

The major allergen content of allergenic preparations reflects their biological activity

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The content of major allergens in biologically standardized allergenic preparations of birch, mite (*Der p*), cat, *Alternaria* (*Alt a*) and ragweed (*Amb e*) was determined. It was found fairly constant *between species*, i.e. varied within a factor of 2, with the exception of *Alt a 1* in *Alternaria alternata* extract. This variation is allowed by authorities between different batches prepared from the *same species* of allergen. The method for biological standardization (BS) prescribed in the Nordic Guidelines has, for common inhalant allergens, been shown to give reproducible results between regions of Europe. However, it is difficult to define patients suitable for BS of most food allergens as well as less common inhalant allergens. Therefore we propose that, in the future, BS is replaced by determination of well-established major allergens and that 1 ng of major allergen is given the value of 1 Biological Unit.

Clinical aspects

Clinicians have had difficulties in understanding differences and similarities between units used by manufacturers for labelling of allergenic extracts. Biological standardization is time-consuming and expensive. Probably therefore, and to avoid comparison with extracts prepared by other manufacturers, most manufacturers have used their own units and few of them have used the biological units as defined by the Nordic Guidelines or FDA. Determination of the amount of major allergen by ELISA is simple and cheap. However, the biological relevance of major allergen content has not been established. Our results clearly indicate the possibility of replacing biological units by major allergen content, provided the composition of allergens is adequate. The major allergen content can easily be declared by all manufacturers. In the future, manufacturers should be forced to declare the major allergen content, thus making it easier for clinicians to compare extracts from different suppliers.

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The purpose of allergen standardization is to provide reference preparations with known and reproducible composition and potency for calibration of manufactured and experimental extracts for diagnostic and therapeutic use (6). Various physicochemical methods have been applied for standardization of allergen preparations, such as weight/volume and protein nitrogen units (PNU). However, these techniques give no information about the allergenic activity of the preparations in humans. Different concepts for standardization of allergenic extracts have been advanced by scientists (1, 6, 8), allergen producers, and regulatory agencies (3, 19): biological standardization (BS)/potency determination by skin testing (1, 3), using regression line analysis (1, 3, 11); molecular characterization by CIE,

SDS-gPAGE, IEF (16, 17); semi-quantitative determination of allergens by CRIE or immunoblotting (4, 5, 16, 17); determination of individual allergens by ELISA, SRID or RIA (17, 19); or any combination of these techniques (1, 3, 11, 17, 20). Different regulatory agencies have taken different approaches and there are currently 3 systems of units: International Units (IU) of WHO/IUIS (20); Biological Units (BU) of the Nordic Guidelines (19), and the Allergy Unit (AU) of FDA (3, 20). The Subcommittee on Allergen Standardization within the International Union of Immunological Societies (IUIS) has prepared a number of international standard preparations (ISP) in collaboration with allergen specialists (16). These ISP are intended to serve as yardsticks for calibration of national standards and

manufacturers' standards. Using IU, BU or AU will allow comparison of results obtained in research laboratories and in clinical trials.

Determination of major allergens can be performed with high accuracy and has therefore been favoured by immuno-chemists. Biological standardization has been favoured by clinicians, since it has a biological basis and aims to equilibrate the potency between allergenic preparations prepared from different types of common inhalant allergens. During the last decade it has been discussed whether major allergen determination can replace BS.

This study was performed to investigate the content of major allergen in previously BS allergenic preparations (8) prepared from different source materials of common inhalant allergens and to find whether there exists any relation between BU and major allergen content.

Material and methods

For each allergen at least 20 adult patients consecutively attending a university allergy clinic were involved (8). The subjects had a positive clinical history of asthma and/or rhino-conjunctivitis and positive skin prick test to the allergen in question. The inclusion criteria were those published previously (8, 11, 19).

Allergenic preparations of birch (*Bet v*), mite (*Der p*), cat (*Fel d*), mould (*Alt a*), and short ragweed

(*Amb e*) were prepared according to Dreborg et al. (11). Carefully selected raw materials were delivered by Allergon AB (Ängelholm, Sweden). Extraction was performed by continuous shaking at +4°C for 20 h. The antigenic/allergenic composition was determined by CIE/CRIE (24) or by immunoblotting (4, 5). Anti-sera were produced according to Harboe & Ingild (13). The protein content was determined by amino acid analysis (23) and the carbohydrate content by gas liquid chromatography (22). The material was freeze-dried in vials containing about 10 mg dry weight or 3–6 mg protein. The biological activity was established according to the Nordic Guidelines as described below. The procedure for establishment of a biologically standardized in-house reference (IHR) preparation is illustrated in Fig. 1.

Biological standardization aims at equilibrating the activity in humans of reference extracts prepared from different inhalant allergen source materials (Fig. 2) (1, 8, 19). When preparing extracts for clinical use, the potency can be adjusted to a suitable strength by direct dilution or concentrated by freeze-drying and subsequent reconstitution in a smaller volume (12) (Figs. 1 and 3). The potency of the BS allergens investigated in this study were adjusted to the same biological activity (100 000 BU/ml). The BU unit has been shown to be reproducible between regions in Europe, i.e. the sensitivity of adults attending university out-patient clinics is approximately the same (8, 12). Further, the skin response

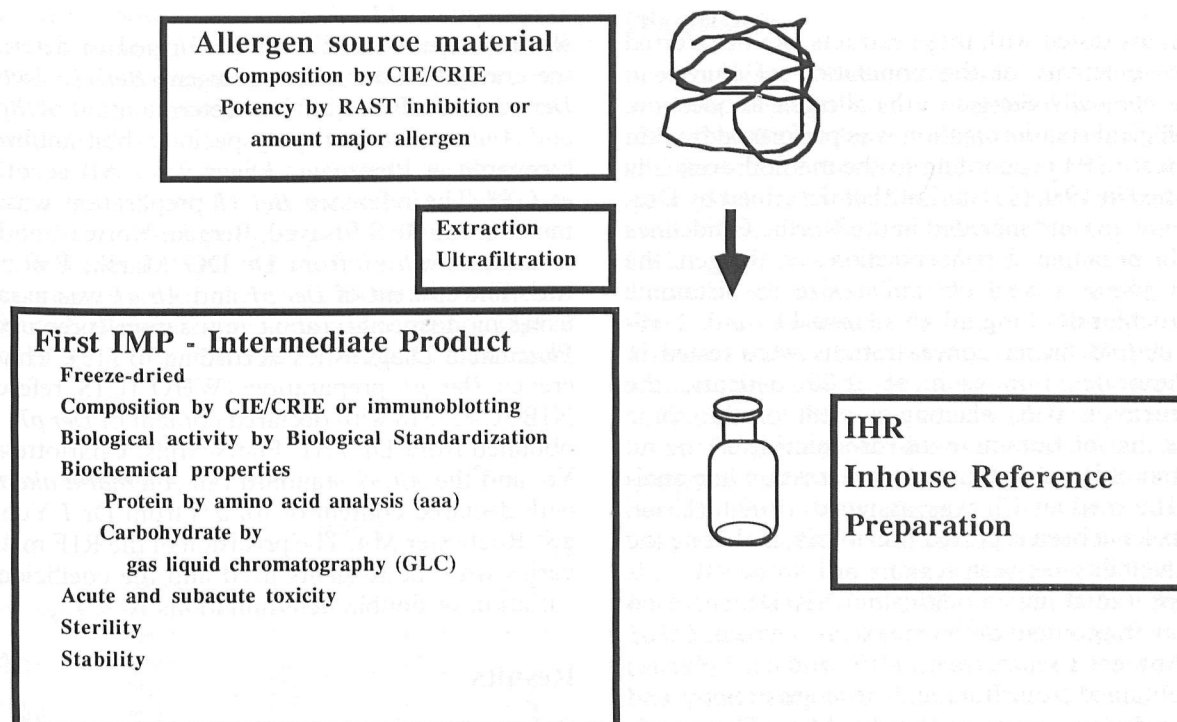


Fig. 1. Raw materials were carefully selected by potency determination and investigation of the composition. After extraction the material was freeze-dried. This intermediate product (IMP) is assigned in-house reference preparation (IHR) (Modified after 12).

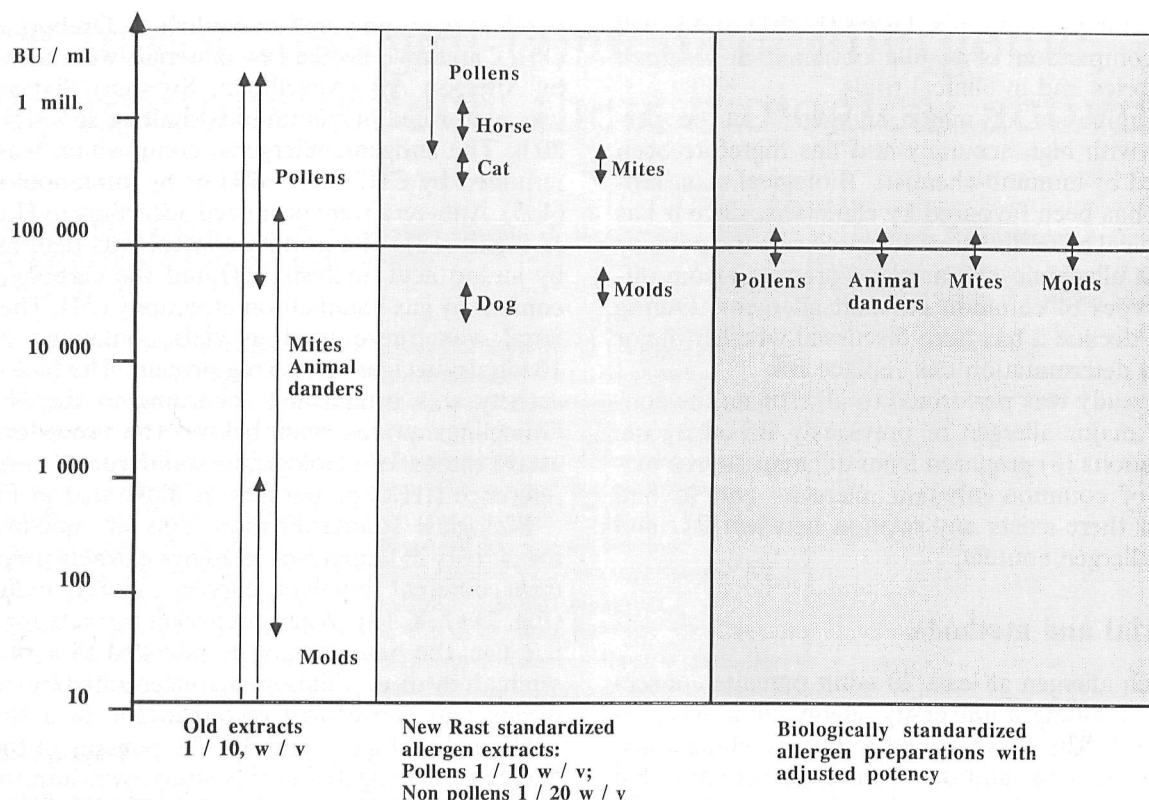


Fig. 2. Non-standardized allergens on the market in Europe 10–15 years ago, varied considerably in activity (left).

The potency of allergens produced from the same allergen source material can be adjusted to the same level by using, e.g. RAST inhibition or determination of major allergen content, and then concentration or dilution. The variation in potency between batches can then be kept within narrow limits (centre). In addition to this batch-to-batch standardization, BS aims at minimizing the difference in activity between allergens prepared from different allergen source materials (right). (Modified after 1, 12).

in humans tested with these extracts can be referred to the sensitivity of the population of European adults, clinically allergic to the allergen in question.

Biological standardization was performed by skin prick test (SPT) according to the method originally published in 1986 (11) and further described by Dreborg et al. (8) and included in the Nordic Guidelines (20). In principle, 3 concentrations of allergen, the lowest giving a weal of similar size as histamine dihydrochloride 1 mg/ml (5.43 mmol/l) and 2 10- and 100-fold higher concentrations were tested in quadruplicate. For each of ≥ 20 patients, the concentration (Ch) eliciting a weal of the same size as that of histamine dihydrochloride 10 mg/ml (54.3 mmol/l) was estimated by regression line analysis. The median Ch was assigned 10 000 BU/ml. Results have been reported previously, including the reproducibility between regions in Europe (8).

Single radial immunodiffusion (SRID) was used to assay the content of the major cat allergen, *Fel d1* (14). Anti-cat 1 serum (*anti-Fel d1*) and cat 1 (*Fel d1*) were obtained from Bureau of Biologics (Food and Drug Administration, Bethesda, Md). The coefficient of variation on double determinations using SRID is $< 3\%$ for *Fel d1* assay. Rocket immuno-

electrophoresis (RIE) was performed to determine the content of the major allergens *Bet v1*, *Amb e1*, *Der p1* and *Alt a1* (2). For determination of *Bet v1* and *Amb e1*, we used polyspecific rabbit antibodies (prepared at Pharmacia Diagnostics AB according to (13)). The reference *Bet v1* preparation was obtained from Dr S Elsayed, Bergen, Norway, and the reference *Amb e1* from Dr DG Marsh, Baltimore Md. The content of *Der p1* and *Alt a1* was assayed using monospecific rabbit anti-serum (prepared at Pharmacia Diagnostics according to (13)). The reference *Der p1* preparation (WHO/IUIS reference NIBSC 82/518 with declared content of *Der p1*) was obtained from Dr TAE Platts-Mills, Charlottesville Va, and the *Alt a1* standard (an *Alternaria alternata* with declared content of *Alt a1*) from Dr J Yunginger, Rochester Mn. The precision of the RIE method varies with the reagents used and the coefficient of variation of double determinations is $< 5\%$.

Results

Reference preparations of birch (*Bet v*), mite (*Der p*), cat (*Fel d*), mould (*Alt a*), and short ragweed (*Amb e*) were established by careful selection of raw ma-

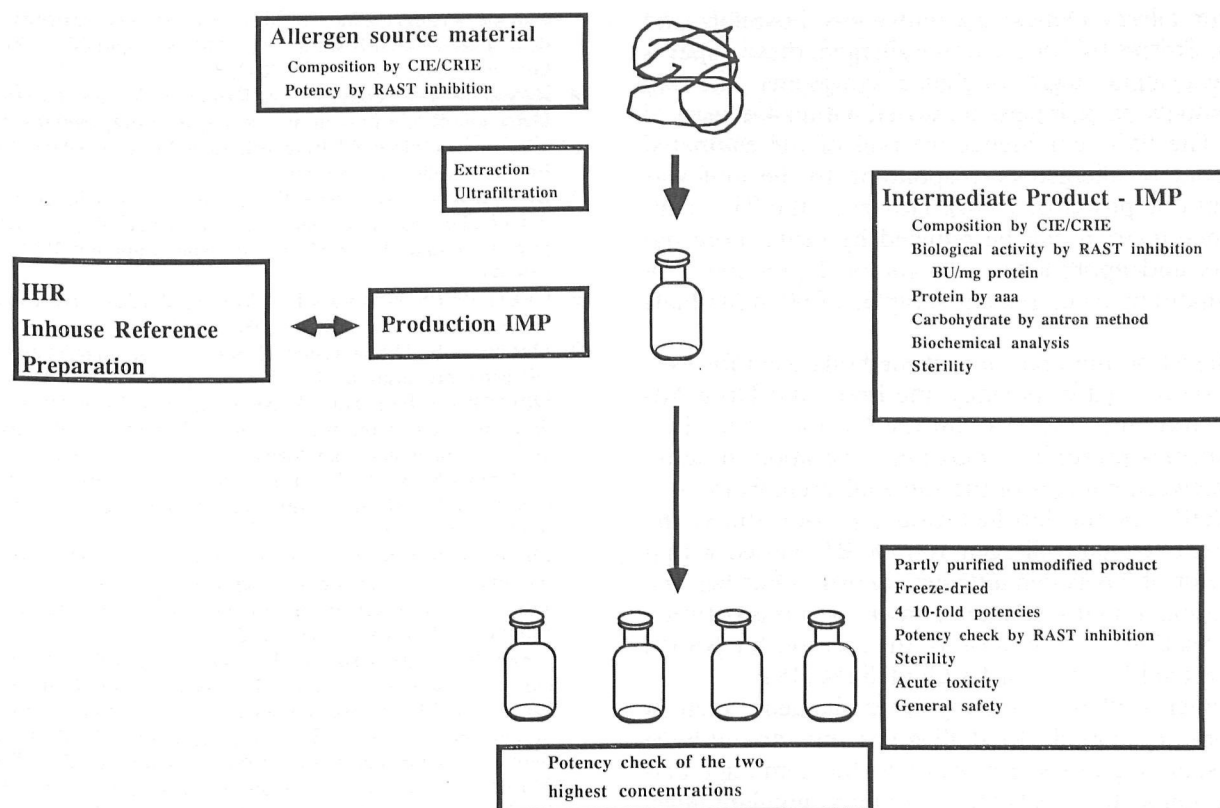


Fig. 3. New batches of allergenic preparations should be produced with the same raw material as those used for production of the IHS. The potency can then be determined by, e.g. RAST inhibition, and the final potency adjusted to a suitable predetermined potency by freeze-drying and dilution (Modified after 12).

terial with known and reproducible composition (11, 12). The biological activity of the allergenic preparations as determined by skin prick testing according to the Nordic Guidelines (19), has been reported elsewhere (8).

The amount of major allergen in allergenic preparations/100 000 BU, as measured by SRID or RIE and expressed in μg or μmol /100 000 BU are given in Table 1. With the exception of *Alternaria*, the content of major allergen/100 000 BU in the investigated allergenic preparations varied within a factor of 2, i.e. from 50 to 200% of the median value.

Discussion

A prerequisite for standardization of allergen preparations prepared from well-defined source materials is that the content of major and minor allergens and the relation between these allergens is constant from batch to batch. The allergenic composition is most often checked by immunoblotting or CIE/CRIE and the activity of the standard can be "transferred" to new batches by e.g. RAST inhibition or determination of major allergen content. These methods allow comparison of allergen preparations prepared from the same source material.

The aim of BS is to equilibrate the potency between allergenic preparations prepared from *different* allergenic source materials (1, 8, 19). By including consecutive patients allergic to the allergen in question, the amount of allergenic material needed to elicit a weal of the same size as that of histamine dihydrochloride 10 mg/ml, i.e. 10 000 BU/ml, has been found to be similar in different regions of Europe (8, 9, 10), thus the results have been shown to be reproducible over time and between regions. Biological standardization of common inhalant allergens is based on the assumption that patients with clinical symptoms of similar severity are referred to

Table 1.

Allergen	Major allergen	Major allergen/ 100 000 BU		Major allergen ng/BU
		μg	$\approx \mu\text{mol}$	
Birch	<i>Bet v 1</i>	100	5	1
Mite, <i>Der p</i>	<i>Der p 1</i>	60	2.5	0.5
Cat	<i>Fel d 1</i>	200	7	2
Mould, <i>Alt a</i>	<i>Alt a 1</i>	500	25	5
Ragweed	<i>Amb e 1</i>	75	2	0.75
Median		100	5	1

major allergy clinics, e.g. university hospitals and that, irrespective of causative allergen, these patients have a similar degree of clinical symptoms. The skin sensitivity of such patients varied within 4 powers of 10. The 95% confidence interval of the estimated amount of allergen corresponding to the unit was about one power of 10 (8). However, the 95% confidence interval can be reduced by using more patients and approaches a factor of 2 (50–200% of estimate) by using patient samples of 60 individuals (7).

Based on the precision of methods used for estimation or relative potency, the Food and Drug Administration in the US and the Nordic Council on Medicines prescribe a maximum variation in activity between batches of the same allergen, from 50% to 200% of the labelled value. In our study, the content of major allergen/100 000 BU varied within a factor of 2 between *different* allergens. Further, the variation in major allergen content was less between species than the variation of the method for BS (8) prescribed by the Nordic Guidelines (19).

Patients allergic to many other allergens, such as laboratory animals, food allergens, etc., do not have the same type of symptoms and the same age distribution as those allergic to common inhalant allergens. The typical cow's milk-sensitive patient is a child who exhibits a more or less severe reaction to cow's milk formula at the age of 3–6 months and who often tolerates high amounts of cow's milk within a few years. The symptoms in such patients are quite different from those in adult patients allergic to common inhalant allergens. Thus, the same patient inclusion criteria can not be used for BS of food allergens. Neither is it possible to include the few adults who are sensitive to cow's milk. They belong to a very special group of individuals who can not be compared to patients with rhinitis or asthma on exposure to grass pollen or dog dander.

Our data suggest that it would be possible to use determination of major allergen content as a substitute for BS, and it is suggested that 100 000 BU/ml is defined as 100 µg of major allergen/ml or, better, that 1 BU/ml is defined as 1 ng of major allergen/ml, provided the distribution of allergenic molecules is that of a relevant well-defined raw material.

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