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# Evaluation of Allergen Vaccine Potency

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The development of reliable and clinically relevant potency assays is essential to the practice of safe and effective allergen-specific immunotherapy. Allergen standardization in the United States is based on the establishment of a national reference assigned with a biological potency unit to which manufacturers' products are compared using validated relative potency assays. This ensures, at least with standardized allergen vaccines, comparability between lots used in clinical practice. Recent progress in the ability to measure the specific allergen content of allergen vaccines has led to its application in monitoring consistency and characterizing allergen preparations. More recently, the "major allergen" content of allergen vaccines has become a means to compare extracts from different manufacturers and to recommend immunotherapy dosing regimens. At the same time, qualitative differences exist between manufacturers' products, and most allergen vaccines used in clinical practice are nonstandardized. Therefore, this approach can be confusing and is misleading. The establishment of additional allergen reference standards and the development of reliable, accurate, and clinically relevant potency assays are urgently needed.

## Introduction

The main purpose for determining the potency of allergen vaccines is to ensure their safety and efficacy for allergy immunotherapy. Numerous in vivo and in vitro potency assays have been developed for this purpose, and the suitability of the various potency assays depends on their intended use. For example, distribution of US-licensed standardized allergen vaccines requires the use of US Food and Drug Administration (FDA)-approved reference methods and standards [1]. In addition, potency measurements suitable for evaluating manufacturers' stock concentrates are not necessarily suitable for products that have been diluted or mixed with other allergen products. In these cases, the assays must be modified or optimized to achieve the necessary performance characteristics [2]. Allergen vaccines used in clinical practice are complex antigenic mixtures to which patients demonstrate varying degrees of allergic responses and sensitivities. Thus, for most allergen vaccines, a combination of assays designed to measure both the overall allergenicity as well as specific allergen content is necessary to evaluate their potency.

The earliest attempt for evaluating the potency of allergen vaccines was introduced by Noon [3], who arbitrarily designated 1 gram of pollen as containing 1 million Noon units. An allergenic extract prepared by extracting 1 gram of pollen in 10 mL of extraction fluid would contain 100,000 Noon units. This system assumes that pollen derived from different sources have the same specific activity, which is not the case. Unfortunately, this weight by volume system is still widely used in clinical practice. When it became known that most allergens were proteins, the protein nitrogen unit (PNU) was introduced [4]. One milligram of protein nitrogen is equivalent to 100,000 PNU. The PNU is determined with precipitation of proteins by phosphotungstic acid and measurement of nitrogen by the micro-Kjeldahl method. Alternative assays for measuring total protein content of allergen vaccines are preferable to PNU because of convenience, equipment requirements, sensitivity, and the amount of sample needed. Regardless of the improved precision and accuracy afforded by these total protein assays, it must be remembered that potency estimates based on total protein content are apt to fail because all proteins are not allergenic, and allergens can deteriorate without any change in their protein content.

Biological or in vivo methods for evaluating the potency of allergen vaccines are used for the direct measurement of the total allergenic activity in specific patients and are an established approach for allergen characterization and standardization. Once an accurate biological potency unit is assigned to a standard, it can be applied to assess the clinical safety and efficacy of allergen vaccines in terms of defined doses and to calibrate subsequent manufacturers' batches. The major impact of this approach has been the improved safety and consistency of allergen vaccines, which in turn should reduce the potential for inducing serious adverse reactions in allergic patients.

Laboratory or in vitro methods of determining the potency of allergen vaccines are based on their chemical and immunologic properties, mainly related to their immunoglobulin (Ig)E-binding capacity. Non-IgE–based

assays based on the quantitation of specific allergenic components (eg, major allergen assays) may be useful, but do not necessarily ensure equivalent IgE-binding capacity or biologic potency of different production batches. In cases in which the concentration of a single major allergenic component is predictive of the overall allergenic activity of a product, or when the product is a recombinant or purified allergen, specific allergen assays have proved useful in allergen standardization.

## In Vivo Potency Assays

Quantitative skin testing in sensitized patients is a well-established approach for determining the biological potency of allergen reference standards and estimating relative potencies between allergen vaccines. Two skin test methods have been employed successfully to assign biologic units of potency to reference allergen vaccines. In one method, vaccines are evaluated for their ability to induce a sum of erythema diameters that is equivalent to 50 mm after intradermal titration. The second is related to the wheal size induced by histamine in prick testing.

The $ID_{50}EAL$ method (Intradermal Dilution for 50 mm sum of erythema diameters determines bioequivalent allergy units) is used for assigning potency units to reference preparations in the United States [5,6]. At least 15 highly sensitive adult subjects with a history of allergic disease related to exposure to the allergen of interest are initially selected. Quantitative intradermal skin tests are performed using 0.05 mL of threefold serial dilutions of the prospective reference extract and the dose and the sum of erythema diameters (longest plus midpoint orthogonal diameters) as the response. The dose-response line is generated using four serial threefold dilutions with graded erythema responses ($\Sigma E$), which bracket $\Sigma E = 50$ mm and include the end point where $\Sigma E = 0$. The best-fit regression lines for each subject are calculated using the formula $Y = I + mX$, where $Y$ is the sum of erythema diameters ($\Sigma E$) at each dose, $I$ is the $y$-intercept, $m$ is the slope, and $X$ is the logarithm to the base 3 of dose. If the best-fit line yields a high correlation coefficient ($r > 0.90$) and slope ($m \geq 13$), then the subject sensitivity ($D_{50} = (50-I)/m$) is determined for each test subject. The mean $D_{50}$ and standard deviation (SD) of the mean are calculated, and if the SD is within acceptable limits, the BAU/mL of the prospective reference is calculated using the formula, $BAU/mL = 3 - (14 - \text{Mean } D_{50}) \bullet 100{,}000$.

In European countries, a potency unit based on the dose of allergen vaccine resulting in a skin wheal response comparable in size to the wheal elicited by a 10 mg/mL concentration of histamine dihydrochloride is widely used [7–9]. This unit, originally called the histamine equivalent by prick (HEP), is correlated to the BU (biological units), which is now more widely used to label allergen products in Europe. At least 20 consecutive adults with clinical sensitivity are recruited for prick testing with at least three serial 10-fold dilutions of allergen up to approximately 4 mg of protein or 1 mg of major allergen and histamine dihydrochloride solutions (1 mg/mL and 10 mg/mL). Using the median wheal area or median of mean diameters for each concentration, the slope of the dose-response line according to the log/log model is calculated and the concentration eliciting the same wheal reaction size as that of 10 mg/mL histamine dihydrochloride in the same subject is determined. The median $CH_{10}$ among all test subjects is calculated and is assigned 10,000 BU/mL.

Quantitative skin testing has traditionally played a paramount role in allergen standardization because it is the most efficient method of evaluating allergenic potency directly in sensitized patients. The procedures must follow precise protocols, including patient selection criteria, medications to be withheld prior to skin testing, use of the correct skin test device and technique, and use of appropriate statistical analyses [5–11]. An underlying assumption for using BAUs or BUs as potency units for allergen vaccines is that their efficacy and safety are related to their allergenic activity. This is probably true for traditional allergen vaccines that are produced from native allergens and are qualitatively similar to diagnostic products used for skin testing. However, the chemical modification of allergens by formaldehyde or glutaraldehyde treatment used to manufacture some therapeutic allergen vaccines (ie, "allergoids") can profoundly reduce their allergenicity without changing their immunogenicity [12,13]. Newer generations of hypoallergenic vaccines that employ recombinant technologies, allergen fragments, or peptides may totally lack allergenic activity [14–16]. In these cases, their allergenic potency serves only to indicate their safety profile, and may not be useful in predicting clinical efficacy.

## In Vitro Potency Assays

Once reference vaccines are assigned potency units, subsequent production batches can be compared to the reference by a parallel-line skin test bioassay or a suitably validated in vitro potency assay [6,17]. For those allergen products for which a major allergenic component has been identified and shown to be predictive of relative potency by bioassay, the unit content of these major allergens may serve as the basis for standardization. The FDA has accepted the measurement of Amb a 1 and Fel d 1 content for short-ragweed pollen and cat extracts, respectively, as valid potency assays. For products containing multiple major allergens or when a single allergenic component can not be shown to be predictive of in vivo relative potency, alternative assays must be developed [1].

The IgE–enzyme-linked immunosorbent assay (ELISA) inhibition has proven to be an ideal platform for evaluating allergen preparations [2,18,19]. It requires a well-characterized allergosorbent (solid-phase), a reference serum pool obtained from allergic donors, and a

reference allergen preparation. In allergen standardization, the reference allergen preparation is used to prepare the allergosorbent, and the serum pool, which serves as the source of allergen-specific IgE antibodies, is characterized to show that all relevant IgE specificities are represented. Under ideal conditions, the dose-response curves of the reference and test-allergen preparations are parallel, which provides evidence of qualitative similarity between them. A parallel-line assay employing regression analysis can then be used to calculate the relative potency of the test preparation with respect to the reference. The first step for a typical protocol for conducting a relative potency assay is to coat plastic microtiter wells with an optimal coating dilution of the reference allergen to prepare the allergosorbent. Serial threefold dilutions of the reference- and test-allergen preparations are prepared in an appropriate buffer to serve as inhibitors. Each dilution of inhibitor is mixed with a constant dilution of the allergic serum pool and coincubated in duplicate wells. After incubation, an enzyme-linked anti-human IgE reagent is added to each well, and a chromogenic substrate is added to detect IgE binding in the wells. A microplate reader is used to measure the optical density (OD) of the color that develops, and the percentage of inhibition of binding is calculated for each dilution using the formula: % inhibition = $(A_1 - A_2 / A_1) \times 100$, where $A_1$ is the average OD of the duplicate positive control wells without inhibitor and $A_2$ is the average OD of the wells with inhibitor. Best-fit lines are constructed using at least four points between 10% and 90% inhibition: $y = a = bx$, where, $y$ is the % inhibition, $a$ is the $y$-intercept, $b$ is the slope, and $x$ is the logarithm to the base 3 of the inhibitor concentration. The correlation coefficient of the regression lines must be 0.95 or greater, and the reference and test lines must be parallel at $P = 0.01$ by Student's t test. Using the pooled slope and the two new intercepts, the log3 relative potency is calculated: $(I_t - I_r) / B$, where $I_t$ and $I_r$ are the test and reference $y$-intercepts, respectively, and $B$ is the pooled slope. The relative potency (RP) is given by $10^w$, and potency units can be assigned to the test preparation by multiplying the RP value with the reference potency. In this way, manufacturers can ensure accurate labeling of their products and ensure batch-to-batch consistency of standardized allergen vaccines. Additionally, the assay can be used for stability studies, quality control of raw materials, and in-process applications. ELISA inhibitions using allergen-specific polyclonal [20] or monoclonal [21] IgG antibodies derived from laboratory animals have also been used to estimate relative concentrations of major and minor allergens in allergen preparations.

Novel functional in vitro assays based on mediator release from sensitized basophils have been developed to measure the cellular responses to allergens in addition to their IgE-binding activity. The utility of this assay format has been shown by incorporating monoclonal IgE antibodies specific for birch pollen allergens in mediator release experiments [22]. More recently, the rat basophilic leukemia cell line was transfected with the gene encoding the human high-affinity IgE receptor allowing for the measurement of mediator release employing cells sensitized with allergen-specific human IgE antibodies [23]. Biological assay systems such as these have the advantage over strictly immunochemical ones that only measure the IgE-binding capacity of allergen-derived molecules.

When pure allergens have been isolated and characterized, their concentration in allergen vaccines can be determined using a gel diffusion technique such as radial immunodiffusion or immunoelectrophoresis. Alternatively, when greater sensitivity or specificity is required, two-site immunoassays employing monoclonal antibodies have commonly been used. The major difficulties for these types of assays have been the need for purified reference allergens and monospecific antibodies. The advantages of using monoclonal antibodies for this purpose are their monospecificity, homogeneity, and inexhaustible supply. They can be adapted for use in direct-bind ELISA, two-site immunoassay, or ELISA inhibition formats. Their potential utility for allergen vaccine potency determinations and standardization is restricted only by their fine specificity. Their specificity may be too fine to detect multiple allergenic epitopes of a given allergen molecule, and a mixture of monoclonal antibodies may be required to properly evaluate an allergen preparation.

The radial immunodiffusion (RID) technique of Mancini has been adapted successfully to measure the Amb a 1 content in short ragweed pollen extracts [24], Lol p 1 in perennial ryegrass pollen extracts [25], and Fel d 1 in cat extracts [26]. In each of these cases, the in vitro reactivity of these extracts as determined by specific allergen content correlated with their biological potency. Thus, the Amb a 1 and Fel d 1 contents are the basis for potency labeling of short-ragweed and cat-allergen vaccines, respectively, in the United States. The method of RID is based on the diffusion of antigen from a circular well into a homogeneous gel containing specific antiserum. A ring of precipitated antigen and antibody complexes forms and grows until equilibrium is reached. The diameters of the rings are a function of antigen concentration. RID is relatively simple to perform and requires little or no investment in laboratory equipment. The time required to obtain results, especially if repeated tests are necessary, may be an inconvenience. The detection limits of immunodiffusion assays are in the microgram range and are suitable for measuring allergens in stock concentrates. At dilutions greater than 1:10 v/v of concentrate or with sample preparations in the nanogram range, more sensitive immunoassays must be employed.

When compared to the RID, a typical ELISA can detect allergens at 1000 times lower concentrations. This enhanced sensitivity can be useful when evaluating diluted vaccines, performing stability studies, and measuring allergen levels in environmental samples. The double-bind ELISA, sometimes called the "sandwich" ELISA, overcomes many of the

problems inherent to the direct-bind ELISA and evaluation of complex mixtures of allergens such as crude extracts. A specific antibody referred to as the capture antibody is used to coat microtiter plate wells. Serial dilutions of the allergen containing preparation are added to duplicate antibody-coated wells. An enzyme-labeled second antibody directed toward a different antigen epitope of the captured antigen is added, completing the "sandwich." Data reduction is performed essentially as described for the direct-bind ELISA. The allergen specificity of the double-bind ELISA is determined by the capture antibody, and the performance characteristics of the assay depend on the proper selection of the antibody pair. An optimal combination frequently consists of a monoclonal capture antibody and a polyclonal second antibody to confer maximum specificity and sensitivity. A large number of double-bind ELISAs have been developed for a variety of important allergens [27–37], and because of the increased sensitivity over the traditional RID, this assay format is currently the most widely used for specific allergen measurements in allergen preparations.

The performance characteristics of in vitro potency assays will depend on the assay format, choice of antibody detection reagents, and reference allergen standards. It is often difficult to assess how these variables account for the observed differences in assay results using the same materials, format, or reagents [2,19]. These differences may have several explanations, including the interaction of the allergen with the solid phase, differences in the structural requirements for antigen-antibody binding in the solid and fluid phases, or differences in determinant valency of the target allergens. Identifying the sources of such variability and establishing the clinical utility of in vitro assays will be critical to the validation of allergen potency assays and selection of allergen reference standards [38•,39••].

## Conclusions

The development and application of accurate, sensitive, and clinically relevant assays for evaluating the potency of allergen vaccines are paramount to the quality and consistency of allergy immunotherapy. In recent years, much progress has been made in the analytical approaches used for measuring specific allergens. The sensitivity and specificity of these assays frequently exceed what is necessary for routine evaluations. The use of potency units based on major-allergen content is highly attractive, as it could facilitate communication, allow direct comparisons between allergen products, and define the basis for a unified approach to allergen standardization [40•].

By comparison, allergen vaccines have not qualitatively changed for decades. They remain highly heterogeneous mixtures containing multiple allergenic and nonallergenic components. Mixtures containing cross-reacting, chemically modified, or denatured antigens present additional challenges in assay development and validation. Qualifications of new reference standards and reagents continue to be a major obstacle in implementing assays across laboratories. The FDA has addressed this problem in its allergen standardization program [1]. However, most allergen vaccines used today in clinical practice still are not standardized. The adoption of universal reference standards, reagents, and assay formats is needed if results between laboratories are to be compared. Until then, clinical recommendations based on "major allergen" or arbitrary units derived from nonvalidated assays will continue to be confusing and misleading.

## References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance


1. Morrow KS, Slater JE: **Regulatory aspects of allergen vaccines in the US.** *Clin Rev Allergy Immunol* 2001, 21:141–152.
2. Grier TJ: **Laboratory methods for allergen extract analysis and quality control.** *Clin Rev Allergy Immunol* 2001, 21:111–140.
3. Noon L: **Prophylactic inoculation against hay fever.** *Lancet* 1911, i:1572–1573.
4. May JC, Sih JT, Miller JR, Seligmann EB Jr: **Optimization of parameters in protein nitrogen unit precipitation procedure for allergenic extracts.** *J Allergy Clin Immunol* 1979, 63:87–97.
5. Turkeltaub PC: **Biological standardization.** *Arb Paul Ehrlich Institut* 1997, 91:145–156.
6. Turkeltaub PC, Rastogi SC, Baer H, et al.: **A standardized quantitative skin-test assay of allergen potency and stability: studies on the allergen dose-response curve and effect of wheal, erythema, and patient selection on assay results.** *J Allergy Clin Immunol* 1982, 70:343–352.
7. Malling HJ: **Skin prick testing in biological standardization of allergenic products.** *Arb Paul Ehrlich Institut* 1997, 91:157–163.
8. Dreborg S: **Precision of biological standardization of allergenic preparations.** *Allergy* 1992, 47:291–294.
9. Dreborg S, Sjogren I, Eriksson NE, Einarsson R: **Selection of patients for biological standardization as exemplified by standardization of mugwort, goosefoot and English plantain pollen allergen extracts/preparations.** *Allergy* 1987, 42:485–495.
10. Rabin RL, Slater JE, Lachenbrunch P, Pastor RW: **Sample size considerations for establishing bioequivalence of allergen formulations.** *Arb Paul Ehrlich Institut* 2002, 94:24–33.
11. Casas R, Ferrandiz R, Wihl JA, et al.: **Biologic activity of Dermatophagoides siboney and Blomia tropicalis allergens in exposed and unexposed mite-allergic individuals. Effect of patient selection on the biologic standardization of mite extracts.** *Allergy* 1999, 54:392–396.
12. Cromwell O, Weber B, Kahlert H, Fiebig H: **Standardization in quality control of modified end-products.** *Arb Paul Ehrlich Insitut* 1999, 93:93–100.
13. Ibarrola I, Sanz ML, Gamboa PM, et al.: **Biological characteristization of gluaraldehyde-modified Parietaria judaica pollen extracts.** *Clin Exp Allergy* 2004, 34:303–309.
14. Ferreira F, Briza P, Infuhr D, et al.: **Modified recombinant allergen for safer immunotherapy.** Inflamm Allergy Drug Targets 2006 5:5–14.
15. Niederberger V, Valenta R: **Molecular approaches for new vaccines against allergy.** *Expert Rev Vaccines* 2006, 5:103–110.
16. Alexander C, Kay AB, Larche M: **Peptide-based vaccines in the treatment of specific allergy.** *Curr Drug Targets Inflamm Allergy* 2002, 1:353–361.

17. Turkeltaub PC: Allergen vaccine unitage based on biological standardization: clinical significance. In *Allergen and Allergen Immunotherapy*. Edited by Lockey R, Bukantz SC. New York: Marcel Dekker; 1999:321–340.
18. Food and Drug Administration: ELISA competition assay: quantitative determination of relative potency of allergenic extracts. In *Methods of the Allergenic Products Testing Laboratory*. CBER/FDA; 1998.
19. Esch RE: Allergen source materials and quality control of allergenic extracts. *Methods* 1997, 13:2–13.
20. Dobrovolskaia E, Gam A, Slater JE: Competition enzyme-linked immunosorbent assay (ELISA) can be a sensitive method for the specific detection of small quantities of allergen in a complex mixture. *Clin Exp Allergy* 2006, 36:525–530.
21. Afferni C, Pini C, Tinghino R, et al.: Use of monoclonal antibodies in the standardization of Parietaria judaica allergenic extracts. *Biologicals* 1995, 23:239–247.
22. Kaul S, Scheurer S, Danz N, et al.: Monoclonal IgE antibodies against birch pollen allergens: novel tools for biological standardization of allergens. *J Allergy Clin Immunol* 2003, 111:1262–1268.
23. Vogel L, Luttkopf D, Hatahet L, et al.: Development of a functional in vitro assay as a novel tool for the standardization of allergen extracts in the human system. *Allergy* 2005, 60:1021–1028.
24. Baer H, Godfrey H, Maloney CJ, et al.: The potency and antigen E content of commercially prepared ragweed extracts. *J Allergy* 1970, 45:347–354.
25. Baer H, Maloney CJ, Norman PS, Marsh DG: The potency and Group I antigen content of six commercially prepared grass pollen extracts. *J Allergy Clin Immunol* 1974, 54:157–164.
26. Anderson MC, Baer H: Allergenically active components of cat allergen extracts. *J Immunol* 1981, 127:972–975.
27. Duffort O, Calabozo B, Gonzalez R, et al.: Monoclonal antibody-based ELISA to quantify the major allergen of Cynodon dactylon (Bermuda grass) pollen, Cyn d 1. *Int Arch Allergy Immunol* 2004, 135:277–283.
28. Ramirez J, Obispo TM, Duffort D, et al.: Group 5 determination in Pooideae grass pollen extracts by monoclonal antibody-based ELISA. Correlation with biologic activity. *Allergy* 1997, 52:806–813.
29. Jimeno L, Duffort O, Serrano C, et al.: Monoclonal antibody-based ELISA to quantify the major allergen of Artemisia vulgaris pollen, Art v 1. *Allergy* 2004, 59:995–1001.
30. Arilla MC, Eraso E, Ibarrola I, et al.: Monoclonal antibody-based method measuring olive pollen major allergen Ole e 1. *Ann Allergy Asthma Immunol* 2002, 89:83–89.
31. Arilla, MC, Ibarrola I, Garcia R, et al.: Quantification of the major allergen from cypress (Cupressus arizonica) pollen, Cup a 1, by monoclonal antibody-based ELISA. *Int Arch Allergy Immunol* 2004, 134:10–16.
32. Arilla MC, Ibarrola I, Mir A, et al.: Development of a sandwich-type ELISA for measuring Pla a 1, the major allergen of Platanus acerifolia pollen. *Int Arch Allergy Immunol* 2005, 138:127–133.
33. Yi FC, Lee BW, Cheong N, Chua KY: Quantification of Blot t 5 in mite and dust extracts by two-site ELISA. *Allergy* 2005, 60:108–112.
34. Jimeno L, Duffort O, Serrano C, et al.: Monoclonal antibody-based ELISA to quantify the major allergen of Artemisia vulgaris pollen, Art v 1. *Allergy* 2004, 59:995–1001.
35. Arilla MC, Gonzalez-Rioja R, Ibarrola I, et al.: A sensitive monoclonal antibody-based enzyme-linked immunosorbent assay to quantify Parietaria judaica major allergens, Par j 1 and Par j 2. *Clin Exp Allergy* 2006, 36:87–93.
36. Portnoy J, Pacheco F, Upadrasthta, Barnes C: A double monoclonal antibody assay for the Alternaria allergen GP70. *Ann Allergy* 1993, 71:401–407.
37. Vailes L, Sridhara S, Cromwell O, et al.: Quantitation of the major fungal allergens, Alt a 1 and Asp f 1, in commercial allergenic products. *J Allergy Clin Immunol* 2001, 107:641–646.
38.• Grier TJ, Hazelhurst DM, Duncan EA, et al.: Major allergen measurements: sources of variability, validation, quality assurance, and utility for laboratories, manufacturers, and clinics. *Allergy Asthma Proc* 2002, 23:125–131.
This paper discusses the sources of variability and differences in major allergen concentrations reported by different testing laboratories.
39.•• Esch RE: Manufacturing and standardizing fungal allergen products. *J Allergy Clin Immunol* 2004, 113:210–215.
This paper discusses the difficulties associated with selecting reference standards and potency assay methods when significant qualitative differences exist among allergen vaccines.
40.• van Ree R, Dorpema JW, Veiths S: Allergy vaccines: a need for standardization in mass units of major allergen. *Pharmeuropa Bio* 2005, 1:27–30.
This paper discusses the initiative in Europe to facilitate the development of international reference standards and standardization protocols for expressing vaccine potency by major allergen content.