay (22).

mean diameter (12). Provided a suitable concentration is used, results correlate well to those obtained with the method prescribed by the Nordic Council on Medicines (29). Before any biological standardization has been performed it is very difficult to estimate the most suitable concentration. Therefore, Dreborg (9,10) proposed a method (Fig. 2c) that utilizes allergen of high potency, that is reconstituted and diluted ten-fold five times (to six ten-fold concentrations). The patient is first tested with the second ten-fold dilution (third highest concentration) and the reference, both in duplicate: If the weal obtained is similar to that of the histamine reference (histamine HCl 10 mg/ml), then no more tests are needed. If the weal is larger or smaller, additional tests are performed. All tests (4 to a maximum of 12 SPT) can be performed on the volar aspect of the forearm. Results with this method also correlate well (9,10) with results obtained with the method prescribed in the Nordic Guidelines (29). Despite only one concentration is used for calculations, no negative results will be obtained in clinically sensitive patients, provided the stock solution has high enough potency. According to the Nordic Guidelines, methods correlating with the prescribed method can be used, and therefore this simple method is recommended.

4.1.1.1.3. Other Biological Units

There are several other biological units, used by manufacturers.

The best documented of these, is the Biological Unit/ml (BU/ml) of Brighton, used by Abello, Madrid. In principle, no reference substance is used. Instead, the concentration eliciting an allergen weal with 75 mm² area is estimated. The concentration is calculated by regression line analysis, based on the mean weal area, obtained with each concentration, using a sample of patients (Fig. 2 d). In publications using this method several centres have participated (6). Therefore differences in technique may have been reduced, provided participating centres used both traumatic and non-traumatic techniques or the technique used was similar to that of all clinicians working with the same standardized material. However, it has been demonstrated, that the size of weals obtained with histamine HCl 10 mg/ml (13) and timothy allergen preparation (9) differ considerably between centres.

The method described by Maasch et al (25) was similar to the method used by Dreborg and Grimmer (19), i.e. it aims at determination of the concentration eliciting a weal of the same size as that of histamine HCl 1 mg/ml according to the Nordic Guidelines 1980 (28). Data obtained with these methods multiplied by ten, correlate well with results obtained with the method prescribed in the second edition of the Nordic Guidelines (29). The Unit described by Constable et al (7) estimates the concentration of allergen eliciting a weal of the same size as histamine HCl 10 mg/ml. However, the Nordic BU/ml is not used. The manufacturer uses another Unit called AUR, which means Activity Unit by RAST instead of the Nordic BU/ml for labelling of their products. The methods mentioned in this paragraph, all use 3- to 5-fold dilutions, six three-fold dilutions give a 243-fold difference between the highest and the lowest concentration. Then the potency of the extract is crucial and must be high enough to avoid negative results, since the skin sensitivity of patients with clinical allergy differs at least four powers of ten between the most and the least

sensitive patient in samples used for BS, i.e. 10 000 times (10,13,14,16,18). The risk of negative ST results in clinically sensitive patients is high, when extracts with low potency are used.

For estimation of the Index or Reactivity (IR) used in France (4), a procedure similar to that described by Dreborg and Grimmer (16) is used. However, codein phosphate 9 mg/ml is used as reference solution instead of histmaine HCl 1 mg/ml. The French and the Nordic methods have not been correlated.

4.1.1.2. PRECISION OF ESTIMATE WITHIN PATIENT

Provided there are no systematic errors, the precision of the estimate within patients is of little interest, as the biological units described are based on the sensitivity of samples of patients.

4.1.1.3. REPEATABILITY OF THE BU BETWEEN REGIONS

The unit, 10 000 BU/ml, has been shown to be reproducible between regions/samples of patients (10,13,16,19). Also when slightly different methods for SPT have been used by other investigators (7,25).

4.1.1.4. POTENCY OF ESTABLISHED ISP IN BU/ML

Allergen preparations biologically standardized according to the Nordic Guidelines (26) have been calibrated (23) by RAST inhibition against International Standard Preparations (ISP)(31). International Standard Preparations have been set up with the aim to obtain yardsticks with known and relevant composition. Their potency has been set to 100 000 International Units/ampoule (31). When ISP were calibrated by RAST inhibition against BS allergen preparations, the biological potency varied considerably: Timothy $\approx$ 5400000 BU/ml, ragweed $\approx$ 1700000 BU/ml, *Der. p.* $\approx$ 13000 BU/ml, dog $\approx$ 130000 BU/ml, *Alternaria* $\approx$ 40000 BU/ml, birch $\approx$ 340000 BU/ml and Bermuda grass $\approx$ 900000 BU/ml. These potencies correspond roughly to what has been

found with other modern standardized extracts 1/10 (pollens) or 1/20 (non pollens) w/v (17), with exception for the mite ISP (13000 compared to $\approx$ 600000 BU/ml). The International Standard Preparations will be standardized according to the Nordic (10,17,29) and the US (2,34,35) protocols, starting 1990 (4). Thus, soon, new data will be available.

4.1.1.5. RECOMMENDATIONS FOR ESTIMATION OF BIOLOGICAL ACTIVITY OF ALLERGENIC PREPARATIONS BY SPT

Since there is only one BU that has been thoroughly documented (10,13,14,17), the Biological Unit prescribed by the Nordic Council on Medicines (29), this Unit is, at present recommended for BS of allergen preparations, intended for diagnostic use. However, it would be more convenient to manufacturers and scientists to prescribe the simple (second) method described by Dreborg (9,10), that correlates to the Nordic method (9). Whether, in the future it will be possible to document the biological value of determination of the molar content of major allergens in allergen preparations intended for diagnostic purpose is as yet not clear.

4.1.2. *Biological standardization by ICT*

The only method using ICT has been developed by the Office of Biologics Research and Review Branch (OBRR) within the Food and Drug Administration (FDA), USA. There are no such methods described from Europe.

4.1.2.1. THE METHOD OF OBRR

The ID50EAL method described by Turkeltaub (Fig. 3) (34) is in principle based on the rapid increase of the area of the erythema induced by intracutaneous tests at low response levels, i.e. erythemas with a diameter less than 30 mm in diameter (weals less than 10 mm in diameter). An erythema with a diameter less than that of the weal is given the diameter 0 mm. The model used is $\ln/3\log$, i.e. linear representation of the increase in the sum of longest and midpoint orthogonal erythema diameters and three-fold increases in allergen concentration.

Only single allergen tests are performed with at least four concentrations of allergen. No reference substance is used, since the ICT method is more precise than the SPT method, and varies little between investigators (30). Patients with high sensitivity are selected. This is done for safety reasons, as the ICT method is often used for diagnostic purpose in the US. Thus a safe starting dose for intracutaneous tests and even for immunotherapy can be prescribed based on this type of biological standardization.

4.1.2.2. DEFINITION OF THE ALLERGY UNIT (AU/ML)

The concentration necessary for induction of an erythema with a sum of the longest and the midpoint orthogonal diameters = 50 mm (Σ E50), is estimated in a sample of at least five individuals highly sensitive to the allergen in question. The precision of the estimate within patients has been found to be high and only highly sensitive patients are included. Therefore, it is only necessary to include five patients. An allergen preparation is given the potency 100 000 Allergy Units (100 000 AU/ml), provided the median sensitive patient reacts with a Σ E50 to the 15th 3-fold dilution of allergen and 10 000 AU/ml when the median sensitive patient reacts with to the 12th three-fold dilution. There are no published results on the repeatability of results between regions. The OBRR has pooled a lot of data from different manufacturers using the OBRR references to obtain an estimate of the potency of these references (35). This method will be compared with the Nordic method using SPT (29) in the study set up by the Subcommittee on Allergen Standardization within the IUIS to estimate the biological activity of ISP (4). The Subcommittee on Skin Tests within EAACI recommends that the Nordic method is utilized when the biological activity of allergens is estimated by ICT.

4.1.2.3. RECOMMENDATIONS FOR BIOLOGICAL STANDARDIZATION BY ICT

Biological standardization by ICT is not recommended in Europe. However, Europeans

should be aware of the existence of the US system.

4.2 ESTIMATION OF THE RELATIVE POTENCY OF ALLERGENIC PREPARATIONS

Estimation of the relative potency of allergenic preparations is used for determination of the potency of allergenic preparations in relation to a standard, e.g. an in-house reference (IHR) preparation is calibrated against a national or international standard or an allergen extract stored in a stability study, against the IHR. The standard procedure involves estimation of the difference in dilution factor of an allergenic extract in relation to a standard to obtain the same skin response. This is most often done by parallel line bioassay (24). The principle is illustrated in Fig. 3. However, in many papers the difference in potency between two allergen extracts is by giving the difference in weal size obtained when these extracts are used for skin testing. The weal size elicited by a strong extract is larger than that of a weak extract. However, since the dose response relationship of allergens is best fitted to a log/log model (see 4.1.1.1.) and the slope (b) is flat, i.e. 0.4 when using the weal area and 0.2 when using the diameter as a measure of weal size (3,10,14,16-19), the size of the weal is not a good measure of potency. Thus, as illustrated in Fig. 2, a difference in weal diameter of 4 mm indicates $\approx$ 10-fold difference in potency if the difference is between 6.8 mm and 10.8 mm but more than 100-fold difference in potency if the weal diameter obtained with the two extracts are 3 and 7 mm.

4.2.1. Estimation of relative potency by SPT

The difference in potency between two allergen preparations can be estimated by parallel line bioassay according to Finney (24), as proposed by Malling (26). The proposed method has not been validated. However, according to non-published data, the precision is good with 95 % confidence limits being $\approx$ 70-130 % of the estimated difference in potency (21). In principle the same procedure is used as for biological standardization, with two extracts at the same

time and applying statistical methods for parallel line bioassay. The method is also similar to the methods described by Dreborg et al (15) for estimation of changes in skin sensitivity during immunotherapy, with the modification that two extracts are compared at the same time (See Chapter 5).

4.2.2. *Estimation of relative potency by ICT*

The same principles are used with ICT as described for SPT.

4.2.2.1. METHODS

There exists only one method that has been thoroughly validated, the method described by Turkeltaub et al (36). This method has been revised and a detailed protocol can be obtained from Dr Paul Turkeltaub*. In principle four 3-fold dilutions of the standard and the extract under investigation are tested (single tests) by ICT, the longest and the midpoint orthogonal diameters are measured and the sum of erythema diameters used for calculations. The difference in potency is calculated by parallel line bioassay. As mentioned in Chapter 3 the slope of the erythema dose response relationship is steep and the 0-point is always included (erythema diameters less than those of the weal). Regression lines are always steep and parallel. The precision of the estimate is good. Therefore, provided the composition of allergenic preparations under investigation is similar, only three patients must be tested to obtain a reliable result. The method has been compared to ELISA inhibition assay by Dolen et al (8). The principle is given in Fig. 3.

4.2.2.2 RECOMMENDATIONS FOR ESTIMATION OF THE RELATIVE POTENCY OF ALLERGENIC PREPARATIONS BY ICT

The OBRR method is recommended for estimation of the relative potency of allergenic preparations by ICT.

4.3 RECOMMENDATIONS FOR ESTIMATION OF THE RELATIVE POTENCY OF ALLERGENIC PREPARATIONS BY SKIN TESTS

The OBRR method using ICT is recommended as the standard method for estimation of the relative potency of allergenic preparations in relation to a given standard. Provided a sample of at least 20 patients is included, the simple methods described for estimation of changes in skin sensitivity by SPT (See Chapter 5) may be used, but further validation for the purpose of estimation of relative potency are needed.

4.4. BIOLOGICAL STANDARDIZATION (BS) OF ALLERGENIC PREPARATIONS FOR IT

It has been proposed that biological standardization by skin tests should not be valid for allergenic preparations used for immunotherapy (38). Therefore some scientists and manufacturers use the molar content of major allergen(s) for standardization of such products. Provided it was possible to determine the amount of most components and equilibrate the amount of these components in the preparation to the same molar concentration, then this system would be superior to BS both for diagnostic and therapeutic allergenic preparations. However, at present it is impossible to determine the amount of all relevant allergens and still more difficult to equilibrate the amount of these allergens in a mixture. Changes in skin sensitivity during IT are relatively easy to estimate, and are less influenced by hyperreactivity than are shock organ challenge tests. Therefore, as proposed by Dreborg (10) it might be suitable to introduce a unit based on the therapeutic effect as measured by decrease in skin sensitivity, e.g. 100 000 Therapeutic Units (TU/ml) = decrease of skin sensitivity by 10 times. Such data should be derived from trials using several top doses and the dose effect (change in skin sensitivity) relationship calculated. Data are already available from at least two trials using two and three different top doses of allergen (22).

* OBRR, FDA, Bethesda, Maryland, USA

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5. Evaluation of changes in skin sensitivity by means of skin tests

The same principles as described under 4.1 are also valid for estimation of changes in skin sensitivity. Due to the log/log dose response relationship of allergens, the most common measure of change in skin sensitivity, change of weal size (1,16,21), is not valid. So called end point titration has a low precision and should not be used (18). Instead, methods based on parallel line bioassay (6,9,12), estimating the change in concentration needed to obtain the same weal response before and after treatment, should be preferred. Changes in skin reactivity given in change in concentration of allergen inducing a certain skin response, e.g. by parallel line assay, methods estimating the concentration eliciting a skin response corresponding to that of a reference substance such as codeine (23) or histamine (9), can easily be compared to e.g. changes in concentration of allergen inducing a positive challenge test in shock organs or changes in antibody titers. All these measures of sensitization show a normal distribution after logarithmic transformation (5,7,8,9).

There are a few reports showing decreased skin reactivity as measured by the size of the LPR (4,12,18).

5.1 SKIN PRICK TEST (SPT) METHODS FOR ESTIMATION OF CHANGE IN SKIN SENSITIVITY

5.1.1. *Methods for estimation of change of early phase reaction (EPR)*

5.1.1.1. WEAL SIZE

Most authors evaluating the change in skin sensitivity induced by immunotherapy or pharmaceu-

tical treatment have used the change in weal size to illustrate changes in skin reactivity. Due to the log/log relationship between changes in skin prick test response and allergen concentration only very large differences in skin sensitivity will be detected when using a single fixed concentration of allergen. The decrease in absolute size (21) or in % of size (1, 16) after treatment compared to before treatment is therefore not recommended as the method of choice. Furthermore, a limited decrease in weal size indicates a marked decrease in skin sensitivity, e.g. a tenfold decrease in skin sensitivity means a reduction in weal diameter from 6.8 mm to 4.5 mm and a hundred-fold decrease corresponds to a decrease in weal diameter from 6.8 to 3 mm.

5.1.1.2. PARALLEL LINE BIOASSAY

In 1984, Dreborg proposed (6) the use of parallel line bioassay (12) for evaluation of changes in skin sensitivity. Since then there has been several reports using this method (1,2,9,11,15,17,18). Most of these have used the system of Dreborg (6), testing in at least duplicate, at least three ten-fold concentrations of freshly reconstituted allergen preparation from the same batch before and after (immuno)therapy. The principle is illustrated in Fig. 1 a. This method should be considered the reference method.

5.1.1.3. METHODS BASED ON MEDIAN SLOPE

From this relatively complicated method, Dreborg et al (9) derived two other less laborious methods based on the same principle as the simple methods for BS (See Chapter 4). Both are based on median slope (0.4 when the area and 0.2 when the mean diameter of allergen induced weals are used). This is correct, provided changes are inves-

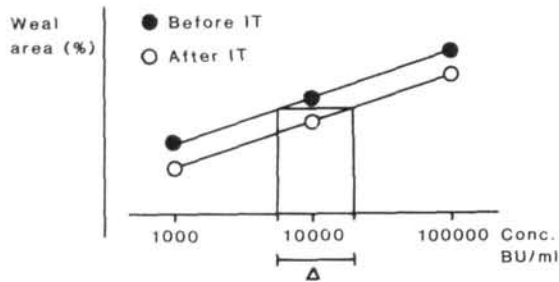


Figure 1 a. The principle of estimation in change in skin sensitivity by parallel line bioassay (4,8,11). Three concentrations, e.g. 1 000, 10 000 and 100 000 BU/ml of a freeze-dried allergen preparation are tested in at least duplicate before and after immunotherapy. The difference in concentration eliciting the same skin response is estimated by regression line analysis. Since skin sensitivity decreases during immunotherapy (or anti-allergic drug therapy) allergens should be 10–100 times stronger after than before therapy. No reference substance is needed.

tigated in samples of patients. The first method uses only one and the same concentration of allergen and histamine reference tested in duplicate before and after therapy. The change in allergen concentration needed to elicit a weal of the same size as a reference substance, e.g. histamine HCl 10 mg/ml (Fig. 1 b) is estimated. The other method is also based on the same principle. First, only one concentration of allergen is tested in at least duplicate. If the weals are of similar size as that of the reference then no further tests are performed, if smaller, then two ten- and hundred-fold higher concentrations of allergen are tested. Opposite,

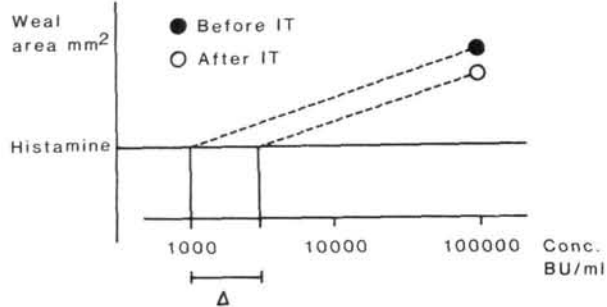


Figure 1 b. One and the same concentration of allergen and histamine 1 or 10 mg/ml is tested before and after therapy. Provided the allergen concentrations are high enough, results will be obtained from all patients. Since many extracts are weak, patients often turn negative, which makes evaluation difficult. The influence of the approximation of the allergen slope (0.4) can be pronounced, if most patients react with a weal much larger than that of histamine.

when the first concentration induces weals larger than those of the reference, then two ten- and hundred-fold lower concentrations of allergen are tested. Only results obtained with one of these concentrations, that inducing a weal most similar in size to that of histamine HCl 10 mg/ml are used for calculations (Fig. 1 c).

The drawback with the first method is that in some patients the concentration of allergen may be too high and especially after immunotherapy many patients will turn totally skin negative if the allergen concentration used is too low. The second method is a little more laborious, but has the advantage, that provided the concentration of allergen of the stock solution is high enough, changes in skin sensitivity can be estimated in all patients without any risk of inducing too large reactions.

5.1.1.4. ALLERGEN CONCENTRATION ELICITING A WEAL SIMILAR TO THAT OF A REFERENCE SUBSTANCE

The end point defined as the allergen concentration giving a weal size similar to that of the positive control, 9 % codeine phosphate, has been used as a measure of skin sensitivity in studies of allergen immunotherapy using house dust miet (23), grass pollen (24,25) and Alternaria (26) allergen extracts. Moreover, with this method a significant correlation was found between the end

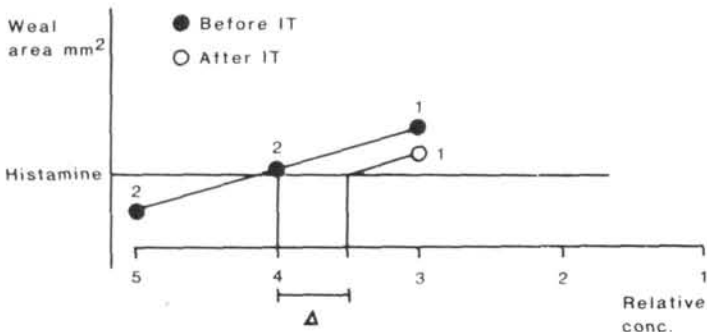


Figure 1 c. The simple method proposed by Dreborg et al (8). First one concentration (second dilution, third concentration) of allergen and the histamine reference are tested in at least duplicate. Provided the allergen weal has a size similar to that of the histamine reference, then no further tests are needed. If the response to allergen deviates much from that to histamine, then two ten and hundred-fold higher or lower concentrations of allergen are tested.

point titer and the severity of the pollen season (25).

5.1.1.5. PRECISION OF THE ESTIMATE

The two simple methods correlate well to results obtained by parallel line bioassay in the same patient (9).

5.1.1.6. DOSE EFFECT (CHANGE IN SKIN SENSITIVITY) RELATIONSHIP

Using parallel line bioassay or the two simplified methods related above, the decrease in skin sensitivity during one year of immunotherapy varies between a few times and 300 times (8,15,17). In two studies, the effect of different top doses of allergen has been studied. Results fit roughly to a linear dose response relationship ($\log [\text{change in skin sensitivity}] = a + b \log [\text{top dose of allergens}]$) (12), but further studies are needed.

5.1.2. *Methods for estimation of change in late phase reaction (LPR) by SPT*

There are no methods in the literature for estimation of changes in LPR by SPT.

5.1.3. *Recommendations for estimation of change in skin sensitivity by SPT*

The Subcommittee recommends that changes in concentration of allergen is used as a measure of change in skin sensitivity and that parallel line bioassay is used as the standard method. Provided more than 10 patients are included in clinical trials the second method proposed by Dreborg (9) (with several allergen concentrations) is convenient. For practical purpose the first method of Dreborg et al (9) may be used in the routine with or without transformation of weal size to change in concentration inducing a weal of the same size as histamine HCl 10 mg/ml (9).

Alternatively the concentration of allergen inducing a weal response similar to that of a reference substance can be used (23).

5.2. INTRACUTANEOUS TEST METHODS FOR ESTIMATION OF CHANGE IN SKIN SENSITIVITY

5.2.1. *Methods for estimation of change in early phase reaction (EPR) by ICT*

When ICT has been used for estimation of the decrease in skin sensitivity, then weal size or ICT end point has been the most common parameters studied. However, the method employed by Turkeltaub et al for estimation of the relative potency of allergenic preparations in relation to a standard (23) can be adopted for this purpose. The same freeze-dried allergen preparation should be used before and after immunotherapy (or other form of therapy). The change in allergen concentration for induction of Σ 50 mm of erythema diameters should be estimated. As mentioned in chapter 4, the slope of the erythema dose response relationship is steep. This method might be the most precise method, suitable for estimation of changes in skin sensitivity in small samples of patients, but further studies are needed.

5.2.2. *Methods for estimation of change in late phase reaction (LPR) by ICT*

5.2.2.1. METHODS

Pienkowski et al (20) and Fling et al (13) have described methods. The concentration recommended for ICT (20) was 10 times the concentration eliciting a 4 + reaction according to their system. Both groups draw the contours of the swelling at 4 and 8 hrs (20) and 6 hrs (13), respectively. Then, the drawings were transferred to a record sheet with a translucent tape. The diameters were measured with a ruler (20) and the area was estimated by a digitizer (13), respectively. The contours of the LPR swelling is in most cases vague. Therefore, it is not possible to refer to any well defined method.

5.2.2.2. DOSE RESPONSE RELATIONSHIP

The difference in size of the LPR between patients treated with a potent ragweed extract and un-

treated patients was significant ($p < 0.001$, 20 and 67 mm in diameter, respectively, at 8 hrs) in the trial of Pienkowski et al (20). In the trial of Fling et al (13), comparing a mountain cedar extract to placebo, the difference between decrease in LPR was also significant ($p < 0.005$). Furthermore, Dieges et al (4) found that the late phase reaction totally disappeared in patients given grass pollen IT.

5.2.3 Recommendations for estimation of change in skin sensitivity by ICT

The Subcommittee recommends that the method of Turkeltaub et al proposed for estimation of differences in potency between allergen preparations is adopted also for estimation of changes in skin reactivity. Detailed methods for estimation of changes in LPR must be further evaluated.

5.3 RECOMMENDATIONS FOR ESTIMATION OF CHANGES IN SKIN SENSITIVITY DURING THERAPY

The Subcommittee recommends that the methods described under SPT methods are employed for estimation of changes in skin sensitivity, either spontaneous changes or changes induced by drug therapy or immunotherapy. Further methodological studies are needed before ICT methods are generally employed for this purpose. Estimation of changes of LPR are probably of great importance. However, before any recommendations are made, the dose response relationship must be studied and methods for estimation of change in LPR must be validated.

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6. Skin tests used for epidemiological studies

6.1. PURPOSE OF SKIN TESTING IN EPIDEMIOLOGICAL STUDIES

The purpose of skin testing in epidemiological studies has been to find out the prevalence of skin test positivity to common allergens in the general population or in groups of patients lacking a clinical history of atopy. In epidemiological follow up studies of samples of patients, skin tests have been used as a tool to study the development of sensitization and natural desensitization. It has also been suggested (51) that it might be valuable to advise symptom-free persons with positive skin tests, i.e. with latent allergy (41) that they may belong to a risk group which is at risk of developing atopic symptoms (58,51). Whether it is advisable to start prophylactic measures and avoidance of allergen exposure in these subjects should be further evaluated. As yet, the predictive value of positive skin tests is dubious. This is especially true for positive tests with pollen extracts.

6.2. NOMENCLATURE

Prevalence	the number (%) of patients with positive ST in the sample (population) studied
Incidence	the number of new cases with positive ST in the sample (population) studied
Cumulative incidence	the number of subjects in the sample (population) who have been ST positive during a given period of time
Atopic diseases	Diseases such as hayfever, asthma, urticaria, eczema, food allergy when based on IgE-antibody interaction

Atopic individual	A person prone to develop IgE mediated allergic disease when exposed to certain antigens (allergens)
Sensitization	Immunologic response with production of IgE antibodies, conceivably resulting in atopic disease
ED50	Median effective dose

6.3. ESTIMATES OF SENSITIZATION OF A SAMPLE (POPULATION)

6.3.1. Prevalence

Prevalence, i.e. the percentage of individuals showing a positive SPT to a certain allergen, or in some studies (30), two or several positive SPT's to any of the allergens tested has been used as a criterion of sensitization.

6.3.2. Median effective dose (ED50)

The median threshold concentration (with 95 % confidence interval) in the sample studied has been used as a measure of the sensitivity of that sample (8). This measure can be compared to the ED50 in other samples. In fact the Nordic Biological Unit 10 000 BU/ml (11,15,46) is the ED50 of patient samples referred to University Allergy outpatient Clinics (or similar).

6.4. INFLUENCE OF DIFFERENT FACTORS ON RESULTS

A number of factors influence the result of epidemiologic studies using skin tests for esti-

mation of the prevalence of sensitization: The cut off limit of the method; The technique employed and how it is performed; The precision of the method in the hands of the investigator (nurse, assistant); The composition and potency of allergenic extract employed.

6.4.1. *Cut off limit of the method - detection limit*

An SPT reaction is considered positive when the weal diameter is ≥ 3 mm in diameter ($= \geq 7$ mm²)(reviewed in 11). However, in some studies a cut off limit of ≥ 2 mm in diameter (3 mm²) has been used (34). Then the background response, i.e. the weal response to diluent in the same sample of patients should be carefully documented. The difference between 2 and 3 mm in mean diameter denotes a 10-fold difference in skin sensitivity (13).

6.4.2. *Cut off limit - number of positive SPT*

When the prevalence of sensitization is studied, then some investigators have used one positive SPT as an indication of atopy, others two or several positive tests. Haahtela and Jakomæki (30) found that the prevalence of atopy decreased from 49 to 34% when the limit for atopy was changed from one to two positive SPT.

6.4.3. *Precision of the method*

Precision of the SPT (and ICT) methods vary with technique (4,6,37), investigator / nurse / assistant performing the test (4,15). Therefore, the precision of the technique used in the hands of the investigator / nurse / assistant must be documented, preferably by performing at least duplicate tests with each test solution (or at least the reference substance)(11). Using the weal area as a measure of weal size, and transforming the data ($^{10}\log(\text{area mm}^2 + 1)$), the coefficient of variation (c.v. % = s.d. x 100 / mean) should be less than 40% (20% when the mean diameter is used). This limit seems reasonable, since 15% (7%) can

be achieved by skilled investigators (15), although much lower precision, i.e. higher c.v. %, has been recorded (15). This means that - 1 s.d. may not exceed 1.5 mm when the mean diameter is 4 mm (lower limit of the mean diameter 2.5 mm) and not 1.6 mm when the diameter is 5 mm (lower limit 3.5 mm). Similarly, - 2 s.d. = 1,7 and 1,9 mm, respectively (lower limit 2.3 and 3.1 mm, for 4 and 5 mm weals, respectively). This is a crucial point as those with a true weal diameter of 4 mm, all have allergen specific IgE attached to their skin MC. However, many weals will be recorded as negative despite the individuals are sensitized. Thus, differences in precision may account for differences in recorded prevalence between samples of patients with the same true prevalence.

6.4.4. *Technique and performance*

It has been demonstrated that the size of the weal differs between techniques in the hands of the same investigator (4,6), i.e. a technique that induces smaller weals, gives a lower prevalence than does a technique that induces larger weals (6,47). The technique is of greatest importance in patients with a skin sensitivity at the border of positivity. However, differences in performance between investigators using the same technique and test solution should also be considered. Differences in weal size as large as 3.5 versus 7 mm in mean diameter when using the same allergen solution, have been registered (4,11).

6.4.5. *Potency and composition of extract*

The higher the potency of the allergenic extract used, the more patients will show a positive reaction, i.e. sensitization (12,18)(Fig. 1 and 3, Chapter 3). It has been documented in biological standardization trials that patients attending a University Allergy Outpatient Clinic, all will get a positive reaction with 100 000 BU/ml(15-17). When the same extract was used in a diagnostic trial using subjects from the general population, i.e. including also individuals only slightly allergic, then 1 mill. BU/ml was needed to diag-

nose all patients, whereas 10 000 BU/ml showed a high specificity (18). These examples clearly demonstrate the necessity of using allergenic preparations with known potency, relevant to the question raised in the study.

When an allergenic preparation with only one component, to which some people are highly allergic, is used for diagnosis, only some of the patients clinically sensitive to the allergen will give a positive test. Thus, 98 % of dog sensitive patients were positive when SPT was performed with a well characterized dog allergen preparation (50) prepared from the best allergen source material, dog dander, whereas only ≈ 20 % were positive with one of the allergens of dog dander, dog serum albumin (12,44).

6.4.6. *Prevalence of clinical allergy*

When the aim of an epidemiological investigation is to study the prevalence of sensitization, then it is recommended to use an extract with defined and high potency and relevant composition. Provided the aim permits it, one major allergen can be used. When the aim is to study the prevalence of clinical allergy, then the concentration used should be documented regarding its diagnostic capacity in a suitable sample of patients, preferably with characteristics similar to those of the sample studied in the epidemiological study. However, the prevalence of sensitization and/or clinical allergy is often higher in samples studied in diagnostic trials, than in epidemiological studies, because many patients are needed when the prevalence of disease is low (as for example in the general population). Then, corrections must be made for the lower prevalence of atopic disease, otherwise many individuals without disease will be diagnosed ("false positives", patients with latent allergy (41)).

6.5. PREVALENCE OF SENSITIZATION TO COMMON ALLERGENS AND ATOPY, RESPECTIVELY

6.5.1. *General considerations*

The prevalence of positive skin tests to defined allergens and of atopy defined e.g. as two

positive skin tests using a battery of allergen extracts known to be of importance in the region, has been extensively studied (1-3,5,7-10,19-36,38-43,52)(Table 1). The quality of allergens employed was unsatisfactory in most, but not all studies, the potency was ill defined and skin test techniques and criteria for positivity varied between studies. Therefore, comparison of results between these studies is difficult. The prevalence of positive skin tests varied considerably i.e. from 19 to 70 %. These differences do probably not reflect differences in prevalence, but rather variation in technique, potency etc., which highly stresses the need for new studies with defined materials, techniques, documented precision etc. Extracts should be stored freeze-dried for follow up studies and for calibration against standards with known potency and composition, or for comparison with extracts used by other investigators or extracts used in the marketplace.

6.5.2. *Variation with age*

Sensitization varies with age (34,35,45,48,51). Sensitization to foods is common in infants and young children, but often disappears within a few years (34,51), whereas sensitization to inhalants is more common in older children and adults (34,35,51). The skin reactivity seems to be more pronounced in older children and young adults than during the two first years of life (45,48,51). Skassa-Brociek et al (48) found a lower sensitivity to histamine in young and elderly individuals. However, Stucky et al (49) found that individuals with skin sensitivity to many allergens had a higher skin sensitivity to histamine. It is well known that the number of patients with atopic skin sensitization is lower in young children and the elderly. Therefore, the difference in reactivity to histamine may be an expression of the degree of allergen sensitization, varying with age. This interpretation is favoured by the findings that children with sensitization to foods showed similar skin reactivity to histamine irrespective of age (14).

Table 1

Prevalence of immediate skin test reactivity in "normal" subjects and in general populations according to selected studies

Country and study	Number, type and age of subjects	Skin test method	Number of allergens tested	Criteria for a positive reaction (weal mm) reactions (one or more)(%)	Prevalence of positive
<i>USA</i>					
Grow and Herman (1936)	150 normals 20-30 yrs	ic.	13	5	55.5
Curran and Goldman (1961)	100 normals 16 and over	scratch ic.	9 5-10	weal and flare	9
Lindblad and Farr (1961)	100 normals 16-81	ic.	5 1:100 1:10 1:1	5	4 24 71
Hagy and Settipane (1969)	1243 students 16-20	scratch	15	weal and erythema	30.9
Barbee et al. (1976)	3102 general population	prick	5	1	34
Brown et al. (1979)	307 allergic and non-allergic 9-78	prick	14	1 5	68.7 47.9
Friedhoff et al. (1981)	406 factory workers	prick	variety of crude extr.		♀ 7 ♂ 29
Barke, R. et al. (1987)	1333 cohort > 3 y. followed 8.1 y.	prick	5 W/w	> contr.	init. 39.1% follow up 50.7%
Gergen, P. et al. (1987)	16.204 6-74	prick	8	erythema > 10.5 mm =	20.2%
<i>Italy</i>					
Corbo, G. et al. (1988)	295 11-14 y.	prick	4	2.5 mm ²	♀ 18% ♂ 32%
<i>Canada</i>					
Chang-Yeung, M. et al. (1985)	3353 workers 3 diff. groups	prick	common allergens		22.4
<i>United Kingdom</i>					
Herxheimer et al. (1954)	100 normals 5-75	prick	12	3	34
Greenberg et al. (1970)	121 factory workers	prick	7	1	21
Godfrey and Griffiths (1976)	303 schoolchildren, 8-14	prick	2	3	33.7
D'Souza and Davies (1977)	1267 general populations, 45-70	prick	4	1	24.3
How et al. (1978)	288 factory workers	prick	3	1	38.8

Table 1 continued

Prevalence of immediate skin test reactivity in "normal" subjects and in general populations according to selected studies

Country and study	Number, type and age of subjects	Skin test method	Number of al- lergens tested	Criteria for a positive reaction (weal mm) reactions (one or more)(%)	Prevalence of positive
<i>Australia and Papua New Guinea</i>					
Andersson (1979)	335 general population	prick	11	3	19
Woolcock et al (1978)	505 general po- pulation, 20-69	prick	8	2	44.3
<i>Denmark</i>					
Johnson and Mygind (1978)	127 medical students, 21-35	prick	21	3	29.9
<i>Hungary</i>					
Cserhati et al. (1983)	300 negat. fam. hist.	prick	comm. allergens	> contr.	64 %
<i>Finland</i>					
Haahtela (1979)	218 adoles- cents, 14-16	prick	8	3	31
Haahtela and Jokela (1979)	295 conscripts, 18-19	prick	16	3	50
Haahtela et al. (1980)	708 adoles- cents, 15-17	prick	16	3	49
Haahtela (1980) (thesis)	1217 young people, 14-19	prick	16	3	46
Haahtela (1988)	1640 male smokers 50-69	prick	7	3	26 1 10 2

6.5.3. Prevalence of clinical allergy

As mentioned in Chapter 3, many patients show positive skin tests, provided the potency of allergens used is high, i.e. they are sensitized but not clinically allergic, they have latent allergy (41). Van Asperen et al (51) showed that those with clinical allergy were more sensitive than those without clinical symptoms at contact with the allergen in question. Horak (38) followed a group of children with positive skin tests but without clinical symptoms. He found that over a period of 5 years, more than 50 % acquired rhino-conjunctivitis and an additional 5 % asthma. The prognostic value of such data is high, provided standardized allergens with known potency are used, i.e. the results can be utilized by practicing allergists/pediatricians.

6.6. RECOMMENDATIONS FOR FUTURE EPIDEMIOLOGICAL STUDIES

The skin prick test, scratch and ICT methods are all possible to use for epidemiological studies. However, in epidemiological investigations in which a large number of individuals are studied, and skin tests are used as a diagnostic tool, the test method should be reproducible and easy to perform. The recording of skin reactions (weal size) should be simple and it should be easy to obtain an estimate of skin sensitivity. Due to its simplicity, safety and painlessness in relation to the ICT method and higher precision in relation to the scratch test method, the SPT method should be preferred in epidemiological studies. Among SPT methods, the standardized method using a needle of the fsterballe type should be preferred (See Chap-

ter 2 and 3). Allergenic preparations used in epidemiologic studies should fulfil the criteria given in Chapter 2. Preferably, the diagnostic value of such extracts should be documented in a sample/population with known prevalence of disease (disease defined as positive history, positive challenge test at a certain concentration etc.) and including all patients in the general population with clinical allergy to the allergen in question.

Future epidemiological studies using skin tests should

- be performed with the SPT/puncture technique and a needle of the Østerballe type,
- use allergen extracts which are freeze-dried and at least one vial should be stored frozen for future comparison with later developed standards,
- use allergens known to be of importance or probably of importance in the region, with a composition reflecting that of the naturally occurring allergen source material, or for some purposes, well defined major allergens
- have a diagnostic capacity of the concentration chosen documented in relation to clinical allergy, when the possible clinical relevance of an allergen is addressed
- be documented regarding the precision of the technique of the investigator/assistant, preferably by performing tests in duplicate with each test solution,
- be performed at a defined time of the year and time of the day (See Chapter 2)
- have weal sizes documented in a permanent manner (See Chapter 2),
- use ≥ 3 mm diameter as cut off limit for weal size and the presentation of distribution of sensitivities in the sample should be by weal area and/or mean diameter, complemented with the corresponding end point concentration, estimated by an equation using the mean slope for allergen (0.4 for area, 0.2 for diameter when the mean diameter is used as measure of weal size):

$$\text{End point conc in e.g. IU} = \text{Tested conc} \\ \times [3/\text{actual mean weal diameter}]^5$$

where 3 denotes the cut off limit for weal diameter and 5 is derived from $1/b$ (b slope; bestimated on the basis of weal diameters = 0.2).

The above mentioned guidelines should be followed. If it is not possible to follow them in all details, the reason should be given and commented on.

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