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Allergen Source Materials and Quality Control of Allergenic Extracts

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Allergen extracts are prepared from a wide variety of source materials including pollens, fungi, arthropods, animal danders, foods, and dusts. The composition of allergen extracts can vary depending on the allergen source, manufacturing process, and storage conditions. Allergen-specific immunoglobulin E (IgE) assays and skin tests employ a variety of allergen-containing reagents that confer specificity on the test. Given that the allergen source materials are heterogenous mixtures of proteins, glycoproteins, carbohydrates, and other substances that are not allergenic, it is not unexpected that variability exists between test results obtained with different allergen-containing reagents. Variability within a single manufacturer's allergen product can be controlled by using reproducible extraction and processing procedures, single large lots of allergen source materials, and solid-phase supports. These controls do not, however, ensure the consistency of products between manufacturers or laboratories because allergen source materials, manufacturing procedures, and acceptance criteria for allergen reagents may vary.

Hundreds of allergen-containing reagents are routinely employed for skin testing and immunoglobulin E (IgE) antibody assays. Allergen products are classified by the manufacturer into different allergen groups, designated by a coding system (e.g., W1 or weed 1 for short ragweed or *Ambrosia artemisiifolia* pollen). Different manufacturers may assign a different code for the same allergen or they may use the same code to define allergen products derived from different species of the same genus or different allergen sources all together. Thus, the use of this type of coding system to assign allergen specificity should be avoided to minimize confusion when comparing products from different manufacturers. Allergen specificities should include the complete allergen name (genus and species), and each manufacturer should make this available when requested. The WHO/IUIS Allergen Nomenclature Subcommittee, an international effort of scientists to promote uniformity in allergen nomenclature, provides information regarding primary allergen sources and characterized purified allergens and maintains a detailed list of currently characterized purified allergens that are recognized by the organization. Allergens are designated according to the accepted taxonomic name of their source, using the first three letters of the genus, space, the first letter of the species, space, and an Arabic number. The numbers are assigned on the basis of their order of identification, and the same number is generally used to designate homologous allergens of related species: Der p 1 refers to the first dust mite allergen identified from *Dermatophagoides pteronyssinus* and Der f 1 refers to the homologous allergen from *Dermatophagoides farinae*. The recently revised nomenclature for allergens includes a proposed system for allergen genes, mRNAs, cDNAs, and recombinant and synthetic peptides of allergenic interest (1).

ALLERGEN STANDARDIZATION

The earliest method of standardizing allergen extracts was introduced by Noon (2). He arbitrarily

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designated 1 g of pollen as containing 1 million Noon units: A 1/10 (w/v) extract prepared by extracting 1 g of pollen in 10 mL of extraction fluid would contain 100,000 Noon units/mL. This weight-by-volume (w/v) system has been extended to virtually all allergen source materials and is still one of the most widely used in clinical practice. When it became obvious that most allergens were proteins, the protein nitrogen unit (PNU) was proposed as a method of standardizing allergen extracts (3). One milligram of protein nitrogen is equivalent to 100,000 PNU. The assay has been standardized by the U.S. Food and Drug Administration and is routinely used by all U.S. manufacturers. The limitations of both the w/v and the PNU systems become apparent when one considers the fact that (i) changing extraction conditions can lead to significant variability in the composition and potency of allergen extracts, (ii) most proteins in extracts are not allergens, and (iii) extracts can deteriorate without any change in their protein content.

The discovery of IgE antibodies and the development of immunoassays that measure allergen-specific IgE led to the use of (radioallosorbent test) RAST and (enzyme-linked immunosorbent assay (ELISA) inhibition procedures in allergen standardization (4). In this assay, dilutions of fluid-phase reference and test allergen extracts are allowed to compete with the solid-

TABLE 1

Current Status of Standardized Allergen Extracts in the United States

Allergen extract	Allergen source (Genus/species)	Major allergens
Standardized extracts, approved by FDA		
Honey bee venom	*Apis mellifera*	Api m 1 (phospholipase A_2) Api m 2 (hyaluronidase) Api m 4 (mellitin)
Yellow jacket venom	*Vespula* spp. (*V. vulgaris, V. maculifrons, V. germanica, V. pensylvanica, V. squamosa, V. flavopilosa*)	Ves 1 (phospholipase A_1) Ves 2 (hyaluronidase) Ves 5 (antigen 5)
Paper wasp venom	*Polistes* spp. (*P. annularis, P. exclamans, P. fuscatus, P. metricus*)	Pol 1 (phospholipase A_1) Pol 2 (hyaluronidase) Pol 5 (antigen 5)
Yellow hornet venom	*Dolichovespula arenaria*	Dol a 1 (phospholipase A_1) Dol a 2 (hyaluronidase) Dol a 5 (antigen 5)
White-faced hornet venom	*Dolichovespula maculata*	Dol m 1 (phospholipase A_1) Dol m 2 (hyaluronidase) Dol m 5 (antigen 5)
Short ragweed pollen	*Ambrosia artemisiifolia*	Amb a 1 (antigen E)
Cat hair	*Felis domesticus*	Fel d 1 (cat antigen 1)
Cat pelt	*Felis domesticus*	Fel d 1 (cat antigen 1)
Dermatophagoides farinae	*Dermatophagoides farinae*	Der f 1, Der f 2
Dermatophagoides pteronyssinus	*Dermatophagoides pteronyssinus*	Der p 1, Der p 2
Manufacturers' applications pending FDA approval		
Bermuda grass pollen extract	*Cynodon dactylon*	Cyn d 1
Perennial ryegrass pollen	*Lolium perenne*	Lol p 1, Lol p 5
Orchard grass pollen	*Dactylis glomerate*	Dac g 1, Dac g 4, Dac g 5
Timothy grass pollen	*Phleum pratense*	Phl p 1, Phl p 5
Kentucky bluegrass pollen	*Poa pratensis*	Poa p 1, Poa p 5
Meadow fescue pollen	*Festuca elatior*	Fes e 1
Sweet vernal grass pollen	*Anthoxanthum odoratum*	Ant o 1
Red top grass pollen	*Agrostis alba*	Agr a 1
American cockroach	*Periplaneta americana*	Per a 1
German cockroach	*Blatella germanica*	Bla g 1, Bla g 2
Oriental cockroach	*Blatta orientalis*	Bla o 1
Latex (rubber)	*Hevea brasilensis*	Hev b 1 (rubber elongation factor), Hev b 5

phase reference allergosorbent for binding to IgE antibodies from a pool of allergic serum. The assay results have been correlated with the overall biological potency of extracts, but the approach is limited by the ability of the IgE to bind the allergen and the variability of the specificity and concentration of the IgE in serum from the selected patients.

The identification and purification of major allergenic components from a number of important allergen sources have allowed for the direct measurement of specific allergen content in allergen extracts. Methods based on polyclonal and monoclonal antibodies recognizing major allergens have been successfully employed for extract standardization (5–7). This approach is not dependent upon the variation in the specificity of patient's IgE antibodies, but requires the measurement of most if not all relevant allergens (major and minor) in an extract. If important allergens that are responsible for clinical symptoms in some patients are not measured, diagnostic and therapeutic efficacy may not be predictable.

Two methods based upon quantitative skin testing are currently used for allergen extract standardization. In the U.S. method (8), extracts are evaluated for their ability to induce a mean erythema diameter sum of 50 mm after intradermal injection (ID_{50}EAL, or intradermal dilution for 50-mm sum of erythema determines the allergy unit), and in the Nordic method (9), extract potency is related to the wheal size induced by histamine in prick testing. The HEP (histamine equivalent by prick testing) unit, equivalent to the wheal reaction elicited by a 10 mg/mL histamine phosphate solution, is assigned 1000 biological units (BU). The ID_{50}EAL system is advocated by the U.S. FDA as the preferred method of biological standardization and is currently being used to establish U.S. reference allergen extracts. Briefly, a series of threefold dilutions of a candidate reference extract is prepared, and 0.05 mL is administered intradermally to 15–20 selected allergic subjects. The dose–response data are used to calculate the dilution, D_{50}, required to elicit a 50-mm sum of erythema for each subject tested. The mean dilution required to produce a 50-mm sum of erythema is calculated and the bioequivalent allergy unit (BAU) is assigned for the extract wherein an extract with a D_{50} of 14.0 is assigned 100,000 BAU/mL. Both methods provide an approximation of an extract's allergenic potency in terms of reaction size. Skin testing methods can be cumbersome, time-consuming, and prone to imprecision; the patient selection criteria and availability of suitable numbers of patients (especially for esoteric allergens) can limit the validity of some results.

Skin testing gives a direct biological estimate of potency in allergic subjects; *in vitro* assays (e.g., ELISA inhibition) have wide applicability and are good predictors of overall allergenic potency, and major allergen assays are precise, accurate, and easy to perform on a routine basis. The Noon (w/v) unit, PNU, and a variety of analytical techniques such as isoelectric focusing, SDS–PAGE, crossed-immunoelectrophoresis, and immunoblotting have proven their utility in the qualitative analysis of allergen extracts. While each of these methods have made and will continue to make essential contributions toward the standardization and quality control effort, problems still exist that are inherent to allergenic extracts.

Only a limited number of standardized allergen extract preparations have been approved by the U.S. FDA for commercial distribution (Table 1). The process of standardizing allergen extracts is a time-consuming process of characterizing prospective reference allergen extract preparations, identifying major and minor allergenic components, measuring biologic potency in clinically characterized sensitized human subjects, developing *in vitro* assays that predict biological potency, obtaining licenses for commercial distribution, and putting in place a lot release system to monitor the regulatory compliance

TABLE 2

Amb a 1 Content of Short Ragweed Pollen Lots (1980–1995)

Pollen collection year	Amb a 1 content[a] (units per gram of pollen)
1980	586–788
1981	879–1023
1984	467–623
1985	350–362
1986	415–530
1987	112–229
1988	300–467
1989	329–436
1990	358–377
1991	482–527
1992	254–365
1994	387–456
1995	472–567

[a] Amb a 1 content determined by radial immunodiffusion of 1/10 w/v pollen extracts and reported in terms of units/mL based on CBER/FDA reference extract wherein 1 unit is equivalent to approximately 1 μg.

TABLE 3

Mite Group 1 and 2 Allergen Content of Standardized *D. farinae* Extracts[a]

Manufacturer	Der f 1	Der f 2	Der f 1 + Der f 2	Der f 1:Der f 2
A	46.0	67.0	113.0	0.69
B	81.0	95.0	176.0	0.85
C	120.0	87.0	207.0	1.38
D	8.0	113.0	121.0	0.07

[a] Based on results reported in Ref. (23).

by each allergen product manufacturer. On average, this process has taken approximately 10 years from the initial discovery of a major allergen to commercialization of a standardized extract.

ALLERGEN SOURCE MATERIALS

Table 2 shows the variation in the major allergen (Amb a 1) content of short ragweed pollen collected between 1980 and 1995 from the same location and extracted identically. Extracts prepared from pollen collected in 1981 contained almost 10 times more Amb a 1 than those prepared from pollen collected in 1987. By measuring the major allergen content of short ragweed pollen lots prior to extraction and selectively combining them, one can reproducibly manufacture short ragweed extracts (1/10 w/v) with a consistent Amb a 1 content. This practice increases the reproducibility of extract potency in spite of the variability of the major allergen content of the source material and can be applied to other pollen source materials for which the major allergens can be measured.

When specific allergen content cannot be measured, qualitative methods can be employed to monitor the consistency and reproducibility of allergen source materials and extracts. Isoelectric focusing and SDS–PAGE banding patterns are compared to reference extracts to establish the identity and protein composition of allergen extracts. This method is especially important with grass pollen extracts, since microscopic examination of pollen grains cannot be used to differentiate the different grass species. Furthermore, the major pollen allergens of most grass species are highly cross-reactive, and species-specific antibody reagents for each grass species are not available.

Pure fungal cultures can be sustained in the laboratory, but their composition may vary depending on the culture conditions, the strain used, and whether the fungal mat and the spent medium are harvested. Some strains of *Alternaria alternata* will produce significant quantities of the major allergen Alt a 1 only under certain conditions, and some strains will not produce detectable quantities under any condition. A standardization approach based on the selection of *A. alternata* strains to develop a single *Alternaria* preparation that contains the full reportoire of relevant allergens has been proposed (10). Since no single strain appears to be suitable for this,

TABLE 4

Fel d 1 and Albumin Content of Standardized Cat Extracts

Manufacturer	Product	Fel d 1[a]	Albumin[b]	Fel d 1: Albumin
A	Hair	14.3	11.2	1.28
B	Hair	17.3	84.1	0.21
C	Hair	14.9	122.1	0.12
D	Pelt	15.2	1645.1	0.009
E	Pelt	16.3	5656.8	0.002

[a] Fel d 1 content determined by radial immunodiffusion and reported in terms of units/mL based on the CBER/FDA reference antigen wherein 1 unit is equivalent to approximately 4 μg.

[b] Cat albumin content determined by radial immunodiffusion and reported in terms of μg/mL based a purified cat albumin reference.

multiple strains will most likely have to be incorporated.

Dermatophagoides mites are grown as pure cultures; however, qualitative differences can be detected among extracts prepared from source materials from different producers. Some batches of source materials may contain more fecal material than others and thus lead to extracts with a higher specific Group 1 allergen content. Removal of the growth medium may not be complete during harvesting, leading to extracts containing varying amounts of extraneous proteins. Table 3 shows the Der f 1 and Der f 2 content of four different manufacturers' standardized (10,000 AU/mL) *D. farinae* extracts. The results suggest that although biological standardization may ensure batch-to-batch consistency of lots produced by one manufacturer using mite source materials prepared in a reproducible manner, consistency between lots from different manufacturers (using qualitatively different source materials) may not be ensured. In practice, this means that switching from one manufacturer's standardized mite extract to another's must take into account the possible differences in specific allergen composition that could exist.

The allergen composition of extracts derived from animal sources can also vary greatly. For example, cat extracts are prepared from the hair, dander, skin scrapings, or pelts or from combinations of these sources. Extract from these sources can yield equivalent biological potencies when calibrated according to their major allergen (Fel d 1) content, but will differ in the concentration of another important allergen, cat albumin. Thus, the albumin concentrations of standardized (10,000 BAU/mL) cat extracts containing approximately 15 Fel d 1 units/mL may vary 10- to 500-fold (Table 4). To reflect this significant variation in albumin concentration, cat extracts are labeled as either cat hair or pelt.

STABILITY OF ALLERGEN EXTRACTS

Extract manufacturing procedures may vary, and extraction conditions, such as the buffer used, extraction temperature, and extraction time, and the incorporation of stabilizers can influence final product quality. Storage conditions, especially temperature and extract dilution, can also affect product quality and potency (11). Endogenous proteases can lead to the degradation of some allergens during storage (12). The most effective methods for stabiliz-

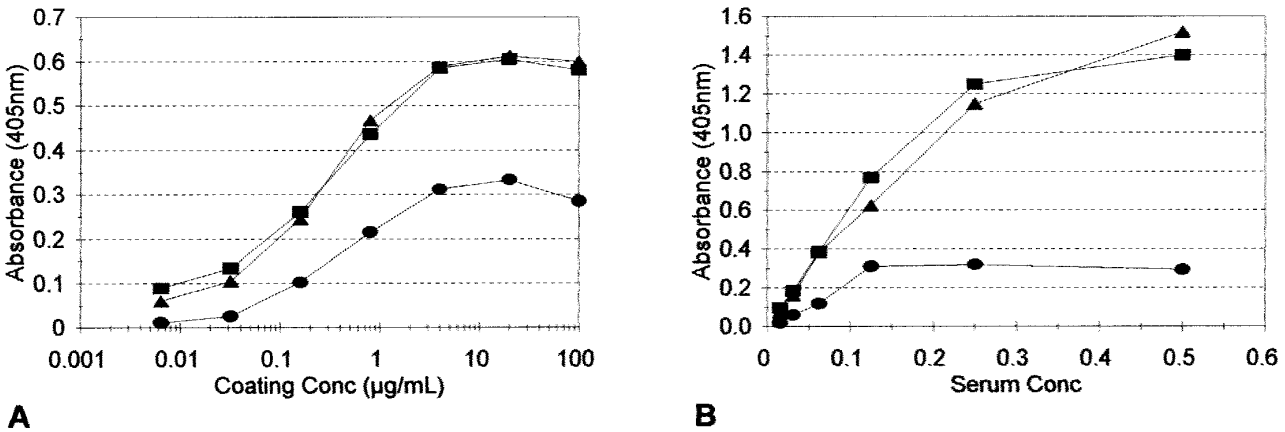

FIG. 1. (A) Coating titrations of crude (●), dialyzed (▲), and G25 void (■) extract fractions prepared from a single lot of cat dander source material. Fivefold serial dilutions starting with a 100 μg protein/mL preparation were made in carbonate coating buffer, pH 9.6, and used to coat Corning EIA microtiter plate wells. The source of IgE antibodies was a serum pool obtained from 18 history and skin test-positive subjects (ZE-P3), and the serum pool was applied to the allergosorbent at a dilution of 1:10. The IgE bound was detected using an alkaline phosphatase-labeled anti-human IgE second antibody (Sanofi Diagnostics Pasteur, Chaska MN) and *p*-nitrophenyl phosphate substrate. The absorbance at 405 nm was measured after incubation with substrate for 30 min. (B) IgE antibody titrations of the control anti-cat serum pool ZE-P3 using crude (●), dialyzed (▲), and G25 void (■) allergosorbents. Twofold serial dilutions of the serum pool were incubated with Corning EIA microtiter plate wells coated with the cat dander extract at 10 μg protein/mL, and the bound IgE antibodies were detected as described for A.

ing extracts are to freeze-dry them or to formulate them in 50% glycerin.

Because allergen extracts contain both heat-stable and unstable allergens and the patient's reactivity toward each allergen varies, stability studies with partially degraded extracts may yield unpredictable and equivocal results. For this reason, a conservative approach to the design of stability studies would be to assign the shelf-life of allergen-containing reagents based on the activity of the most labile allergenic component in the extract. While this approach may ensure that all allergens within a given extract remain reactive, one could argue that a single allergen molecule should not represent the diagnostic utility of a mixture that contains other major and minor allergens that may be useful in detecting IgE antibodies directed against other major and minor allergens in the same extract.

VARIABILITY OF ALLERGEN SOURCE MATERIALS AND ALLERGEN-SPECIFIC IgE ASSAYS

Whether the immunoassay is designed to detect and measure specific IgE antibodies in serum samples or to measure the potency of allergenic extracts, the allergen-containing reagent is critical for obtaining results that are reproducible and comparable between laboratories. A common practice used

TABLE 5

Reactivity of Control Sera to Cat Allergosorbents Prepared from Pelt, Dander, and Saliva Source Materials[a]

Control serum No.	Pelt	Dander	Saliva	Pelt/dander
1	96.6	41.3	1.6	73.8
2	73.7	132.8	103.8	84.1
3	134.3	92.3	139.4	109.5
4	2.9	73.3	1.9	39.4
5	1.2	90.4	1.6	52.2
6	54.0	0.4	0.3	42.0
7	80.9	70.0	35.2	63.4
8	0.8	60.5	27.8	31.8
9	111.0	111.2	103.2	100.3
10	133.8	127.7	30.1	117.4
11	105.6	96.7	27.2	79.3
12	145.0	147.1	131.7	140.1
13	68.5	78.0	17.6	67.6
14	0.0	50.6	2.2	24.2
15	113.1	155.1	109.6	121.2
16	0.1	67.0	45.5	28.1
17	100.5	114.9	73.0	101.6
18	21.3	67.8	3.5	35.4
NC	0.5	0.3	1.9	0.4
POS	100.0	100.0	100.0	100.0
Anti-cat albumin	1231	212	5	855
Anti-Fel d 1	312	924	298	655

[a] Results for individual allergic sera reported as relative units with respect to the positive control pool (POS) using the formula, units = $(T/R) \times 100$, where T is the absorbance units obtained with the individual test serum sample, and R is the absorbance units obtained with the positive control serum pool from the same assay. A negative control serum pool (NC) obtained from history and skin test-negative subjects were included as a measure of nonspecific binding. Results for the allergen-specific control sera are reported as arbritary units based on the absorbance units (×1000) measured after 30 min of incubation with *p*-nitrophenyl phosphate substrate. The Fel d 1 and cat albumin concentrations of the allergen extracts used to prepare the allergosorbents were determined by radial immunodiffusion (11) using the CBER/FDA reference Fel d 1 (1 unit = 4 μg) and Sigma cat albumin for standards and precipitating sheep anti-Fel d 1 (CBER/FDA) and rabbit anti-cat albumin (Greer) antisera: Cat pelt, 0.20 μg/mL Fel d 1, 4.80 μg/mL albumin; cat dander, 2.20 μg/mL Fel d 1, 0.10 μg/mL albumin; cat saliva, 1.66 μg/mL Fel d 1, <0.01 μg/mL albumin; cat pelt/dander, 2.30 μg/mL Fel d 1, 2.45 μg/mL albumin.

in research as well as in commercial laboratories is to prepare allergen allergosorbents by either passive adsorption or covalent binding of allergens to a solid-phase support. The allergen sources used include extracts prepared from a wide variety of raw materials known to contain IgE-binding proteins or antigens. In most applications, it is desirable that all relevant allergenic epitopes be available on the solid phase. The specificity of the allergosorbents is often assumed to be defined by the composition of the extract and its source without confirming the successful coupling of the relevant antigens to the solid phase. Quality control procedures using individual control serum samples and animal antisera with defined specificities can be performed to operationally demonstrate the specificity of an allergosorbent. Examples are presented for the preparation of a cat allergen extract allergosorbent.

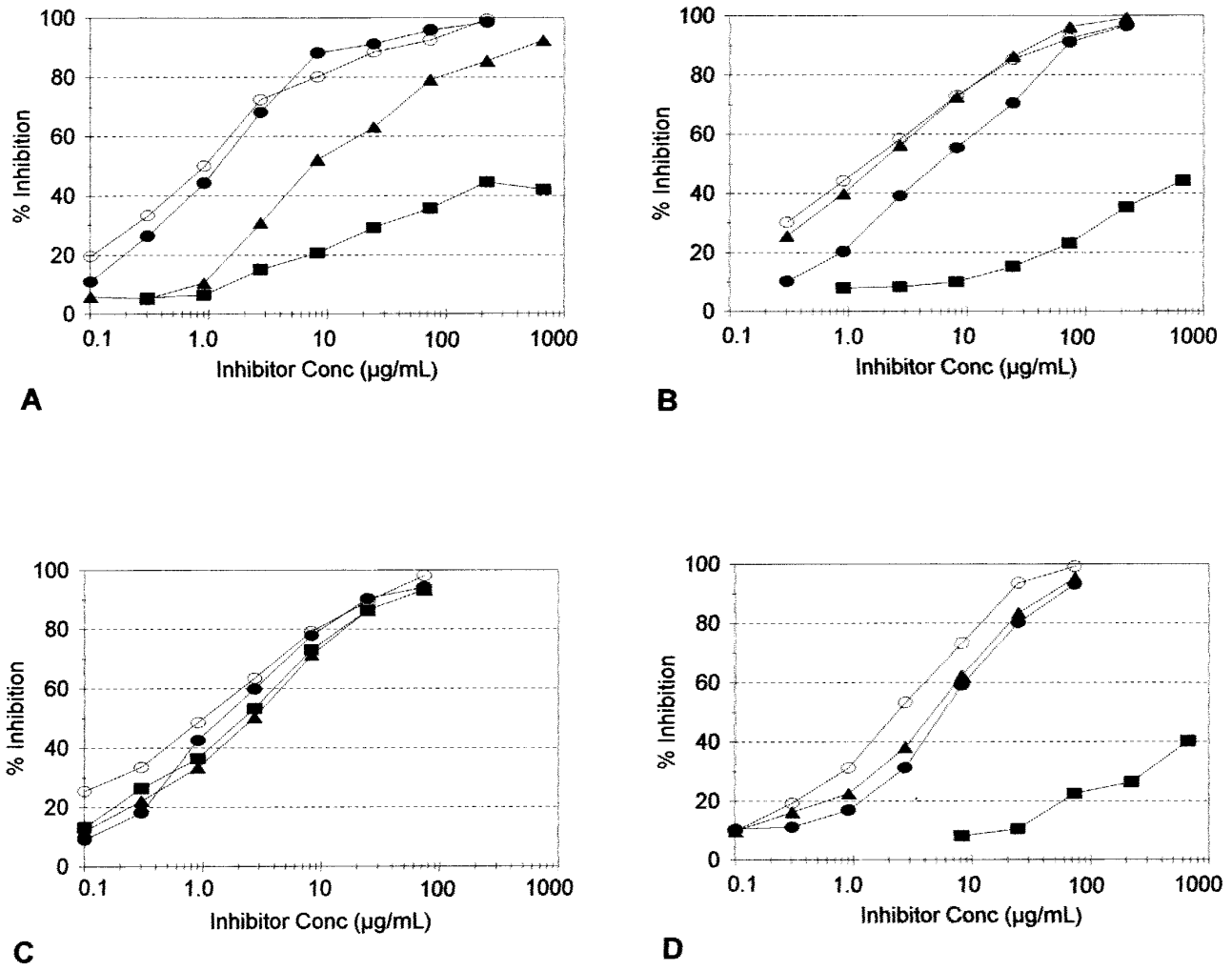

FIG. 2. ELISA inhibition assay employing cat dander (A), cat pelt (B), cat saliva (C), and cat pelt/dander (D) allergosorbents. Dialyzed extracts prepared from cat dander (●), pelt (▲), saliva (■), and cat pelt/dander (○) were co-incubated in the microtiter plate wells at various concentrations with the control serum pool ZE-P3 (at a final dilution of 1:10) for 6–8 h before the addition of the alkaline phosphatase-labeled anti-human IgE second antibody. The absorbance at 405 nm was measured 30 min after addition of the *p*-nitrophenyl phosphate substrate, and the percentage inhibition of IgE binding was calculated using the formula, % inhibition $= [(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance measured in the absence of inhibitor, and A_1 is the absorbance measured in the presence of inhibitor. A grass pollen extract was used as a nonspecific inhibitor control and was found to inhibit IgE binding $<20\%$ at up to 1 mg/mL protein concentrations (data not shown).

Several source materials can be used to produce highly reproducible and specific reagents for the measurement of cat allergen-specific IgE antibodies. Cat dander, saliva, and pelt are known to contain detectable levels of allergens that bind IgE antibodies from cat-sensitive individuals and are thus frequently used as raw materials for the production of allergosorbents in specific IgE antibody immunoassays (13–16). More recently, attempts have been made to utilize purified native and recombinant Fel d 1, the major allergen of cat origin, to measure IgE antibody (17–20).

Extracts prepared from these sources were evaluated using the same solid-phase support and a serum pool composed of 18 history and skin test-positive human subjects. Since the plastic solid-phase supports commonly used in enzyme immunoassay applications have limitations in binding capacities, various methods have been investigated to enhance the binding of the specific allergen content of extracts and reduce adsorption of nonallergenic components to the solid phase. Removal of low-molecular-weight contaminants from the extract by either dialysis or Sephadex G-25 gel filtration greatly improves the binding of IgE antibodies to the allergosorbent as indicated in Fig. 1. All extracts give binding curves that indicate that saturation of the solid-phase support is achieved at a protein concentration of about 10 μg/mL. Figure 1A shows a representative experiment conducted with cat dander extracts. Serum titration curves (Fig. 1B) suggest that although conditions of allergen excess could not be achieved with the plastic support, the dynamic range of allergen-specific IgE assays was increased by such procedures.

Each allergosorbent was evaluated for its ability to bind specific IgE antibodies from 18 history and skin-test-positive sera (Table 5). In addition, each solid-phase allergen was examined for the presence of the known cat allergens Fel d 1 and cat albumin using monospecific rabbit polyclonal antibodies. The results were highly variable, and no single allergosorbent yielded a positive reaction in all 18 serum samples. The cat pelt allergosorbent identified one serum sample (No. 6) as positive that was not identified by either the cat dander or the cat saliva allergosorbents, but it failed to detect IgE antibodies in five samples (Nos. 4, 5, 8, 14, and 16). The cat saliva allergosorbent failed to detect IgE antibodies in 6 of 18 samples. The cat dander allergosorbent successfully identified 17 of the 18 samples as positive. Based on the results obtained with the monospecific rabbit antisera, the deficiencies noted with the allergosorbents could be attributed in part to their relative lack of sensitivity toward detecting specific anti-Fel d 1 or anti-cat albumin antibodies. For example, the cat pelt allergosorbent showed reduced binding of anti-Fel d 1 antibodies, while the cat dander allergosorbent showed reduced binding of anti-cat albumin antibodies. The Fel d 1 and albumin content and their ratios in the respective extracts are different; Fel d 1 is the major component in cat dander and saliva extracts, and albumin is the major component in cat pelt extracts. The difficulty in achieving conditions of allergen excess on the allergosorbents, especially with the minor components, may explain the differences observed in the antibody-binding efficiencies. Different solid phases may also favor the adsorption of different allergenic components, and some allergens may not bind at all. Nonetheless, the cat pelt

TABLE 6

Relative Potency Determinations of Cat Allergenic Extracts Prepared from Pelt, Dander, and Saliva Source Materials on Various Cat Allergosorbents[a]

Text extract	Allergosorbent system			
	Pelt	Dander	Saliva	Pelt/dander
Pelt	1.00 (0.68–1.43)	0.10 (0.07–0.14)	0.85 (0.58–1.22)	0.50 (0.34–0.72)
Dander	0.32 (0.22–0.46)	1.00 (0.68–1.43)	1.11 (0.75–1.59)	0.38 (0.26–0.54)
Saliva	Invalid[b]	Invalid[b]	1.00 (0.68–1.43)	Invalid[b]
Pelt/dander	1.07 (0.72–1.53)	1.24 (0.84–1.77)	1.92 (1.31–2.75)	1.00 (0.68–1.43)

[a] Relative potency values were determined by parallel line regression analysis (CBER/FDA Manual of Methods) using a serum pool obtained from 18 history and skin test-positive subjects (ZE-P3). Each extract served as the reference extract in its respective allergosorbent system and by definition was given a relative potency value of 1.00. The 2SD upper and lower limits for $N = 3$ are presented in parentheses.

[b] Saliva extract relative potency assays failed the t test for parallelism at $p = 0.01$ when performed in the heterologous allergosorbent systems and therefore were not valid (see Fig. 2).

and cat hair/dander sources appeared to contain all the allergen specificities that were detectable by IgE antibodies in the human sera. Based on these findings, an allergosorbent that uses an extract mixture with equal amounts of Fel d 1 and albumin concentrations was evaluated. After optimizing for coating using the control serum pool, the sensitivity of the mixed extract allergosorbent was evaluated with the same 18 human sera. In this experiment, all 18 sera were identified as positive for specific IgE antibodies, and both the Fel d 1 and the cat albumin specificities appeared to be adequately represented on the solid phase (Table 5).

ALLERGEN EXTRACT POTENCY DETERMINATIONS BY ELISA INHIBITION ARE DEFINED BY BOTH REFERENCE ALLERGEN AND ANTISERUM CHARACTERISTICS

Immunoassays based on the competition of fluid-phase and solid-phase allergens for specific antibodies are commonly employed for potency estimations of allergenic extracts. The standardization of commercial extracts used in the United States for diagnosis and

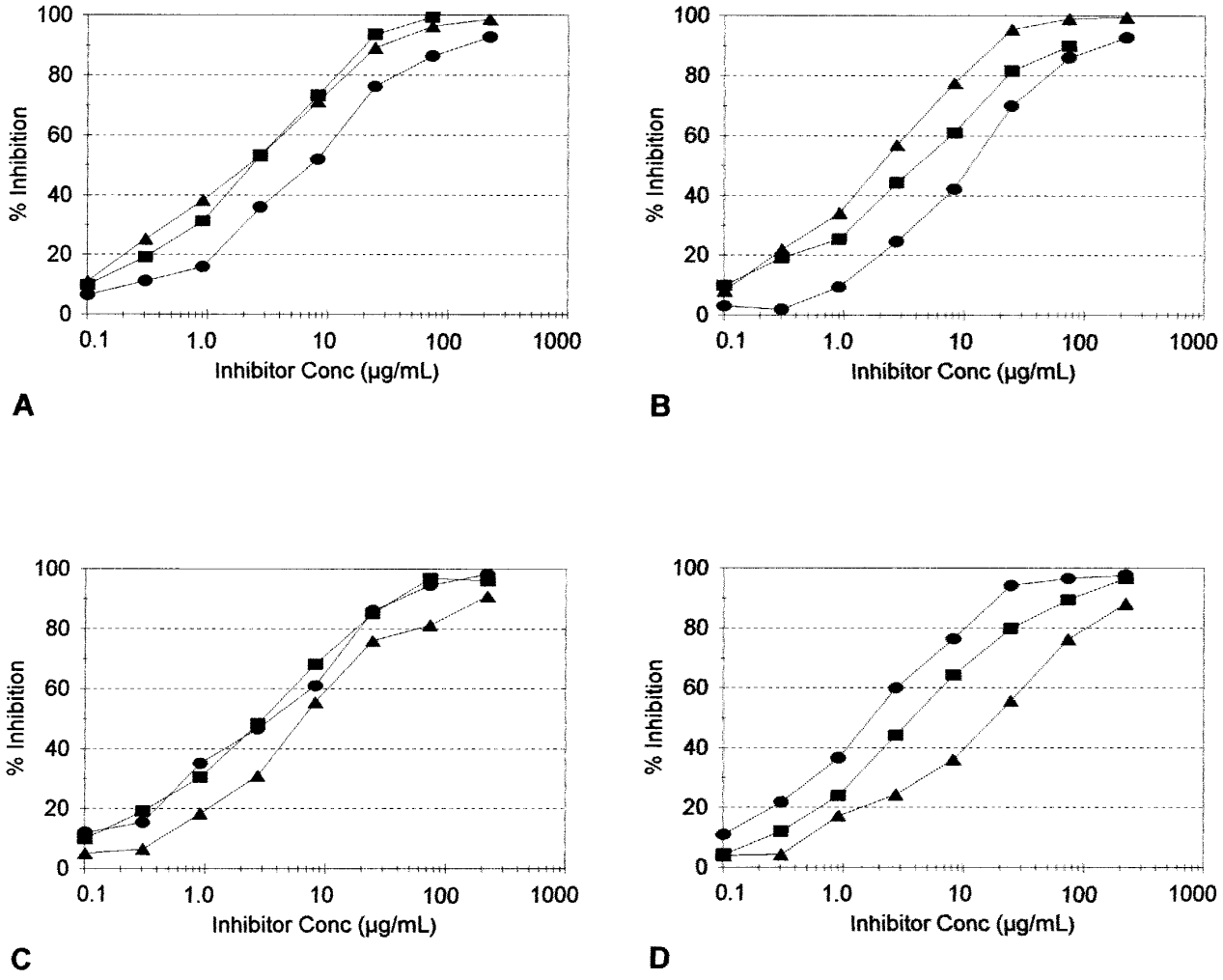

FIG. 3. ELISA inhibition assay employing the cat dander/pelt allergosorbent and two different control sera selected on the basis of reactivity toward cat pelt (A and B) or cat dander (C and D). The assays were conducted at final serum dilutions of either 1:10 (A and C) or 1:80 (B and D). Dialyzed extracts prepared from cat dander (●), pelt (▲), and cat pelt/dander (■) were assayed, and the percentage inhibition of IgE binding was calculated as described in the legend to Fig. 2.

immunotherapy of allergic disease is based on ELISA inhibition assays (22), and modifications of the assay format have been used to detect and measure specific allergen levels in environmental samples (24).

The performance of the cat allergen allergosorbents was evaluated in ELISA inhibitions designed to determine the relative potencies of cat allergen extracts (Fig. 2 and Table 6). Each of the allergen extracts was a potent inhibitor when examined with its homologous allergosorbent. Qualitative differences between extracts could be identified by the lack of parallelism in the dose–response curves generated with the cat saliva extract when tested in the cat pelt and cat dander allergosorbents. The *t* test for parallelism between the regression lines generated by the extract dose–response curves identified gross qualitative differences and was used to establish assay validity. Depending on the allergosorbent used, different relative potencies were calculated for the allergen extracts. For example, when the cat dander allergosorbent system was used, the relative potency of the cat pelt extract was approximately 10% that of the cat dander extract. In contrast, when the homologous cat pelt allergosorbent system was used, the cat pelt extract was about three times as potent as the cat dander extract. Finally, when compared in the mixed cat dander/pelt or cat saliva systems, no significant difference in the potencies of the cat pelt and cat dander extracts could be detected.

These results show that ELISA inhibition assays are sensitive to both qualitative and quantitative differences between extracts derived from cat source materials and underscore the importance of the nature of the allergen allergosorbent when determining allergen extract potency. Ideally, the allergosorbent should (i) represent the allergen specificities present in both the reference and the test extracts, (ii) produce parallel reference and test inhibition regression lines, and (iii) allow for inhibitions approaching 100% for both reference and test extracts. From the perspective of allergenic extract standardization, it might be argued that the use of a single reference allergen extract as the standard for controlling all cat-derived allergen products requires that each manufacturer utilize nearly identical source materials and production procedures. Because different manufacturers or laboratories use different allergen source materials and processing procedures, it appears more appropriate to establish individual in-house reference extract preparations that reflect the compositional and biochemical characteristics of each manufacturer's product.

The choice of an appropriate allergic serum pool can also lead to disparate potency determinations by ELISA inhibition. The reference serum selection criteria employed may favor the detection of some allergenic components over others and bias the potency estimates toward those components as defined by the serum IgE antibody specificities. In the experiment shown in Fig. 3, an ELISA inhibition assay was conducted using the optimized cat pelt/dander allergosorbent and a reference serum pool selected on the basis of reactivity toward cat dander or pelt-derived allergens. The calculated relative potencies of the respective extracts differed by nearly a factor of 3, depending upon the serum pool used (Table 7). Furthermore, the discrepencies were magnified at higher serum dilutions (i.e., 1/80 vs 1/10 dilutions), indicating that minor IgE antibody specificities may have been diluted to extinction. The possibility

TABLE 7

Relative Potency Determinations of Cat Allergenic Extracts Prepared from Pelt and Dander Source Materials Using Different Control Sera[a]

Test extract	Anti-cat pelt serum		Anti-cat dander serum	
	1:10	1:80	1:10	1:80
Pelt	1.22 (0.83–1.74)	1.78 (1.21–2.55)	0.36 (0.25–0.52)	0.28 (0.19–0.40)
Dander	0.35 (0.24–0.50)	0.35 (0.24–0.50)	0.95 (0.65–1.36)	2.48 (1.69–3.55)
Pelt/dander	1.00 (0.68–1.43)	1.00 (0.68–1.43)	1.00 (0.68–1.43)	1.00 (0.68–1.43)

[a] Relative potency values were determined by parallel line regression analysis (CBER/FDA Manual of Methods (20)) using control sera 6 (anti-cat pelt serum) and 5 (anti-cat dander serum) described in Table 3. The cat pelt/dander extract served as the reference extract and by definition was given a relative potency value of 1.00. The 2SD upper and lower limits for $N = 3$ are presented in parentheses.

therefore exists that by diluting the reference sera one may affect not only the sensitivity of the assay, but also its specificity (Fig. 4).

CONCLUSIONS

The inherent variability of natural allergen source materials requires quality control procedures that ensure their identity, purity, and consistent processing. The design and validation of immunoassays for the detection of allergen-specific IgE antibodies or for the measurement of allergen extract potency require the use of well-characterized reference reagents for the preparation of allergosorbents and control antibodies. Uniformity in the use of established allergen nomenclature and allergen extract standardization provide

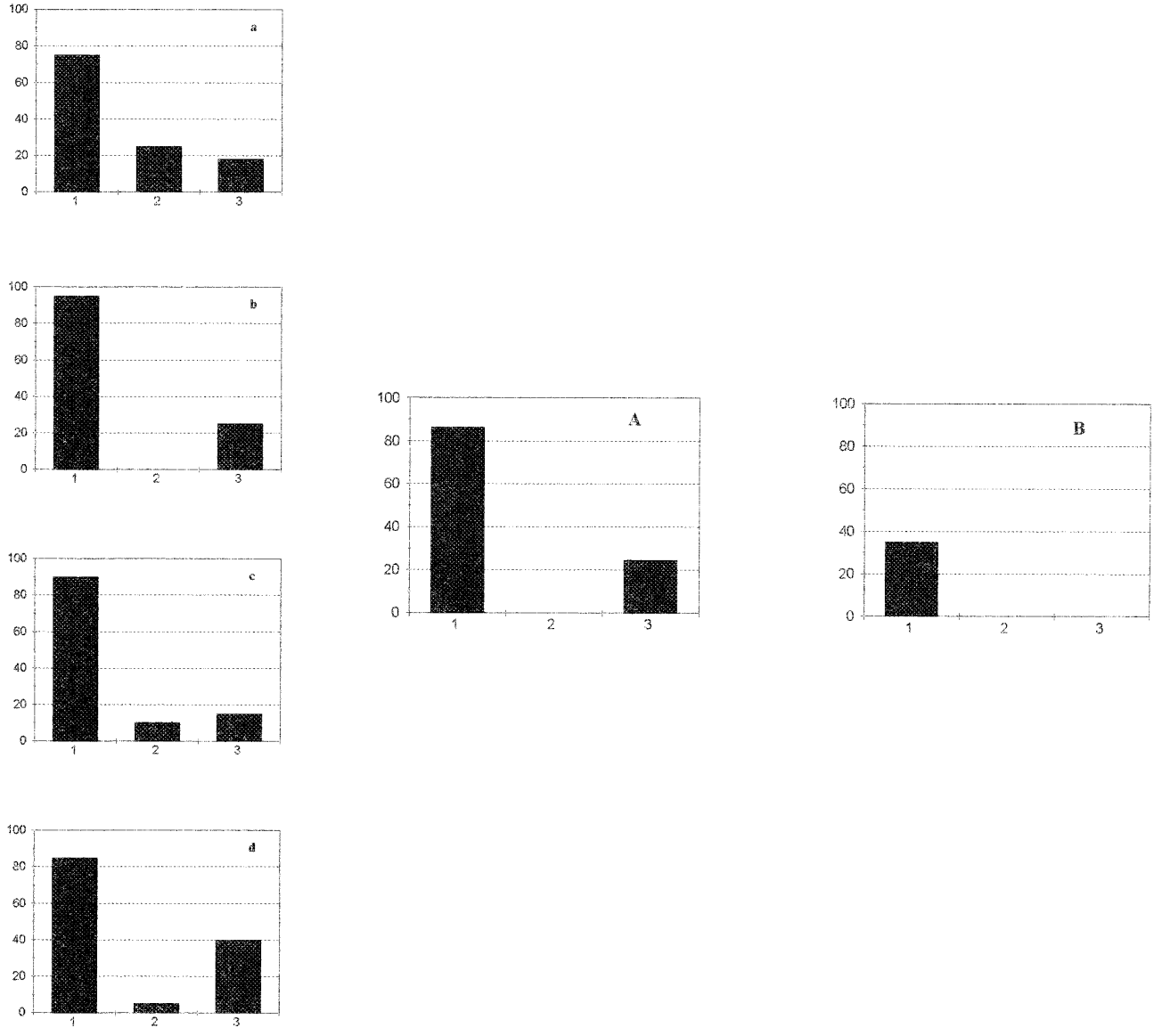

FIG. 4. Effect of pooling of individual sera (A) and dilution (B) on the specificity of control sera used for allergen standardization. The bar graphs illustrate the relative concentrations of IgE antibodies from four individuals (a–d) for three allergen specificities (1–3). When these sera are pooled, the concentration of IgE antibodies directed toward minor allergens becomes underrepresented and the major IgE antibody specificity predominates. Dilution of the pooled sera further dilutes the minor IgE antibody specificities, often beyond the detection limit of IgE antibody assays.

approaches toward comparability and consistency between products from different manufacturers.

The specificity of a particular immunoassay should be defined operationally in terms of the assay format employed because it is difficult to attain conditions of allergen excess on most allergosorbents. This requires the use of a battery of individual positive control sera or monospecific antibody controls directed toward relevant allergenic components. The accuracy of measuring allergen extract potency by an ELISA inhibition assay is dependent on the immunoassay system including the reference extract and the serum pool used. The specificity of ELISA inhibitions can be defined not only by the allergosorbent but also by the IgE antibody specificities that can be altered by dilution. The examples presented here with cat-derived allergen source materials illustrate the difficulties in attaining standardization and conformity between results obtained in different laboratories utilizing different reagents.

Interchangeability between allergen-containing reagents produced by different manufacturers is not a realistic expectation because these products vary in their composition and immunoreactivity (Table 8). A more reasonable goal of allergen standardization may be to define the specificity of each manufacturer's allergen reagents based on major allergen content or on the reactivity toward a common reference serum pool containing IgE antibody specificities to which each allergen-containing reagent can be compared.

TABLE 8

Potential Causes of Allergen Product Heterogeneity

Misidentification, purity and inherent biological variation of source material
Raw material collection and processing conditions
Extraction conditions (buffer, time and temperature)
Variable binding of relevant allergens to labels or solid supports
Variable stability of allergen during storage
Heterogeneous internal allergen references used in quality control
Heterogeneous IgE antibody specificities in human serum references used in quality control
Variations in acceptance criteria used in final reagent validations

REFERENCES

1. World Health Organization/IUIS Allergen Nomenclature Subcommittee (1995) *J. Allergy Clin. Immunol.* **96,** 5–14.
2. Noon, L. (1911) *Lancet* **1,** 1572–1573.
3. Stull, A., Cooke, R. A., and Tenant, J. (1933) *J. Allergy* **4,** 455–467.
4. Yuninger, J. W., Jones, R. T., and Gleich, G. J. (1976) *J. Allergy Clin. Immunol.* **58,** 405–413.
5. Baer, H., Godfrey, H., Maloney, C. J., Norman, P. S., and Lichtenstein, L. M. (1970) *J. Allergy* **45,** 347–354.
6. Baer, H., Maloney, C. J., Norman, P. S., and Marsh, D. G. (1974) *J. Allergy Clin. Immunol.* **54,** 157–164.
7. Chapman, M. D., Heymann, P. W., Wilkins, S. R., Brown, M. J., and Platts-Mills, T. A. E. (1987) *J. Allergy Clin. Immunol.* **80,** 184–194.
8. Turkeltaub, P. C., Rastogi, S. C., and Baer, H. (1986) *in* CBER/FDA Manual of Methods, Bethesda, MD.
9. Nordic Council of Medicines. (1989) *in* Registration of Allergen Preparations, 2nd ed., Nordiska Läkemedelsnämnden, Uppsala, Sweden. [NLM Publication No. 23]
10. Portnoy, J., Pacheco, F., Barnes, C., Upadrasta, B., Crenshaw, R., and Esch, R. E. (1993) *J. Allergy Clin. Immunol.* **91,** 773–782.
11. Nelson, H. S. (1979) *J. Allergy Clin. Immunol.* **63,** 417–425.
12. Esch, R. E. (1991) *Arbeiten aus dem Paul Ehrlich Institut* **85,** 171–179.
13. Anderson, M. C., Baer, H., and Ohman, J. L. (1985) *J. Allergy Clin. Immunol.* **76,** 563–569.
14. Dabrowski, A. J., Van Der Brempt, X., Soler, M., Seguret, N., Lucciani, P., Charpin, D., and Vervloet, D. (1990) *J. Allergy Clin. Immunol.* **86,** 462–465.
15. Lowenstein, H., Lind, P., and Weeke, B. (1985) *Allergy* **40,** 430–441.
16. Ohman, J. L., Baer, H., Anderson, M. C., Leitermann, K., and Brown, P. (1993) *J. Allergy Clin. Immunol.* **72,** 288–293.
17. De Groot, H., Van Swieten, P., Van Leeuwen, J., Lind, P., and Aalberse, R. C. (1988) *J. Allergy Clin. Immunol.* **82,** 778–786.
18. Witteman, A. M., Stapel, S. O., Perdok, G. J., Sjamsoedin, D. H. S., Jansen, H. M., Aalberse, R. C., and van der Zee, J. S. (1996) *J. Allergy Clin. Immunol.* **97,** 16–25.
19. Bond, J. F., Brauer, A. W., Segal, D. B., Nault, A. K., Rogers, B. L., and Kou, M-C. (1993) *Mol. Immunol.* **30,** 1529–1541.
20. Slunt, J. B., Rogers, B. L., and Chapman, M. D. (1995) *J. Allergy Clin. Immunol.* **95,** 1221–1228.
21. Anderson, M. C. (1988) *in* CBER/FDA Manual of Methods, Bethesda, MD.
22. Laboratory of Immunobiochemistry (1994) *in* CBER/FDA Manual of Methods, Bethesda, MD.
23. Chapman, M., and Alshishtawi, M. (1991) *J. Allergy Clin. Immunol.* **87,** 178.
24. Swanson, M. C., Campbell, A. R., Klauck, M. J., and Reed, C. E. (1989) *J. Allergy Clin. Immunol.* **83,** 776–783.