



AMERICAN ACADEMY OF ALLERGY  
ASTHMA & IMMUNOLOGY

## **AAAAI Position Statement: The Use of Standardized Allergen Extracts**

May 1997

From the Committee on Allergen Standardization

*The statement below is not to be construed as dictating an exclusive course of action nor is it intended to replace the medical judgment of healthcare professionals. The unique circumstances of individual patients and environments are to be taken into account in any diagnosis and treatment plan. The above statement reflects clinical and scientific advances as of the date of publication and is subject to change.*

There has been significant progress in allergen standardization in recent years, and a growing number of standardized allergen extracts are now marketed in the United States. Despite this progress, there is still confusion among physicians and allied health professionals about how allergen extracts are standardized and how they should be used in clinical practice. In short, what constitutes a standardized extract? How do these extracts differ from nonstandardized products, what are their advantages, and how should they be used in allergy diagnosis and treatment? The aim of this position statement is to clarify these issues and provide guidance on the use of standardized extracts in clinical practice. This position statement has been approved for publication by the Board of Directors of the American Academy of Allergy, Asthma and Immunology and is based on the latest objective information available on standardized allergens.

### **Current Status**

The Academy has endorsed the use of standardized extracts in two recent position statements, on skin testing and on in vitro testing for IgE antibodies.<sup>1-5</sup> Licensing of allergenic products for clinical use in the United States is regulated by the Food and Drug Administration (FDA), Center for Biologics Evaluation and Research (CBER) (Division of Allergenic Products and Parasitology). The CBER has developed a program of allergen standardization based on assessment of the potency of allergen extracts by using quantitative skin tests and expressing the results in Allergy Units (AU) per milliliter.<sup>6,7</sup> In 1991, CBER introduced the bioequivalent allergy unit (BAU) to replace allergy units in order to distinguish potency labeling on the basis of the results of skin testing from that derived only from results of in vitro testing. The intradermal dilution for 50 mm sum of erythema (ID<sub>50</sub>EAL) system for determining BAU is advocated by the U.S. FDA and is currently being used to establish reference allergen extracts.<sup>7,8</sup>

Briefly, a series of threefold dilutions of a candidate reference extract is prepared, and 0.05 ml is administered intradermally to 15 to 20 highly sensitive allergic subjects. The dose-response data are used to determine the D<sub>50</sub>, or the dilution required to elicit a 50 mm erythema skin test response (sum of orthogonal diameters) for each subject tested.

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The mean  $D_{50}$  is calculated, and the potency of the extract is assigned. An extract with a  $D_{50}$  of 14.0 contains 100,000 BAU/ml.<sup>7,8</sup> In addition to skin testing, standardized extracts licensed through CBER are also evaluated by immunochemical techniques, including competitive binding assays (RAST and ELISA inhibition), isoelectric focusing, gel electrophoresis, and, in some cases, by specific allergen assays.<sup>6</sup> Once BAU have been established by CBER for the reference, manufacturers assign this potency to their standardized lot (if the product is equipotent to the reference) by using in vitro estimates of relative potency (e.g., ELISA inhibition or allergen assay).

To be released as a standardized product, an extract must satisfy criteria of allergenic potency, which are approved by the CBER. Such evidence of allergenic potency is not required for unstandardized extracts, which may show marked variability in allergen content. Some of the most common allergens used in clinical practice are now standardized products: short ragweed, mites, cat hair and pelt, and insect venoms. Other allergens pending standardization by CBER include grass pollens (to be completed by July 1997), tree pollens, giant ragweed, cockroach, latex, dog, and some foods and molds. The Allergen Standardization Committee of the Academy has also compiled a list of candidate extracts for standardization on the basis of a survey of the Academy membership to determine which extracts deserve the highest priority. The list is included as an appendix to this position statement.

## **New Technologies**

In spite of recent progress, it is widely acknowledged that there are several pitfalls associated with current standardization methods. Skin testing depends on the availability of “highly sensitive” patients and how they are identified and selected. IgE antibody–based tests depend on supplies of human sera, which can be difficult to maintain, and on the composition of the serum pool and allergen extract used as reference standards for comparison.<sup>3,6</sup> A critical problem with measuring “total potency,” whether on the basis of biologic or in vitro assays, is that the unitage is arbitrary. Thus for example, extracts standardized in BAU in the United States cannot be directly compared with extracts marketed in biologic units (skin test potency relative to histamine) or in international units in Europe and elsewhere (international units are assigned relative to World Health Organization/International Union of Immunological Societies [WHO/IUIS] reference preparations).<sup>9</sup> The use of different systems of units (e.g., protein nitrogen units, allergy units, BAU, biologic units, and international units) is confusing and underscores the need for a common unitage as part of worldwide standardization efforts.

The rapid development of new technologies for both DNA and protein analysis offers opportunities for improved standardization. Many important allergens from pollen, dust mites, animal danders, insects, and foods have now been cloned and are being expressed as homogeneous recombinant proteins, which in several cases have allergenic activity comparable to that of the natural protein allergen. Other technologies that have potential for improving allergen identification include high-performance liquid chromatography,

capillary electrophoresis, and mass spectrometry.<sup>6</sup> With these new technologies, an allergen extract can be defined by major allergen content in mass units, and the consistency of each lot can be accurately monitored. The application of new technologies to allergen standardization, particularly measurement of well-defined allergens and the use of recombinant allergens, will greatly facilitate objective comparisons of allergen extracts. Measuring specific allergens (or “marker proteins,” as defined by CBER) allows quantitative comparison of the allergen composition of different extracts in absolute units (nanograms or micrograms of specific allergen).<sup>3,6,9,10</sup> Such measurements should focus on proteins of well-established allergenic importance, which fulfill criteria for inclusion in the WHO/IUIS nomenclature (e.g., Fel d 1, Der p 1, Lol p 1). It is important that there should be a *consistent relationship* between the allergen that is being measured and other allergens present in the extract. However, the precise method of allergen measurement is less important. Although monoclonal antibody–based assays are preferred because the reagents are available “in perpetuity” and have defined specificity, tests with polyclonal antibodies may be equally valid, provided that the antibodies are monospecific. At present, measurements of Fel d 1 form the basis for assigning BAU to cat hair and cat pelt extracts by CBER. These measurements have been shown to correlate with skin test potency in BAU. Cat hair and dander or cat pelt extracts marketed at 10,000 BAU are targeted to contain 15.0 FDA units of Fel d 1/per milliliter; (1 unit is ~4 µg of Fel d 1 protein).<sup>6,10</sup> Major allergen measurements have been shown to correlate with estimates of biologic potency on the basis of the European biologic unit system.<sup>11,12</sup>

The introduction of measurements of major allergens as the basis of standardization is now a realistic and desirable goal, which should be encouraged. A key element of this process is the maintenance of reference standards containing known amounts of relevant allergens. Standards for a number of extracts have been produced as part of the WHO/IUIS allergen standardization program, which has been supported by the Academy. Several of these standards (e.g., short ragweed, mite [*Dermatophagoides pteronyssinus*], and dog) contain known amounts of major allergens.<sup>3,9</sup> The allergen content of some CBER reference preparations has also been determined. It is vital that standards with defined allergen content are maintained under stable conditions in approved repositories, such as the CBER, WHO, or the American Type Culture Collection (ATCC) facilities, so that all measurements can be made by reference to a single common standard. In the future, it may be envisioned that recombinant allergens will provide primary standards for allergen analysis, as well as forming the basis for development of new diagnostic and therapeutic allergy products.<sup>13,14</sup>

The most common extracts used in clinical allergy practice are now available as standardized products or are pending standardization (see Appendix). However, there are several hundred extracts currently being marketed (many of which are only used occasionally), and it is neither feasible nor economical to standardize them all. To cover these products in the United States, the CBER is considering the introduction of “Extracts tested for consistency by an in-house reference,” which will be monitored by allergen manufacturers themselves, using methods approved by CBER. This approach is designed to ensure a minimum level of standardization and quality control in otherwise

unstandardized extracts. There will remain a number of extracts that are commercially available but have not been characterized or assessed for allergen content. This is usually because these allergens affect only a small number of patients with allergy (e.g., those sensitive to certain food, insect, or mold allergens) or because the allergens occur in limited geographic areas.

The implementation of new technology into allergenic products will of course depend on convincing allergen manufacturing companies, regulatory authorities, and physicians that these procedures will lead to significant improvements in the quality of allergen extracts and the manufacturing process, which are also cost-effective. The Allergen Products Manufacturers Association (APMA), which represents companies that market allergenic products in the United States, has supported and promoted allergen standardization, and there is no doubt that improvements in the quality of allergen extracts that have occurred over the past 5 years are in large part due to improved manufacturing and testing procedures. Several companies are now measuring specific allergen content in addition to other measures of potency, and increasing use of allergen assays should improve quality control, extraction procedures, and stability testing.

### **Clinical Relevance**

Why should clinicians use standardized extracts? The Academy endorses the use of standardized products because they have been tested for potency both in vivo and in vitro. The extracts show improved consistency both between manufacturers and between batches produced by the same manufacturer. For diagnostic purposes, standardized extracts should elicit more reproducible skin test responses and a lower frequency of false-positive reactions. However, the main benefits of standardized allergens, for both the patient and physician, are improvements in safety and efficacy of extracts used for immunotherapy. Although there are no formal studies comparing the frequency or severity of adverse reactions in standardized or unstandardized products, the Academy believes that there are advantages to using standardized products that are likely to reduce the prevalence of these reactions. The principal advantages are that the biologic effects of standardized extracts have been tested and that the range of major allergen levels in standardized extracts is much less variable than in unstandardized products (twofold to threefold, as compared to 10-fold to >100-fold). As allergists and allied health workers gain experience in using extracts labeled in BAU or allergen content, as opposed to protein nitrogen units or weight per volume, it will be easier to adjust immunotherapy regimens and to compare patients at various stages of immunotherapy with extracts from different manufacturers. Thus it should be possible to tailor allergen doses for patients using an extract marketed at a particular allergen content (BAU or specific allergen level).

The other important benefit of using standardized extracts is therapeutic efficacy. There is good evidence from a series of studies on ragweed, grass, mite, cat, and venom allergens that achieving a maintenance dose of approximately 5 to 20 µg of major allergen per injection is associated with significant improvement in patient symptom

scores.<sup>9,15,16</sup> Thus by knowing the specific allergen content of an extract, the allergist can develop treatment protocols that work toward these clinically effective maintenance doses.

### **Recommendations**

1. In keeping with previous position statements, the Academy recommends the use of standardized extracts (where available) for the diagnosis and treatment of allergic diseases.<sup>4,5</sup> The Academy is committed to working with regulatory authorities, allergen product manufacturers, and academia to promote the use of standardized extracts and to educate physicians and other health professionals in the use of these extracts.
2. The Academy recognizes that the use of different arbitrary units for allergenic activity, both within the United States and in other countries, is not satisfactory. Research should be directed toward developing a common unitage that provides objective measurement of allergen content, and when possible, measuring mass units of specific allergens is recommended. The Academy supports and encourages new technologies that facilitate the identification and quantification of allergens. For standardization purposes, it is crucial that the importance of individual allergens in causing immediate hypersensitivity responses should be clearly defined by carrying out appropriate tests including skin tests, histamine release assays, and serum IgE antibody measurements in a large unselected population of patients with allergy.
3. It should not be assumed that standardized allergens are inherently “safer” than other extracts. However, the goal of standardization is to produce allergenic products that are consistent from manufacturer to manufacturer and on a lot-to-lot basis, and this is likely to reduce the frequency of adverse reactions. There is good evidence that achieving maintenance doses of immunotherapy on the basis of major allergen content, reaching 5 to 20 µg of allergen per injection, has beneficial clinical results. The Academy commends the use of standardized extracts as one approach to improving the efficacy of immunotherapy and supports further controlled trials for establishing clinically effective doses for individual allergens.
4. Extracts without compelling evidence of their allergenic importance should not be used in routine clinical practice.

Further progress in allergen standardization will depend on continued improvement in the exchange of information and development of ideas among regulatory authorities, industry, academia, physicians, and health care workers, as well as on further research on allergens and the relationship between allergen sensitivities and human disease. Education about the benefits of using standardized products is needed, and this will depend on studies that establish their clinical efficacy and utility in clinical practice.

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*AAAAI Position Statements and Work Group Reports are not to be considered to reflect current AAAAI standards or policy after five years from the date of publication. For reference only. May 1997*

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## Appendix

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### Current status of standardized allergen extracts in the United States

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<b>Standardized extracts, approved by FDA</b>	<b>Manufacturer's license applications pending FDA approval</b>	<b>Candidate extracts for standardization</b>
Cat hair	Bermuda grass*	Johnson grass
Cat pelt	Red top grass*	Bahia grass
Mite ( <i>D. farinae</i> )	June grass*	Giant ragweed
Mite ( <i>D. pteronyssinus</i> )	Perennial ryegrass*	Lamb's-quarter
Short ragweed	Orchard grass*	Plantain
Hymenoptera venoms	Timothy grass*	Russian thistle
Honeybee	Meadow fescue*	Mugwort
Yellow hornet	Sweet vernal grass*	Pigweed
White-faced hornet	American cockroach	Oak
Yellow jacket	German cockroach	Box elder
Paper wasp	Oriental cockroach	Elm
	Latex	Mountain cedar
		Ash
		Birch
		<i>Alternaria</i> spp.

*Aspergillus* spp.

*Cladosporium* spp.

*Penicillium* spp.

Fire ant

Dog

Peanut

Egg

Milk

Shrimp

\*These license applications are expected to be approved by July 1997, and at that time only standardized extracts will be marketed.

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