

Status of standardized allergen extracts

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Standardization of allergen extracts was initiated in the mid-1970s, and that effort continues today. Research and standardization of these products have fostered communication concerning appropriate dosing and dose response with standardized allergen extracts, allowing comparison between recommended doses for various allergens and doses that have been proven effective for those allergens. Immunotherapy as a treatment for allergy has also been enhanced by the development of standardized allergen extracts. Recent studies with standardized allergen extracts conclude that allergy symptoms and antiallergic or antiasthmatic medication use in allergic/asthmatic patients can be reduced using immunotherapy with these products, and these desirable effects persist long term, perhaps even permanently, after cessation of immunotherapy. Standardization of allergen extracts has resulted in products that are safer, more stable, and lend themselves to meaningful research in the diagnosis and treatment of allergies. *Curr Opin Otolaryngol Head Neck Surg* 2001, 9:181–185

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Current Opinion in Otolaryngology & Head and Neck Surgery 2001, 9:181–185

Abbreviations

AU	allergy units/milliliter
BAU	bioequivalent allergy units/milliliter
CBER	Center for Biologics Evaluation and Research
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration

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The use of extracts from allergens such as pollens, molds, and foods for the purposes of diagnosing and treating allergy dates back to 1911, when Noon [1] proposed the use of these extracts for “prophylactic inoculation against hay fever.” The subsequent emergence of allergy as a medical specialty led to the recognition of the need for potency standards for allergenic extracts as early as 1933 [2], but the complex and diverse nature of the more than 1000 different extracts offered for sale in the United States has proved to be an imposing obstacle to the achievement of standardization of these products. Unstandardized allergen extracts have been commonly labeled with the weight-per-volume ratio at which the allergen was extracted or by protein nitrogen unit content in an effort to express the relative strength of these products. Neither designation is a reliable measure of biologic or allergenic activity.

The first allergen extract to be standardized was short ragweed pollen and was achieved by the measurement of antigen E (Amb a 1) content in the mid-1970s. Standardization of cat hair and cat pelt extracts was accomplished by assay for cat antigen 1 (fel d 1). House dust mite extracts (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) were standardized by use of a relative potency assay that initially uses intradermal titration skin testing and then a radioallergosorbent inhibition assay to assign units of potency [3]. Radioallergosorbent test inhibition has since been supplanted by competition enzyme-linked immunosorbent assay (ELISA). Standardization of eight grass pollen extracts by ELISA inhibition was mandated by the Food and Drug Administration (FDA) in July 1998, and those standardized grass pollen extracts are commonly used in the United States today. Table 1 lists all standardized allergen extracts and the method(s) by which each extract is standardized [4•].

Use of standardized allergen extracts in clinical practice

Federal regulatory authorities have not issued useful guidance for dosing with standardized allergen extracts [5••], but communication among physicians concerning dosing with these products is now possible as a result of the assignment of meaningful potency units. Nelson [5••] recently reported the results of a study designed to determine the doses of standardized allergen extracts commonly used by board-certified allergists in the United States and compare those doses with doses that have been proven to be effective in double-blind

Table 1. Standardized allergen vaccines currently licensed in the United States

Allergen vaccine	Current lot-release tests
Dust mite (<i>Dermatophagoides farinae</i>)	Competition ELISA, protein*
Dust mite (<i>Dermatophagoides pteronyssinus</i>)	
Cat pelt (<i>Felis domesticus</i>)	Fel d 1 (RID), IEF, protein
Cat hair (<i>F. domesticus</i>)	
Bermuda grass (<i>Cynodon dactylon</i>)	Competition ELISA, IEF, protein*
Red top grass (<i>Agrostis alba</i>)	
June (Kentucky Blue) grass (<i>Poa pratensis</i>)	
Perennial ryegrass (<i>Lolium perenne</i>)	
Orchard grass (<i>Dactylis glomerata</i>)	
Timothy grass (<i>Phleum pratense</i>)	
Meadow fescue grass (<i>Festuca elatior</i>)	
Sweet vernal grass (<i>Anthoxanthum odoratum</i>)	
Short ragweed (<i>Ambrosia artemisiifolia</i>)	Amb a 1 (RID)
Yellow hornet (<i>Vespa</i> species)	Hyaluronidase and phospholipase activity
Wasp (<i>Polistes</i> species)	
Honey bee (<i>Apis mellifera</i>)	
White-faced hornet (<i>Vespa</i> species)	
Yellow jacket (<i>Vespula</i> species)	
Mixed vespid (<i>Vespa</i> and <i>Vespula</i> species)	

*Test for informational purposes only. ELISA, enzyme-linked immunosorbent assay; IEF, isoelectric focusing; RID, radial immunodiffusion. Data from Slater and Pastor [4•].

placebo-controlled studies. This study involved mailing questionnaires to practicing allergists in the United States and asking for their recommended dosing for a hypothetical patient with allergies. Eighty physicians provided responses that contained interpretable data on

maintenance dosing with standardized extracts. Maintenance doses recommended by board-certified allergists appear in Table 2.

The median maintenance dose recommended for grass pollen extracts, with the notable exception of Bermuda grass, ranged from approximately 0.01 to 0.025 mL of 100,000 bioequivalent allergy units/mL (BAU), which was considered comparable with those doses that have been demonstrated to be clinically effective by controlled studies. The reported recommended dose for Bermuda grass was approximately one eighth the amount of the other grasses (in BAUs), which immediately raises some questions, because Bermuda is assigned a maximum potency that is one tenth that of the other standardized grass extracts. Are all allergists in the United States underdosing Bermuda grass or is the assigned BAU value somehow flawed? Unfortunately, there are no controlled studies on Bermuda grass that demonstrate a clinically effective dose for this allergen.

Ragweed maintenance doses were reported in weight-per-volume ratios but lacked a recommended volume of the concentration; therefore, no calculation of the actual dosage could be made from the data published. The author concluded that the doses for ragweed were within the range of reported successful dosing.

The median maintenance dose of house dust mite (*D. farinae*–*D. pteronyssinus* mixed) was reported to be 0.025 mL of 10,000 allergy units/mL (AU) and was considered to be adequate in comparison to doses reported in controlled studies. Data published in this study indicates the effective maintenance dose for *D. pteronyssinus* is 0.05 mL of 10,000 AU, whereas *D. farinae* would

Table 2. Recommended maintenance doses by certified allergists

Extract	Number of responses	Median dose	25th quantile	75th quantile	Lowest dose	Highest dose
Timothy (alone)	31	2500 BAU	1000 BAU	4762 BAU	50 BAU	16,130 BAU
Timothy (plus fescue)	43	960 BAU	750 BAU	2994 BAU	50 BAU	21,212 BAU
Fescue (plus timothy)	43	1754 BAU	625 BAU	4000 BAU	80 BAU	10,000 BAU
Bermuda grass	37	300 BAU	150 BAU	500 BAU	2.7 BAU	1000 BAU
Short ragweed (alone)	11	1:100 wt/vol	1:200 wt/vol	1:100 wt/vol	1:1000 wt/vol	1:60 wt/vol
Short ragweed (plus giant ragweed)	50	1:250 wt/vol	1:533 wt/vol	1:120 wt/vol	1:5000 wt/vol	1:50 wt/vol
Giant ragweed (plus short ragweed)	13	1639 PNU	1000 PNU	2500 PNU	100 PNU	4000 PNU
Giant ragweed (plus short ragweed)	50	1:250 wt/vol	1:500 wt/vol	1:200 wt/vol	1:5000 wt/vol	1:50 wt/vol
<i>Dermatophagoides pteronyssinus</i>	13	1680 PNU	1000 PNU	2500 PNU	100 PNU	4000 PNU
(69 with <i>Dermatophagoides farinae</i>)	70	250 AU	150 AU	500 AU	0.5 AU	1500 AU
<i>D. farinae</i>	74	250 AU	150 AU	500 AU	0.5 AU	1500 AU
(69 with <i>D. pteronyssinus</i>)	71	400 BAU	250 BAU	750 BAU	20 BAU	3000 BAU
Cat	50	1:500 wt/vol	1:1000 wt/vol	1:100 wt/vol	1:5000 wt/vol	1:50 wt/vol
Dog	11	600 PNU	250 PNU	1000 PNU	38 PNU	3000 PNU
<i>Alternaria</i> species	60	1:200 wt/vol	1:333 wt/vol	1:100 wt/vol	1:4000 wt/vol	1:25 wt/vol
	12	1000 PNU	500 PNU	2000 PNU	150 PNU	3000 PNU

AU, allergy units/milliliter; BAU, bioequivalent allergy units/milliliter; PNU, protein nitrogen units; wt/vol, weight/volume. Data from Nelson [5••].

require 0.25 mL of 10,000 AU for an effective maintenance dose. The overwhelming majority of allergists responding used *D. farinae* and *D. pteronyssinus* antigens in an equal parts mix. Sixty-nine allergists reported treatment of *D. farinae* with this equal parts mix; five allergists reported treatment with *D. farinae* antigen individually. Sixty-nine allergists also reported treatment of *D. pteronyssinus* using this same equal parts mix; one allergist reported treatment with *D. pteronyssinus* antigen individually.

The median dose of cat antigen prescribed was 0.04 mL of 10,000 BAU, as opposed to the proven dose of approximately 0.35 mL of 10,000 BAU. The author concluded certified allergists significantly underdose with cat and dog antigens.

Nelson noted that *Alternaria* species was reportedly dosed at the same level as ragweed in this study. There is little data on effective dosing for *Alternaria*, but the author concludes that *Alternaria* allergen is highly unlikely to be as potent as ragweed.

Effectiveness of immunotherapy with standardized allergen extracts

Standardization of allergen extracts appears to have contributed to an increased acceptance of immunotherapy for patients suffering from inhalant allergies. Durham *et al.* [6••] recently reported results from a study designed to determine the long-term efficacy of grass pollen immunotherapy. This study was a randomized, double-blind, placebo-controlled trial involving 40 patients treated for a number of years with standardized grass pollen extract. Immunotherapy was administered to all patients in this group for at least 3 years, after which treatment of 20 patients from this group was discontinued; the entire group of 40 patients was closely monitored for another 3 years, as was the original placebo group. It was demonstrated that immunotherapy effectively reduced allergy symptoms and antiallergic medication use during the pollen season, and significantly improved several measures of immune system response. In addition, it demonstrated these desirable effects persisted at least 3 years after immunotherapy was discontinued. Adkinson [7•], in an editorial appearing in the *New England Journal of Medicine*, concluded that this study provides the best evidence to date that allergen immunotherapy has long-term, perhaps permanent, benefits.

A retrospective study by Cools *et al.* [8••] investigated the long-term effects of immunotherapy with standardized house dust mite (*D. pteronyssinus*) on children with asthma. This study incorporated a control group of asthmatic patients with comparable asthma features who never received immunotherapy. Immunotherapy was initiated during childhood and continued for

approximately 5 years. Patients from the control group and patients treated with immunotherapy were reevaluated approximately 10 years after immunotherapy cessation. It was determined that the risk of frequent asthmatic symptoms was three times higher in the control group than in the immunotherapy-treated group, and the frequent use of antiasthmatic medication was more pronounced in the untreated group, although this difference was not statistically significant. This study concluded that immunotherapy with standardized house dust mite extract has long-term effects on asthmatic symptoms in young adults when the total dose of administered antigen is equal to or greater than 320,000 AU.

Safety of immunotherapy with standardized allergen extracts

The most serious problem in practical immunotherapy is the risk of occasional, potentially life-threatening adverse systemic reactions [9•]. A wide variation in biologic potency of any given allergen extract could pose a substantial hazard in the routine administration of immunotherapy. Slater *et al.* [4•] recently completed a study designed to establish a clinical basis for acceptable potency ranges of standardized allergen extracts. The study concluded that the FDA's lot-release limits for the relative potency of standardized allergen extracts are well within the range of clinically safe variability.

Ohashi *et al.* [9•] studied risk factors for adverse systemic reactions while using a standardized mite allergen extract to treat patients with perennial allergic rhinitis. They completed a retrospective study of 386 patients who had received immunotherapy with standardized *D. farinae* extracts for 5 years (22,722 injections). The study focused on serious or severe systemic reactions that required specific treatment and incorporated testing for total IgE and specific IgE (against *D. farinae*) prior to treatment. A total of 27 systemic reactions occurred in this group of patients and demonstrated an inverse correlation between the onset of symptoms and severity of reactions. Twenty-five of 27 reactions occurred within 20 minutes after injection; no reactions occurred after 30 minutes. The study noted that those patients who had a total IgE greater than 1000 U/ml and asthma or atopic dermatitis had an incidence of systemic reaction of 66.67% (12/18) per patient, whereas 81 patients who had only one of these risk factors demonstrated an incidence of 23.46% per patient. By contrast, the incidence of systemic reaction in the remaining patients who had none of these risk factors was 1.64% per patient. The authors concluded that asthma, atopic dermatitis, and a high level of IgE in serum (but not a high level of specific IgE in serum) are important high-risk factors in possibly inducing severe adverse systemic reactions in patients who receive immunotherapy for perennial allergic rhinitis. The rate of systemic reaction

could be reduced by 75% if patients with high-risk factors are strictly excluded from immunotherapy for allergic rhinitis. This study also demonstrates the need for determination of total IgE serum levels in patients before beginning immunotherapy.

Stability of standardized allergen extracts

Allergen extract stability can be defined as the persistence of adequate quantities of relevant antigens in an allergen extract from the time of initial assay to the time of clinical use. Before standardization of allergen extracts, specific information was scarce concerning the shelf life of solutions used for skin testing and immunotherapy. Expiration dating periods and recommended storage conditions were determined somewhat arbitrarily, and the effect of inclusion of stabilizers such as glycerin was mostly theoretical. The *in vivo* and *in vitro* methods developed for the standardization of allergen extracts now provide accurate scientific means to evaluate the actual shelf life of these products, assign expiration dates, and specify optimal storage conditions.

The Center for Biologics Evaluation and Research (CBER) of the FDA has promulgated rules requiring stability testing of standardized allergen extracts. In contrast to European regulations requiring that these products retain a minimum of only 30% of their initial potency at the end of their shelf life, the FDA requires standardized allergen extracts to be within the same potency range at the end of the dating period as they were at the time of initial product release. The standardization program established by the FDA is designed to prevent a major discrepancy between the last dose patients receive from an old vial of an allergenic product and the first dose they receive from a new vial. Widely varying stability limits could result in significant overdosing and could needlessly result in an allergic reaction during immunotherapy. In addition, because immunotherapy depends on the amount of antigen received, a dose that was 30% of the intended dose could reduce the consistency of effect [10].

The FDA also summarized its position on glycerin as a potency preservative:

The addition of glycerin to an allergenic extract dramatically increases the stability of allergen solution. As a result, all standardized allergen extracts are glycerinated. Prior to CBER's allergen standardization program, only a minority of allergen extracts was sold in glycerin solutions. In fact, aqueous (non-glycerinated) extracts remain the norm among currently marketed non-standardized extracts, in spite of clear evidence of improved stability associated with the addition of glycerin.

It would seem that this clear evidence of improved stability should incite the FDA to action, establishing shortened dating periods for unstandardized aqueous extracts or prohibiting the sale of extracts that do not contain glycerin or another acceptable stabilizer.

A recent study investigated the stability of house dust mite allergens in glycerinated extracts [11]. Researchers have isolated and characterized at least ten allergens contained in *D. farinae* and *D. pteronyssinus* extracts. This study sought to compare the stability of mite allergens in a 50% glycerinated solution stored at different temperatures and to examine the role of protease inhibitors in preventing degradation of these products. Reconstituted lyophilized extracts were stored at -70°C , -20°C , 4°C , and 37°C for 12 months before being compared with freshly reconstituted extracts by ELISA inhibition. They found that the overall potencies of the stored extracts were stable at -20°C and 4°C but noted a loss of the *D. farinae* epitope Der f 1 at 4°C . Storage at 37°C led to an overall loss of potency and allergen content, whereas storage at -70°C was associated with a moderate loss of potency that increased with multiple freeze-thaw cycles. It was also concluded that protease inhibitors demonstrated no effect on allergen stability in this particular study. This study brings into question the sensitivity of competition ELISA when loss of antigens within the extract have been demonstrated by other methods, but ELISA testing indicates that the overall potency has not changed.

Research with allergen extracts

Unstandardized allergen extracts represent a substantial portion of extracts commonly used in the United States today. Manufacturers who wish to provide physicians with consistent allergen extracts have applied methods used for standardization to unstandardized products and developed in-house reference standards that can be used to accurately estimate the potency of these allergen extracts and reduce lot-to-lot potency variability.

Methods that may serve as viable alternatives to current classical methods of allergen extract standardization are being investigated in Europe and show signs of promise as additional tools to estimate biologic potency of allergen products [12,13]. Allergen extracts standardized by European methods (skin testing) are being used to evaluate the effectiveness of sublingual immunotherapy for pollen allergies [14,15].

In the United States, the Allergen Product Manufacturers' Association reports that discussion is underway concerning the next allergen extract candidate for standardization (Willoughby, Personal communication). Although choices of allergens for future standardization have not yet been

agreed upon, we should expect that a continuing effort to produce improved allergen extracts will, in turn, further increase the proficiency with which allergic patients are diagnosed and treated using allergen extracts.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- Of special interest
- Of outstanding interest

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