

Guidelines for the standardization of bronchial provocation tests with allergens

An update by an international committee*

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Many types of bronchial provocation tests (BPT) are employed in the study of asthma for clinical purposes and, more frequently, for research. BPT can be divided into two categories: those designed to measure non-specific bronchial hyperresponsiveness (BH) and those designed to measure the bronchial response to a specific allergen (specific sensitivity). This report is confined to provocation with allergens. Except in occupational asthma, the methods of allergen inhalation challenge (AIC) do not differ from those used for methacholine and histamine, although AIC methods require greater caution.

BPT involves many technical and non-technical factors which should be standardized (15). Several select committees have suggested guidelines for standardization (3, 11, 19, 25), and

most of the recommendations for specific and non-specific challenges are generally accepted by investigators worldwide. Consequently, the present document is not concerned with aspects of AIC that have already been amply dealt with in other documents. However, we feel there is a need for an update because the significant progress that has been made in the field of allergy has had repercussions on specific challenges. Lastly, these guidelines are not concerned with the indications of specific inhalation challenges with allergens but are intended to ensure that safer and well-standardized procedures are undertaken when performing these tests.

Allergenic extract

Allergens for BPT should be well-defined, standardized and stabilized (1). Recent years have seen the advent of more reliable methods for the determination of the allergenic activity of ex-

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Table 1.

Unit	Prescribed by	Units prescribed by authorities		
		Unit based on definition of unit	Characterization of allergens by	Potency between batches by
Allergy unit (AU/ml)	Office of Biologics FDA, USA	Intradermal skin testing 15th 3-fold dilution giving a Σ of erythema diameters = 50 mm = 100000 AU/ml	IEF/Immunoblotting	RAST inhibition
Biological unit (BU/ml)	Nordic Council on Medicines, Nordic Guidelines, 2nd ed, 1989	SPT conc. eliciting weal = that of histamine 10 mg/ml in median sensitive patient = 10000 BU/ml	IMC methods (immunoblotting, CIE/ CRIE, RIEF, etc.)	RAST inhibition

SPT = Skin prick test. IMC = Immunochemical methods.

tracts. Many studies have shown that the standardization of extracts in w/v or PNU does not correspond to the real allergenic activity because of the wide variability of this activity. Valid standardization methods are: RAST inhibition, skin prick test (Biological Units), quantitative immuno-electrophoresis, and, preferably, various combinations of the three (see Tables 1 and 2). The Committee suggests that no single method is sufficient to ensure a standardization of the composition or of the total potency of the allergenic extract in question. Only by combining relevant methods can such a standardization be obtained. However, no method of standardization can compensate for wrongly selected raw material. Therefore, a thorough characterization of the different allergenic source materials is indispensable to ensure the correct selection of the clinically most relevant source material (21, 23, 24, 37, 38, 40, 41, 43). The composition of the raw material, the primary extract as well as the final product, should be documented.

Another factor of considerable interest concerns reference standards of allergen preparations. International standards have been established by WHO (42) for some extracts (*D. pteronyssinus*, short ragweed, timothy grass and birch pollen). Several others (cat, dog, Bermuda grass, rye grass, and *Alternaria alternata*) are awaiting approval. These standards have an assigned potency of 100,000 international units

(IU). The IU are arbitrary (49). These standards should be used as a yardstick against which the potency and the composition of extracts marketed by commercial manufacturers or used by laboratories can be compared. The Committee advises the use of lyophilised extracts comparable when possible with the WHO standards. There are several other units. Nordic authorities, for example, have defined Biological Units (BU; 48). The Nordic Guidelines prescribe biological standardization by skin prick testing and in the US FDA Guidelines prescribe biological standardization by intracutaneous testing (70).

We recommend that allergens quantified by different methods be compared with international reference standards when available. If international standards are not available, it is recommended that vials of allergen preparations used in scientific studies be stored freeze-dried and frozen so as to permit later comparison with other preparations. In any case, an in-house standard (IHS) should be established and the potency of the final product should be labelled after calibration against the IHS.

Another critical factor with respect to extract is the stability of its activity in time. This stability may be maintained with various techniques:

- a) Preparing and storing lyophilised extract to be reconstituted on the day of challenge, using a proper diluent (conventional

method) or by storing the extract as a freeze-dried micronized extract as suggested by Melillo (44, 45). The characteristics of the latter allergen extract are listed in Table 3.

- b) By preparing and storing high concentrations of the extract at a low temperature (4°C), to be diluted immediately before challenge.
- c) By the addition of preservatives (not advisable in BPT).

Aerosol delivery systems and inhalation methods

Investigators may use any delivery system and inhalation method depending on their particular

needs, or preference, but any method should be well standardized.

Currently available methods are:

- a) Jet nebulizer (14, 16) with tidal breathing.
- b) Breath-activated dosimeter (11, 62) attached to a jet nebulizer.

The results obtained with the two systems are comparable (30). In these methods, flow rate, aerosol output with time, and particle sizes should be specified. More recently, another method of delivery has been proposed by Melillo (44, 45). The allergen extract is delivered via a powder inhaler. It permits breath-activated delivery to the bronchi of micronized allergen extract of the appropriate particle size and so

Table 2.

Units used by European manufacturers

Unit	Used by	Unit based on definition of unit	Characterization of allergens by	Potency between batches by
Activity units by RAST (AUR/ml)	Dome/Hollister-Stier	SPT and RAST inhib. 1 SARAH (≈ 10000 BU/ml) = 200 U of reference = 200 AUR by RAST inhib.		IMC methods, RAST inhib.
Allergenic units (AU/ml)	Lofarma	SPT	IMC methods	RAST inhib.
Biological units, (BU/ml)	Abello	SPT Conc. eliciting a mean weal diameter of $75 \text{ mm}^2 = 100 \text{ BU/ml}$	IMC. methods	RAST inhib
Standardized Biological Units (SBE/ml)	Allergopharma	SPT 1000 SEB/ml = 1000 BU/ml acc. to Nordic Guidelines 1st ed. 1980 (ref. substance histamine HCl 1 mg/ml)	IMC methods	RAST inhib.
HEP	ALK	SPT 1 HEP acc. to DAS 76	IMC methods i.e. CIE/CRIE. Major allergen deter. by RIE	Major allergen deter. by Ria RAST inhib. SPT
Index of reactivity, (IR)	Stallergenes	SPT Ref. substance codeine phosph. 9 mg/ml	IMC methods	RAST inhib.
SQ unit	ALK	Clin. efficacy (immunotherapy) 100000 SQ Units is recommended max. dose for immunotherapy*	IMC methods i.e. CIE/CRIE Major allergen deter. by RIE	Major allergen deter. by RIE, RAST inhib. SPT

*Can be related to BU/ml acc. to Nordic Guidelines and amount of major allergen in $\mu\text{g/ml}$ (Aquagen SQ used for BPT)

Table 3.

Characteristics of the allergenic extract	
Physical state:	fine powder
Method of preparation:	freeze-drying and micronization
Particle size:	1-5 microns
Container:	capsule ready to use
Vehicle:	lactose (25 mg/capsule)
Quantitation method:	RAST inhibition
Measure units of allergenic activity:	arbitrary units
Comparison with WHO standard	
HDM:	1 Arbitrary unit = 7.5 WHO int. units
Grass:	not evaluated
Stability over time of allergenic activity:	≥ 15 months

ensures deposition in the bronchi (45). The modality of inhalation is similar to that used for the delivery of sodium cromoglycate dry powder via a powder inhaler. The safety of the method (44), the technique for preparing the extract (27), animal studies (10), sensitivity and specificity (13), reproducibility (18), comparison with skin tests and RAST (52), use in children (6), comparison between a multiple-session and a single-session procedure (59), comparison with the continuous aerosol generation technique (56) and the dosimeter technique (5, 64), and suitability for nasal allergen challenge, have been documented.

Interval between the withdrawal of some drugs and allergen bronchial challenge

It is well known that many drugs affect *in vivo* allergen testing. Drugs may alter the response not only of the skin, but of the other target organs of local provocation such as the nose, conjunctiva and bronchus (57). Therefore, any drug that may potentially affect the response should be withheld prior to the provocation test. The drug suspension interval has not been carefully investigated for all these drugs. Moreover, since earlier guidelines were established, new drugs have come onto the market and hence should be accounted for.

i) *Antihistamines*. Information about the effects of antihistamines on AIC is scarce. A few investigations have shown that the inhibition of allergen-induced bronchoconstriction is achieved only with high doses of antihistamines (66), or with high doses administered intravenously or by inhalation (33, 55). Hence, most of our data come from studies on pharmacokinetics of the different antihistamines and on their inhibiting effects on skin or other target organs (nose, conjunctiva).

In adults the serum elimination half-life values of H_1 -receptor antagonists are extremely variable ranging from 2 h to 5 or more days. Moreover, most of the drugs continue to exert some effect when serum levels are low or even undetectable (65, 66). The duration of the inhibitory effect appears to be coupled with the human half-life of the substance. However, it must be kept in mind that the pharmacological effects of drugs may last longer than the half-life of the substance, which is why the interval of cessation of some drugs must be longer than the indicated half-life. We thus recommend withdrawal times before challenge longer than those indicated in other publications (69). Studies as to duration of tissue (receptor) binding are scarce. As a general guideline, anti-allergic drugs should be avoided for at least 10 half-life times, i.e. for ordinary antihistamines 3 days, and for other antihistamines longer periods, up to 6 weeks for Astemizole.

Azelastine. Suspend administration 2-3 days before challenge (32).

Terfenadine. Suspend administration 3 days before challenge (28).

Mequitazine. No information is available about elimination of the drug (63, 72).

Oxatamide. Suspend administration 5 days before challenge.

Loratadine. Suspend administration at least 10 days before challenge (58, 61).

Cetirizine. Suspend administration 5 days before challenge (4, 66).

Astemizole. Suspend administration 6-8 weeks before challenge (12, 34, 35, 46).

Levocabastine. Suspend administration at least 3 days before challenge.

Ketotifen. Suspend administration 15 days before challenge (71).

ii) *Corticosteroids*. Oral corticosteroids effectively inhibit the development of late responses to allergen inhalation, in contrast to their inability to prevent early reactions. If a patient requires regular use of oral corticosteroids it is necessary to decrease the dose to a minimum level, and to maintain this dose for 2–3 weeks before the challenge in order not to block the development of an eventual late reaction (26). Topical steroids can also prevent early reactions if administered at adequate dosages for at least a week before the challenge (20). Consequently, they block both the early and the late reaction (17). Even though their plasma half-life is very short, lasting from 1.5 h for flunisolide to 15 h for beclomethasone dipropionate (53), their action at local level is significantly more prolonged. The duration of the effect has not been well established. It is essential to ensure, however, that the minimum dose of inhaled and oral corticosteroids is administered in order to obtain clinical stability and to minimize the circadian variation of spirometry. When administered, inhaled corticosteroids should be stopped the night before the test and the daily dose given at the end of challenge day. For subjects taking oral corticosteroids, the dose should not be altered and adequate control days should be done, especially if subjects are taking alternate day corticosteroids. In such a situation, 2 control days should be done. In the case of a negative challenge, the diagnosis of sensitization might, however, not be completely ruled out.

The problem of corticosteroid interruption in relation to AIC is a theoretical rather than practical problem. It is very rare that a patient under long-term treatment with steroids requires AIC. However, should the occasion arise, the test may be performed in well-controlled, inhaled corticosteroid-dependent subjects, for example for research purposes.

iii) β_2 -stimulants. New commercially available slow release oral preparations – terbutalin (50) and salbutamol (2) – should be suspended 48 h before challenge. Administration of common inhaled β_2 -stimulants should be stopped 8 h before challenge (3, 11, 57, 67).

iv) *Theophyllines*. Slow release oral preparations should be suspended 72 h before challenge.

v) *Anticholinergic agents*. Protection against antigen-induced bronchoconstriction by ipratropium bromide (IB), an inhaled atropine derivative, is variable and seems dose-related. Some authors have observed that patients were protected by small doses (80 μ) of IB (38). Others have reported that much larger doses of the same drug (1000 μ) were required to achieve significant inhibition of the effects of antigens on the airways (36, 51). Ipratropium bromide should be stopped 12–24 h before challenge.

vi) *Sodium cromoglycate* inhibits both early and late antigen-induced bronchoconstriction (7, 31, 54). Sodium cromoglycate should be discontinued 12–24 h before challenge (57).

vii) *Nedocromil sodium*. The pharmacokinetics of nedocromil sodium is similar to that of sodium cromoglycate. A single dose (4 mg) of drug taken before antigen challenge prevents both early and late asthmatic responses (60). Nedocromil sodium should be discontinued 12–24 h before challenge.

Drugs not mentioned above should be stopped as indicated in previous guidelines.

Starting and maximum dose for AIC

The starting dose of antigen should be related to the dilution that provoked a positive reaction in skin tests and to the degree of non-specific bronchial reactivity (14).

It is difficult to indicate unequivocally the top dose that should be reached to define a test negative. The top dose should be sufficiently high to elicit a reaction in all sensitized

patients, but not so high as to cause false positives. The higher the dose administered, the lower the specificity, i.e. the more patients without clinical bronchial allergy will be diagnosed (22), e.g. patients suffering from hay fever alone.

It appears from various studies that the maximum dose of antigen differs in relation to the type of extract, its standardization, the delivery system, etc. For instance, Townley et al. (68), and Canavagh et al. (9), using an allergen extract standardized in PNU, found that the concentration of 10,000 PNU is too high to be clinically useful. Spector & Farr (67), using allergen labelled in w/v, found that a concentration as high as 10^{-2} was too high and recommend a top concentration of 1:500. Mosbech, using a *Dermatophagoides pteronyssinus* extract, standardized in biological units (BU/ml), adopts a pulmonary threshold from 1 to 10,000 BU/ml (47). Frølund et al. (29) use 100,000 SQ Units/ml as top dose giving a reproducible test. Melillo uses a top dose of 3200 arbitrary units (45), with *D. pteronyssinus*, while the top dose with grass pollen extract is significantly lower: 80 arbitrary units (5). Therefore, no definite conclusion can be drawn from these examples regarding the highest possible dose to use. It can be said that considering the highest natural exposure over 10–20 h and the additive effect of repeated low doses during a bronchial challenge, further studies are required to determine the top dose for the various AIC methods.

Caution should be exercised when tests are repeated after the administration of drugs that can alter the early response, as this may induce a severe late reaction.

Allergen inhalation challenges and non-specific bronchial hyperresponsiveness

Various investigators have shown that bronchial response to an allergen is related not only to the degree of allergic sensitivity (skin tests and RAST), but also to non-specific bronchial responsiveness (8, 14, 39). On the other hand, non-specific bronchial responsiveness can be enhanced by a positive AIC. Therefore, to give

meaningful information, the non-specific responsiveness should be determined together with the AIC.

The Committee tended to feel that even though useful, it is not necessary to perform the determination of non-specific hyperresponsiveness for general purposes during bronchial provocation with allergens, whereas it is necessary for research purposes or when challenge has to be repeated.

Measurement of the response

The Committee suggests dose-response curves be drawn to evaluate the allergen provocation dose.

a) Early reaction

The Committee recommends that a 20 % fall in FEV₁ be considered positive, even if some investigators consider a fall of 15 % sufficient.

b) Late reaction

The Committee holds that a fall in FEV₁ of 20 % be considered a positive late reaction.

Opinions differ as to the interval in hours from challenge during which functional parameters should be monitored for a late reaction. Most physicians record parameters for 8 h, others for 12 h. The Committee suggests that the standard interval should be 8 h. Subjects with negative early responses should still be followed for 8 h because late reactions can still occur even in the absence of an early reaction. More prolonged recording may be used at the discretion of the investigator in particular circumstances or for research purposes. There is a need for proper control days with sham exposure to take into account circadian variation of airway caliber and where patients are also monitored for at least 8 h.

In conclusion, it is hoped that these guidelines will be useful for both research and clinical use. It is probable that periodic revision of the guidelines will be necessary to take account of new procedures.

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