Major Allergen Measurements: Sources of Variability, Validation, Quality Assurance, and Utility for Laboratories, Manufacturers, and Clinics

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ABSTRACT

*The isolation and characterization of prominent allergenic proteins or glycoproteins is an important step in the development of allergenic extracts exhibiting improved definition, consistency, and clinical utility. Quantitative analyses specific for major allergenic components currently are being performed in numerous corporate and academic laboratories but have not been validated within or across laboratories in a systematic manner. In our laboratory, validation of double-bind (sandwich) ELISA assays for a diverse group of major allergens or extract components revealed a number of critical assay variables and reagent incubation conditions that directly influenced the precision, accuracy, specificity, and robustness of these tests. Data from ELISA methods for six allergens (*Dermatophagoides farinae *Der f 1,* Alternaria *Alt a 1, dog albumin, dog Can f 1, fire ant Sol i 3, and yellow jacket venom Ves 5) showed that up to twofold differences in results were observed when analysts or microplates were varied. Analyses of dog allergens using multiple reagents and concentrations indicated that twofold variations in results also can be produced by distinct combinations of materials or incubations from different assay steps. Data from Can f 1 and egg white analyses produced up to fivefold differences in antigen concentrations based on changes in the capture antibody source (mouse monoclonal versus rabbit polyclonal) or storage buffer. These results suggest that differences in major allergen concentrations reported by different testing laboratories may be related to assay differences as well as extract variations and raise questions as to the accuracy of major allergen concentrations and therapeutic dose recommendations reported at regional and national allergy meetings. Validated double-bind ELISA methods may be well suited for consistency monitoring and standardization of extracts provided that reference materials, reagent qualifications, and interlaboratory comparability are defined precisely.* (Allergy and Asthma Proc 23: 125–131, 2002)

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Allergenic extracts are complex mixtures of macromolecules (proteins, glycoproteins, and polysaccharides) and low molecular weight constituents (pigments and salts).[1] In many cases, individual molecules are present at concentrations approaching saturation levels. As a result, it is not uncommon for some allergenic or nonallergenic components to influence the biological activities or stabilities of specific allergens by direct or indirect interactions. The compositional complexities of most extracts, combined with the broad spectrum of human responses to many provoca-

tive allergens, pose a significant challenge for allergen manufacturers to produce stable extracts of suitable compositions for diverse patient populations.[2] The biochemical properties of proteins and glycoproteins identified as major contributors to immunoglobulin E (IgE)–mediated hypersensitivities can be determined using conventional laboratory methods and promote the development of extract formulations possessing improved definition, consistency, potency, stability, and clinical utility.[3]

Interest in major allergens has increased dramatically in recent years. Major allergen levels are now considered by many allergy practitioners as important markers of extract quality and the future of allergen standardization.[4,5] Quantitative test methods specific for major allergens are developed and performed in numerous laboratories, and the results of these tests are being reported at regional and national allergy meetings along with recommendations for therapeutic doses in terms of micrograms of major allergens. However, generally, it is not known that the allergen concentrations derived from these tests do not always correlate directly with current extract strength units, that test methods for target allergens often have very little in common with one another, and that the quality of these results is compromised by the lack of standardization and validation of major allergen assays across multiple laboratories. In fact, the worst case scenario, different laboratories using different materials and methods to measure the same major allergen, appears to be more common than one in which similar components, procedures, and data analyses are used.

MAJOR ALLERGEN ASSAYS

Differences among major allergen methods measuring the same analyte may include the assay format or configuration, the rare antibody and allergen reagents that define the specificity of the assay, the methods of calibration and quantitation (including purified allergen primary standards and reference extracts as secondary routine use standards), and the actual performance characteristics of these procedures based on classical assay validation criteria (methodological features: sensitivity, specificity, and linearity; performance features: precision, reproducibility, accuracy, robustness, and stability).[6] At present, only two assays for major allergens have been validated across multiple laboratories and are recognized by the U.S. Food and Drug Administration, the radial immunodiffusion (RID) assays used to standardize cat extracts based on Fel d 1 content and short ragweed based on antigen E (Amb a 1) levels.[7,8] All other major allergen assays, particularly the sensitive, high-profile double-bind ELISA methods, have not been subjected to the same degree of scrutiny, and often contain essential components prepared and used by a given laboratory, which are unavailable to other investigators.

In the double-bind or sandwich ELISA format (Fig. 1), major allergens present in a test sample are bound by two antibodies, one adsorbed to the microplate solid phase and a second labeled with biotin (vitamin H). Biotinylated antibodies are detected using the egg white protein avidin (which binds to biotin with the strongest interaction in nature) conjugated to the enzyme alkaline phosphatase (AP), and colorimetric reactions produced by the phosphatase substrate paranitrophenyl phosphate (pNPP) are directly proportional to the concentration of major allergen present in the sample.

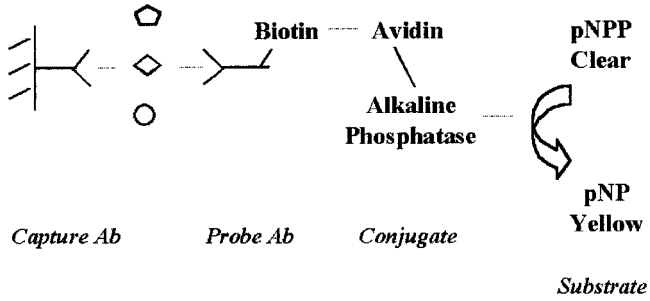

Figure 1. *Double-bind (sandwich) ELISA configuration.*

Double-bind ELISA reactivities are controlled by several critical factors. The capture and probe antibodies may recognize similar or distinct antigenic structures, sequences, or conformations.[9] The use of mouse monoclonal IgG antibodies with distinct specificities is very popular, but polyclonal rabbit IgG antibodies specific for major allergens are effective also.[10] With two antibodies involved in antigen-binding interactions, two independent binding sites must be present on allergen molecules to yield positive reactions. In general, double-bind ELISA assays are extremely sensitive, often achieving nanogram-level sensitivities for major allergens. Although these methods are capable of detecting 100- to 1000-fold lower antigen concentrations than typical RID or ELISA inhibition procedures, dose-response relationships for purified allergens, reference extracts, and test samples must be highly parallel in order to produce accurate results. Deviations from parallel reactions result in assays in which the results obtained vary considerably depending on the dilution factors used for each sample.

These features raise a number of questions and concerns pertaining to the accuracy and reliability of major allergen measurements, including assay variability within a laboratory and between laboratories, the expected reproducibility and precision of these methods, qualification procedures and criteria for new antibody and allergen reagents, and the comparabilities of different assay formats for the same major allergen. To address these concerns, information is presented in this study to identify sources of variability observed during the optimization and implementation of specific ELISA assays for major allergens, to establish the performance characteristics required for an effective quantitative test for these components, and to propose recommendations regarding the development and use of major allergen information for extract manufacturers, laboratories, and clinics.

EXPERIMENTAL PROCEDURES

Allergenic extracts were obtained from current Greer or Hollister-Stier (Spokane, WA) *in vivo* and *in vitro* product inventories. Rabbit antiserums directed against dog albumin, dog Can f 1, or yellow jacket venom Ves 5 were produced in-house by conventional immunization protocols using Freund's complete and incomplete adjuvants. Rabbit anti-Alt a 1 (hypoallergen) antiserum was purchased from Dr. Hari Vijay (Health Canada, Ottawa, Ontario, Canada). Mouse monoclonal antibodies to dust-mite *D. farinae* Der f 1 (6A8-B10) and dog Can f 1 (6E9) were obtained from Dr. Martin Chapman (Indoor Biotechnologies, Charlottesville, VA). Monoclonal antibodies to fire ant *Solenopsis invicta* Sol i 3 (AMS32 and MS2) were provided by Dr. Donald Hoffman (East Carolina University, Greenville, NC). Microtiter plates were sourced from Dynex Technologies (Immulon 2 HB; Chantilly, VA), Costar (Cambridge, MA), and Nunc (MaxiSorp, Roskilde, Denmark). The ELISA probe reagents goat anti-rabbit IgG-AP, AP-labeled avidin (AP-avidin), and pNPP were acquired from Sigma (St. Louis, MO), Zymed (South San Francisco, CA), and Amresco (Solon, OH), respectively. Calf skin gelatin was obtained from ICN (Aurora, OH). Dialysis membranes (SpectraPor-1; 6000–8000 molecular weight cutoff) were a product of Spectrum (Rancho Dominguez, CA). Buffer salts, detergent (Tween 20), and bovine serum albumin were purchased from Sigma.

Serum antibodies (1–2 mL) were concentrated by precipitation with equal volumes of saturated ammonium sulfate for 4 hours at ambient temperatures (20–25°C), followed by dialysis for 12–24 hours at ambient temperature into 2–4 L of phosphate (pH 7.4, phosphate-buffered saline [PBS]) or borate (pH 8.4) buffers containing 0.02% sodium azide. Biotinylated antibodies were prepared by incubating *N*-hydroxysuccinimide–activated biotin (NHS-LC-Biotin; Pierce, Rockford, IL) and antibodies at defined biotin/protein molar ratios in borate buffer for 4 hours at 20–25°C, with reactions terminated by dialysis into 4–8 L of cold (2–8°C) PBS for at least 24 hours at 2–8°C.

Double-bind ELISA procedures were conducted with microtiter plates coated at 2–8°C or 20–25°C (ambient temperatures) with all subsequent incubations at 20–25°C. Plates were coated with serums or antibodies (1:100–1:5000 dilutions) in carbonate (pH 9.6) or phosphate buffers for 12–24 hours and then blocked (if necessary) with 1% bovine serum albumin for 2 hours. Coated plates were then incubated with reference or test antigens at variable concentrations (starting dilutions 1:5–1:2430, two- or threefold serial dilutions) in a high-salt PBS buffer (PBS-THS, PBS–0.5% Tween 20–0.29 M of NaCl) for 3–6 hours, followed by biotinylated antibodies in PBS buffer containing 0.5% Tween 20 and 0.5% gelatin (PBS-T-Gel) for 3–15 hours. Bound antibodies were detected by addition of AP-avidin (1:4000 in PBS-T-Gel for 1 hour) and pNPP (1.0 mg/mL in diethanolamine buffer, pH 9.8 for up to 1 hour). Absorbances were measured at 405 and 600 nm using a Bio-Tek ELx808 (Winooski, VT) microplate reader. Rates of reaction were calculated by subtracting absorbances from early development times from those of later times, and standard curves constructed from these data (absorbance differences versus log concentration of reference antigen) were analyzed by linear regression analyses using a spreadsheet file to produce assay results for test sample dilutions. Mean major allergen concentrations were determined using all dilutions producing absorbances within those of the reference extract range possessing the highest correlation coefficients.

ELISA inhibition and RID assays for Alt a 1 were conducted at ambient temperatures. ELISA plates (Costar) were coated with purified Alt a 1 diluted 1:2000 in carbonate buffer for 12–15 hours and then incubated with rabbit anti-Alt a 1 serum (1:125,000 dilution) in the presence of varying concentrations of purified Alt a 1 (reference) or *Alternaria* extracts (test) in PBS-THS for 4–6 hours. Bound anti-Alt a 1 molecules were detected using goat anti-rabbit IgG-AP (1:4000 dilution in PBS-T-Gel for 15 hours) and pNPP. Absorbance measurements and rates of reaction were determined as described previously for double-bind ELISA. Extents of inhibition were calculated for all test and reference samples, and relative potencies were determined using a parallel-line bioassay algorithm. RID analyses were performed with 1% agarose gels containing 0.16% rabbit anti-Alt a 1 concentrate (ammonium sulfate resuspension). Samples (10 μL) were added to 3-mm holes punched in the gel and allowed to incubate for 72 hours. After removal of excess proteins from the gel by repeated washing and blotting with 0.15 M of NaCl and distilled water, precipitin circles were visualized by Coomassie blue staining and measured with a ruler or calipers. Relative reactivities for RID data were calculated using the same parallel-line bioassay method used for ELISA inhibition.

SINGLE VARIABLES

Initial studies focused on variations in assay results due to individual variables known to exist in many laboratories, such as the analyst. Five different double-bind ELISA assays specific for well-known major allergens (*Alternaria* Alt a 1, *D. farinae* Der f 1, Dog Can f 1, Fire ant Sol i 3, and Yellow jacket venom Ves 5) were performed under identical conditions by each of three analysts experienced in these procedures. All assays were conducted using the optimal materials, reagents, and incubations established during independent validation studies. The results of these experiments (Table I) revealed that mean microgram per milliliter values ($\pm$1 standard deviation) were within a factor of 2, with %CV ranging from 7 to 40%. The highest variabilities were observed with the Sol i 3 assay, which used monoclonal antibodies with distinct specificities to capture and detect antigen.

A second variable that may influence the consistency of ELISA results between laboratories is the microtiter plate.

TABLE I

Variability Due to Analyst in Double-Bind ELISA Assays

Analyte	Extract	Mean ± 1 SD (μg/mL)	%CV
Alternaria Alt a 1	1:10 wt/vol Aqueous	24.1–37.5	21.8
	1:20 wt/vol Glycerinated	13.1–15.0	7.0
D. farinae Der f 1	10,000 AU/mL	80.4–139	26.7
	Freeze-dried	123–184	19.9
Dog Can f 1	1:10 wt/vol Aq epithelia	0.605–0.827	15.5
	1:50 wt/vol Glyc hair/dander	218–288	14.0
Fire ant Sol i 3	1:10 wt/vol Aqueous	2.11–4.97	40.4
	1:20 wt/vol Glycerinated	0.677–1.47	37.0
Yellow jacket Ves 5	Glyc lot 1	152–208	15.4
	Glyc lot 2	109–153	16.8

Average %CV = 20%.

Different laboratories often use microplates produced by different manufacturers based on personal experiences or preferences. It is not unusual for microplates from different sources to possess distinct surface chemistries or binding properties. These differences are of particular concern in double-bind ELISA methods, and it is likely that surface differences that may alter the efficiency or reproducibility of capture antibody adsorption or the availability of antigen-binding sites can contribute significantly to interlaboratory variations in dose-response characteristics and major allergen values. To examine this issue, the primary analyst for each of the methods described in Table I performed these assays on three different microplate products (Dynex Immulon 2 HB, Costar, and Nunc MaxiSorp) used frequently for analyses of allergen extract compositions or potencies. As shown in Table II, variabilities due to microplate were slightly higher (23% overall) compared with differences due to analyst (20%, Table I). The assays exhibiting the largest differences were Der f 1 (mouse monoclonal antibodies as capture and probe reagents) and Can f 1 (mouse monoclonal capture antibody and rabbit polyclonal probe antibody).

MULTIPLE OR INTERACTIVE VARIABLES

Single assay variables such as analysts and microplates are relatively easy to identify and investigate. By comparison, interactive or hidden variables are considerably more difficult to detect and may have a more pronounced effect on assay results. Three examples of variabilities caused by specific combinations of assay parameters are presented in the following text. It is interesting to note that all three cases involve the coating step, supporting the notion that many of the essential properties of major allergen methods are linked to the production of consistent and effective antigen capture surfaces. The first example revealed a close interaction between capture antibodies and biotinylated antibody dilutions during the qualification of a new capture antibody in an ELISA assay for dog albumin. In these studies, two lots of rabbit anti-dog albumin capture antibody and two concentrations of biotinylated rabbit anti-dog albumin antibody were examined with two different lots of 1:10 wt/vol aqueous dog epithelia extracts. The results of these experiments (Table III) showed that at the

TABLE II

Variability Due to Microplate in Double-Bind ELISA Assays

Analyte	Extract	Mean ± 1 SD (μg/mL)	%CV
Alternaria Alt a 1	1:10 wt/vol Aqueous	20.9–34.3	24.2
	1:20 wt/vol Glycerinated	9.8–17.0	26.8
D. farinae Der f 1	10,000 AU/mL	60.2–161	41.4
	Freeze-dried	135–175	12.9
Dog Can f 1	1:10 wt/vol Aq epithelia	0.60–0.84	16.4
	1:50 wt/vol Glyc hair/dander	191–358	30.5
Fire ant Sol i 3	1:10 wt/vol Aqueous	1.84–3.18	26.8
	1:20 wt/vol Glycerinated	0.88–1.14	13.2
Yellow jacket Ves 5	Glyc lot 1	146–225	21.2
	Glyc lot 2	113–167	19.2

Average %CV = 23%.

TABLE III

Variability Due to Capture Antibody Lot + Biotinylated Antibody Dilution in Dog Albumin Double-Bind ELISA Assay

Biotinylated Antibody Dilution	Dog Extract Lot No.	Mean ± 1 SD (μg/mL)		
		Capture Antibody Lot A	Capture Antibody Lot B	% of Lot A
1:5000	1	546–656	490–654	90–100
	2	259–645	289–589	91–112
1:1000	1	726–1040	539–717	69–74
	2	645–959	382–764	59–80

optimal dilution of biotinylated antibody (1:5000), a new capture antibody (lot B) detected allergen levels similar to those observed using the current coating reagent (lot A). However, at a higher concentration of biotinylated antibody (1:1000), results with lot B yielded values that were 20–40% lower than those determined with lot A. These data illustrate the importance of including multiple concentrations and lots of reagents in assay optimization and quality management (reagent qualification) procedures.

A second example of interactive variables was found between capture antibody source and microplate location for a dog Can f 1–specific ELISA. The use of capture antibodies from a variety of sources is not uncommon for many laboratories performing similar tests, and differences in major allergen content of extract samples may be sensitive to the specificity or avidity of the binding interactions with these reagents.[11] It also is assumed by many that ELISA reactivities are comparable across all locations on a microplate, but investigators experienced in the development and validation of ELISA methods, particularly double-bind ELISA assays with extremely low (nanogram) limits of quantitation, have learned that this is not always the case. In the Can f 1 studies, assays using allergen-specific mouse monoclonal or rabbit polyclonal capture antibodies were optimized, calibrated (using purified Can f 1), and validated independently and were examined at two plate locations (left half, columns 1–6 and right half, columns 7–12) with two types of dog extract (epithelia and hair/dander). The resulting data (Table IV) indicated that for the epithelial extract, both mouse and rabbit capture reagents produced similar allergen concentrations on both sides of the plates. The hair/dander extract with mouse antibody coating also yielded comparable results on the left and right sides of the plate and were related closely to results on the left sides of plates coated with rabbit antibody. However, hair/dander extract values were significantly lower on the right sides of the rabbit antibody–coated plates compared with the left sides. These data revealed that unexpected differences in plate surfaces may produce noticeable changes in capture antibody properties and suggested that antibodies from different sources may not exhibit optimal solid-phase adsorptions or assay performance characteristics on the same microplate surfaces. Investigations of different microplates with the rabbit anti-Can f 1 capture antibody are warranted to increase the robustness of allergen quantitation for this assay.

Another example of a subtle difference in materials or reagents that translates into considerable variations in test results can be found with storage buffers used for capture antibodies in the egg white double-bind ELISA assay. The selection of a solution formulation for most biochemical reagents usually involves conditions (materials and concentrations) that provide optimal stability and compatibility for the method of use. The potential for macromolecules such as antibodies to change their three-dimensional conformations in response to changes in their environments is well documented.[12,13] These changes may produce differences in activity and specificity if the regions undergoing physical or chemical modifications are involved in ligand-binding interactions or are altered on immobilization onto a micro-

TABLE IV

Variability Due to Capture Antibody Source + Plate Location in Dog Can f 1 Double-Bind ELISA Assay

Dog Extract	Capture Antibody Source	Mean ± 1 SD (μg/mL)		
		Plate Columns 1–6	Plate Columns 7–12	% of Columns 1–6
Epithelia	Mouse	0.60–0.81	0.56–0.78	93–96
	Rabbit	0.57–0.80	0.62–0.70	88–109
Hair/dander	Mouse	215–287	179–283	83–99
	Rabbit	234–374	40–142	17–38

plate solid phase. In the egg white studies, rabbit anti-chicken egg white antibodies (specific primarily for ovalbumin) dialyzed and stored in two different buffers at 2–8°C (borate, pH 8.4, and phosphate, pH 7.4) were used as capture antibodies to assess the concentrations of egg white components in three extracts (chicken egg white, whole chicken egg, and chicken egg yolk). The expected order of reactivity (egg white > whole egg > egg yolk, based on the relative proportions of egg white components in these extracts) was observed in assays using capture antibodies in phosphate buffer (Table V). However, the same capture antibody stored in borate buffer did not reveal these differences but resulted in threefold differences in microgram per milliliter values for whole egg extracts and fivefold differences in egg yolk extract reactivities. These data showed that storage conditions may play a critical role in the expression of specific capture antibody structures or properties and supported the notion that relatively minor changes in the formulation of a key assay reagent can exert a strong influence on the specificity of ELISA methods for major allergens.

ASSAY FORMAT DIFFERENCES

Quantitative methods for major allergens developed and performed in different laboratories often use distinct assay formats and reagents. Objective comparisons between these procedures and reagents are conducted occasionally but usually are not reported or shared with other investigators. As a result, the potential exists for differences in major allergen values reported by different laboratories to be related to assay variations rather than extract inconsistencies or deficiencies. To investigate the influence of assay configuration on major allergen reactivities, a single antibody preparation has been used to detect *Alternaria* Alt a 1 antigens in extracts prepared from four distinct *A. alternata* strains by four different formats, three quantitative procedures (RID, ELISA inhibition, and double-bind ELISA), and one semiquantitative method (sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting).[14] The results of these tests (Table VI) indicated that no consistent relationships exist among the four extracts examined by ELISA and RID assays. The extent of variability due to assay format can be assessed by comparing the reactions observed with extract 4 with those found with reference extract 1. Extract 4 possessed 60% higher potency compared with extract 1 by RID but exhibited only 50% of the potency of extract 1 by ELISA inhibition and expressed virtually no reaction by double-bind ELISA. Immunoblot results for intact Alt a 1 molecules (30- to 35-kDa bands) agreed most closely with RID results, suggesting that these methods may detect related antigenic structures. It is clear from these results that the concentrations of Alt a 1 determined for *Alternaria* extracts depend directly on the test method used for these analyses.

CONCLUSIONS

The results described in this summary indicate that major allergen values within 25% of one another are not likely to be significantly different, based on the documented precision of most double-bind ELISA methods performed by one or multiple analysts. However, the use of different microtiter plates, reagents, or assay formats are more likely to produce variations in test sample results. Critical comparisons of antibodies from several sources (if available) in a variety of combinations may elucidate the strengths and deficiencies of multiple assay formats. Inconsistencies across a given plate can be addressed by examining microplate source, buffers or diluents, and incubation conditions. To distinguish differences caused by assay or reagent characteristics from those linked directly to product compositions, it may be necessary to include additional information such as the testing laboratory, assay type and configuration, and antibody source(s) with all major allergen test results. Because of the likelihood of encountering hidden or interactive variables in major allergen determinations, the design and execution of qualification experiments are critical for confirming optimal conditions and smooth replacements of new materials or reagents over extended periods of times. Monitoring or tracking of the reactivities of product lots and control samples, reagent properties, and dose-response curves for assay standards is an effective means of identifying potential drifts in assay performance and implementing suitable corrections.

TABLE V

Variability Due to Capture Antibody Storage Buffer in Egg White Double-Bind ELISA Assay

Capture Antibody Storage Buffer	Extract	Mean ± 1 SD (μg/mL)	% of Egg White (μg/mL)
Borate	Egg white	3416–3830	100
	Whole egg	3381–5789	88–169
	Egg yolk	2660–3798	69–111
Phosphate	Egg white	2044–3888	100
	Whole egg	1243–2073	32–101
	Egg yolk	477–757	12–37

TABLE VI

Variability Due to Assay Format in Alternaria Alt a 1 Analyses

Extract/Strain	Relative Potency vs. Extract/Strain #1			Immunoblot Reactivity
	RID	ELISA Inhibition	Double-Bind ELISA	
1	1.00	1.00	1.00	Moderate (2+)
2	0.26	0.73	0.17	Faint (+/−)
3	0.00	0.22	0.043	Faint (+/−)
4	1.60	0.52	0.004	Strong (4+)

The development of sensitive quantitative methods for prominent allergenic proteins is a fundamental component of current efforts dedicated to advancing the quality and consistency of allergen extracts and to improving their use as diagnostic and therapeutic agents in the allergy clinic. Close collaborations among manufacturers, laboratories, and clinics are essential to the achievement of these goals. Analytical laboratories develop the tools and techniques necessary to ensure that a given method yields reliable and predictable results. Allergen manufacturers optimize raw materials and extraction, processing, and storage conditions, which promote the desired compositions and properties of these products. Clinicians contribute the outcomes of skin testing and immunotherapy procedures with patients who have allergies. In addition, it is important for manufacturers and allergy professionals to share information and first-hand knowledge addressing the stability and compatibility of extracts as well as the relationships between current dose units and major allergen levels.

In spite of advances made within a single laboratory, it is expected that the selection of optimal assay reagents and their conditions of use may continue to vary from one laboratory to another. In addition, the physical and immunochemical properties of critical reagents currently used for major allergen measurements (antibody preparations, purified allergen, or reference extract standards) and the methods used for assay development (calibrations with purified allergens and reference extracts, validation of optimal materials and conditions, and qualifications of replacement reagents) are not standardized or performed according to consistent guidelines and specifications. Based on numerous studies investigating the impact of reagent differences on assay results, including the data described in this study, it is likely that assays from different laboratories for the same allergenic protein may produce very different results, particularly those using a sensitive test format such as double-bind ELISA.[11,15,16] The availability of common primary (pure allergen) or secondary (reference extract) standards for these methods would permit direct comparisons of test results and reagent/format properties and represent an important step in the standardization of extracts based on major allergen content. However, the true comparability of these tests must await critical, independent comparisons of multiple standard materials, assay procedures, reagent combinations, and performance characteristics. Until these studies are completed, the accuracy and reliability of major allergen values reported by different groups may remain in doubt.

REFERENCES

1. Ipsen H, Klysner SS, Larsen JN, et al. Allergenic extracts. In Allergy Principles and Practice, 4th ed. Middleton E, Reed CE, Ellis EF, et al (Eds.). St. Louis, MO: Mosby-Year Book, Inc., 529–553, 1993.
2. Baldo BA. Standardization of allergens. Examination of existing procedures and the likely impact of new techniques on the quality control of extracts. Allergy 38:533–546, 1983.
3. Dreborg S, and Einarsson R. The major allergen content of allergenic preparations reflects their biological activity. Allergy 47:418–423, 1992.
4. Chapman MD, Esch R, De Vries Y, and Liu D. Position statement: the use of standardized allergen extracts. J Allergy Clin Immunol 99:583–586, 1997.
5. Nelson HS. The use of standardized extracts in allergen immunotherapy. J Allergy Clin Immunol 106:41–45, 2000.
6. Department of Health and Human Services, Food and Drug Administration. International Conference on Harmonization guidelines on the validation of analytical procedures. Fed Regist 62:27464–27467, 1997.
7. Methods of allergenic products testing laboratory. Center for Biologics Evaluation and Research, Food and Drug Administration Docket No. 94N-0012, 1993.
8. Kordash TR, and Williamson SL. Standardization of allergenic extracts: a manufacturer's perspective. Allergy Proc 14:201–206, 1993.
9. Chapman MD, Vailes LD, and Ichikawa K. Immunoassays for indoor allergens. Clin Rev Allergy Immunol 18:285–300, 2000.
10. Aalberse RC, and van Ree R. Monoclonal antibody assays for allergens: pick your antibodies with care! J Allergy Clin Immunol 99: 583–586, 1997.
11. Batard T, Bukovec F, Berrouet C, et al. Demonstration of a partially cryptic epitope of the major cat allergen Fel d 1: consequences for mAb-based standardization of cat extracts. J Allergy Clin Immunol 106:669–676, 2000.
12. Kabat EA. Structural Concepts in Immunology and Immunochemistry. New York: Holt, Rinehart and Winston, 1976.
13. Yelton DE, and Scharff MD. Monoclonal antibodies: a powerful new tool in biology and medicine. Ann Rev Biochem 50:657–680, 1981.
14. Grier TJ, Alexandre MD, Crenshaw RC, et al. Alt a 1 determinations comparing five assay formats produce different results. J Allergy Clin Immunol 99:S81, 1997.
15. Renstrom A, Gordon S, Hollander A, et al. Comparison of methods to assess airborne rat and mouse allergen levels. II. Factors influencing antigen detection. Allergy 54:150–157, 1999.
16. Liu T, and Lin Y. The epitope stability of group 1 and group 2 allergens in mite extracts. Ann Allergy Asthma Immunol 80:177–183, 1998. □