

Regulatory Aspects of Allergen Vaccines in the US

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Introduction

Allergen vaccines and other biologics were first regulated by the Hygienic Laboratory of the Public Health and Marine Hospital Service. In 1930, the Hygienic Laboratory was renamed the National Institute (singular) of Health (NIH). The NIH continued to regulate biologics (beginning in 1955, through its Division of Biologics Standards) for over 40 years. In 1972, regulatory authority over biologics was transferred to the Bureau of Biologics at the Food and Drug Administration (FDA). In 1982, the FDA merged the Bureau of Biologics and the Bureau of Drugs into a single Center for Drugs and Biologics; five years later, the entities that regulated drugs and biologics were once again separated, and the Center for Biologics Evaluation and Research (CBER) assumed responsibility for allergenics regulation (1,2).

CBER's authority to regulate allergen extracts derives from two laws enacted by Congress, the Food, Drug and Cosmetic Act of 1938 and the Public Health Service Act of 1944. The specific regulations that govern CBER's regulation of allergens appear in part 680 of Title 21 of the Code of Federal Regulations (21 CFR 680), although other parts of 21 CFR also apply to allergen regulation. Over the past 20 years, two features of CBER's regulatory program have had a significant impact on allergen manufacturers and enhanced the safety of allergen extracts marketed to the American public. The first is the enforcement of current good manufacturing practice (cGMP) standards (21 CFR 210, 211, and 600-680) on the manufacture of allergen products. cGMPs include

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Table 1
Standardized Allergen Vaccines Currently Licensed in the US

Allergen vaccine	Current lot release tests	Labeled Unitage
Dust mite (<i>Dermatophagoides farinae</i>)	Competition ELISA Protein ^a	AU/mL (equivalent to BAUmL)
Dust mite (<i>Dermatophagoides pteronyssinus</i>)		
Cat pelt (<i>Felis domesticus</i>)	Fel d 1 (RID) IEF	BAU/mL 5–9.9 Fel d 1 U/mL = 5000 BAU/mL
Cat hair (<i>Felis domesticus</i>)	Protein ^a	10–19.9 Fel d 1 U/mL = 10,000 BAU/mL
Bermuda grass (<i>Cynodon dactylon</i>)	Competition ELISA IEF	BAU/mL
Red top grass (<i>Agrostis alba</i>)	Protein ^a	
June (Kentucky blue) grass (<i>Poa pratensis</i>)		
Perennial ryegrass (<i>Lolium perenne</i>)		
Orchard grass (<i>Dactylis glomerata</i>)		
Timothy grass (<i>Phleum pratense</i>)		
Meadow fescue grass (<i>Festuca elatior</i>)		
Sweet vernal grass (<i>Anthoxanthum odoratum</i>)		
Short ragweed (<i>Ambrosia artemisiifolia</i>)	Amb a 1 (RID)	Amb a 1 units
Yellow hornet (<i>Vespa spp</i>)	Hyaluronidase and Phospholipase	µg protein
Wasp (<i>Polistes spp</i>)	activity	
Honey bee (<i>Apis mellifera</i>)		
White-faced hornet (<i>Vespa spp</i>)		
Yellow jacket (<i>Vespula spp.</i>)		
Mixed vespid (<i>Vespa</i> + <i>Vespula spp</i>)		

^aTest for informational purposes only.

IEF, isoelectric focusing; RID, radial immunodiffusion.

requirements regarding: organization and personnel, buildings and facilities, equipment, control of components and drug-product containers and closures, production and process controls, holding and distribution, quality control, laboratory controls, and records and reports. cGMPs have been in effect since the 1960s.

A second feature is allergen standardization. 21 CFR 680.3(e) specifies that when a potency test exists for a specific allergenic product, and when CBER has notified manufacturers that the test exists, manufacturers will be required to determine the potency of each lot of the product prior to release. Since the 1980s, 19 allergen extracts have been standardized (*see* Table 1). Much of what follows focuses on these standardized products and the tests that are used to ascertain extract potency.

Allergen Vaccines Currently on the Market (Standardized and Non-standardized)

Allergen vaccines are manufactured and sold worldwide for the diagnosis and treatment of IgE-mediated allergic disease. These vaccines are complex extracts of natural biomaterials. Each extract contains proteins, carbohydrates, enzymes, and pigments of which the allergens—presumably the active ingredients—may constitute only a small proportion (3). Traditionally, allergen vaccines have been labeled either with a designation of extraction ratio (w/v) or with a protein unit designation, which is determined using the Kjeldahl method (protein nitrogen units[PNU]/ml). Unfortunately, there appears to be little correlation between these two designations and biological measures of allergen potency (4,5).

In the absence of a concerted effort to maintain product consistency, lot-to-lot variations in allergen content may be considerable. Product consistency may be enhanced by the inherent nature of the raw materials; for example, pollen and pure-mite extracts generally have greater lot-to-lot consistency than mold, house-dust, and insect extracts. In addition, manufacturers can increase the consistency of their products by controlled collection, storage, and processing of the raw materials; by reproducible and optimized extraction and manufacturing techniques; and by expiration dates based on real-time stability data. However, consistency can only be assured by measuring the potency of each lot of extract, and by marketing only those lots whose potency falls within an acceptable range.

FDA's allergen standardization regulation mandates that when an appropriate potency test exists, manufacturers must test each lot of an allergen extract for potency prior to sale. This regulation takes product consistency one step further by establishing a US standard of potency

for each standardized product. The purpose of allergen standardization is to ensure that the extracts are well-characterized in terms of allergen content and that variation between lots is minimized even among different manufacturers (6). Because standardized extracts are compared to a single, national potency standard, patients and their physicians can switch from one manufacturer's product to another with minimized risk of adverse reaction.

Currently, there are 19 standardized allergen vaccines available from manufacturers in the US (*see* Table 1). For each of these extracts, there is a US standard of potency to which each lot of the extract is compared prior to release for sale to the public. The potency measures, and the assays used to determine these measures, are specified in the approved product license applications of each manufacturer for each product. Manufacturers may use the methods described in CBER's Methods of the Allergen Products Testing Laboratory, or may seek CBER approval to use alternative test methods that provide equally reliable measures of product potency and meet regulatory requirements.

The level of quality control for the 19 standardized allergen vaccines is the exception rather than the rule. In vitro potency tests which correlate with in vivo clinical responses have not been developed for the hundreds of non-standardized extracts available in US product lines. Thus, for most allergen extracts manufactured in the US, consistency cannot be assured by potency testing.

The Basis of Allergen Standardization

Allergen standardization comprises two important components: the selection of a reference preparation of allergenic extract and the selection of the procedures to compare manufactured products to the reference extract (7–9). In the US, the use of a biological model of allergen standardization has permitted the assignment of bioequivalent allergen units for most standardized allergens (8). Once a specific unitage is assigned to a reference, then all allergen extracts from the same source can be assigned units based on the relative potency (RP) with respect to the reference using the established quantitative in vitro potency method (10).

In theory, standardizing an allergen extract might involve purifying each allergen in the extract and establishing with precision the importance of these allergens. However, most allergen extracts are complex mixtures of several relevant allergens of as yet uncertain immunodominance. In addition, an individual allergen may be less "allergenic" in a particular lot owing to instability or denaturation. The choice of the best potency test depends on the allergen vaccine to be standardized. In the absence of data supporting the safety of potency designations

based on single allergen content, a measure of overall allergenicity may be a better predictor of safe dosing. For two allergens (short ragweed and cat hair), data have supported the use of single allergen determinations; for cat pelt and hymenoptera venoms, the presence of two allergens is verified for each lot; for grasses, overall allergenicity is determined.

For initial overall allergenicity assessment, CBER has developed a method using erythema size following serial intradermal testing of highly allergic individuals. Intradermal testing was chosen over prick/puncture testing to achieve greater dosing accuracy; erythema size was chosen over wheal size to achieve greater accuracy in reaction measurements. This method is called IntraDermal dilution for 50 mm sum of Erythema determines the bioequivalent Allergy units ($ID_{50}EAL$), and can be used to compare the allergenicity of extracts regardless of source. Subsequent comparisons of extracts from the same source material are made by a variant analysis called the parallel-line bioassay. Both of these methods are described in CBER's Methods of the Allergens Testing Laboratory and are discussed briefly below.

In the $ID_{50}EAL$ method, allergenic extracts are evaluated in subjects maximally reactive to the respective reference concentrates. Each subject is tested with serial three-fold dilutions of the reference extract. After 15 min, the sum of the longest and midpoint orthogonal diameters of erythema (ΣE) is determined at each dilution, and the log dose producing a 50 mm ΣE response (D_{50}) is calculated (10). The mean D_{50} for 15 highly allergic individuals is used to determine the D_{50} for the extract. Extracts that produce similar D_{50} responses can be considered bioequivalent and are assigned similar units, the bioequivalent allergy unit (BAU). Because the modal D_{50} of a series of extracts was 14 (a 3^{-14} or 1:4.8 million dilution), extracts with a mean D_{50} of 14 were arbitrarily assigned the value of 100,000 BAU/mL (8).

By a similar technique and analysis, bioequivalent doses of test extracts from the same source as the reference extract can be determined by the parallel-line bioassay (11). The inverse ratio of the doses of test extract required to produce identical D_{50} responses to a reference extract is the RP of that extract. This analysis requires that the log dose-response curves of the test extract and the reference extract be parallel; if the two dose-response lines are not parallel, then ratio of skin test doses for identical responses—and the RP—will vary with the dose. In this situation, which strongly suggests compositional differences between the two extracts, the distance between the two lines is different at each dose and a meaningful RP cannot be determined (7,12).

Although skin testing is an essential component of the allergen-standardization program, it is not intended for routine use in the testing of manufactured lots of extracts prior to release. In vitro potency

assays that accurately predict the *in vivo* activity of extracts have been developed (12). Once an *in vivo* assay has been utilized to assign unitage to a reference extract, then an appropriate surrogate *in vitro* assay can be used to assign units to test extracts from the same sources. These methods can be based on quantitation of the total protein content (hymenoptera venoms), the specific allergen content within the allergen extracts (short ragweed and cat), or the inhibition of the binding of IgE from pooled allergic sera to reference allergen (grasses, mites) (13). For the hymenoptera venom allergens, the potency determination is also based on the content of the known principal allergens within the extract, hyaluronidase and phospholipase, which is determined by enzyme activity (*see* Table 1).

The potency units for short ragweed extracts were originally assigned based on their Amb a 1 content. Subsequent data suggested that 1 unit of Amb a 1 is equivalent to 1 mg of Amb a 1, and 350 Amb a 1 U/mL is equivalent to 100,000 BAU/mL. However, the original unitage has been retained. Grass-pollen extracts are labeled in BAU/mL, based on ID₅₀EAL testing. In some cases, the assignment of potency units to standardized allergenic vaccines in the US has changed as better bioequivalence data have become available (10). Cat extracts were originally standardized based on their Fel d 1 content, with arbitrary unitage (AU/mL) tied to the Fel d 1 determinations. Subsequent ID₅₀EAL testing suggested that the 100,000 AU/mL cat extracts, which contained 10–19.9 Fel d 1 U/mL, should be relabeled as 10,000 BAU/mL (14). In addition, 20% of individuals allergic to cat were found to have antibody to non-Fel d 1 proteins (15), and the identification of a cat-albumin band on (isoelectric focusing (IEF) was added as a requirement for cat-pelt extracts. In the mid-1980s dust mite extracts were standardized (in AU/mL) based on radioallergosorbent test (RAST) inhibition assays. Subsequent ID₅₀EAL testing indicated that the arbitrary unitage were statistically bioequivalent to BAU/mL (16); in this case, the original unitage was retained (17).

The identity of an allergen extract may be verified by visualizing the separated allergen proteins based on their size and isoelectric points (3). The IEF assay is an important safety test in the lot release of grass-pollen and cat extracts. The patterns produced by the crude allergen mixtures are reproducible enough to consistently indicate the presence of known allergens, to identify possible contaminants present in the extracts, and to check lot-to-lot variation in the extracts (18). In addition, IEF is used to verify the presence of cat albumin in cat-pelt extracts.

Tests Currently Applied to Standardized Allergens

Several *in vitro* tests have been established for the testing the potency and identity of standardized allergens (*see* Table 1). Tests for

potency include assays for the specific allergen content, for the RP, and for the enzyme activity of allergenic extracts. In addition, the identity of standardized extracts may be tested by the qualitative assessment of allergen content.

The specific allergen content of certain allergenic extracts can be measured by the radial immunodiffusion assay (RID). This assay is currently applied to two standardized allergenic extracts, short ragweed and cat, in which the immunodominant allergens (Amb a 1 and Fel d 1, respectively) have been identified and defined. In this assay, monospecific antiserum is added to an agar solution, which is allowed to solidify. Wells are then cut into the agar; test allergen is placed in the wells. As the specific allergen diffuses out into the agar, a precipitin ring forms, which delineates the equivalence zone for antigen-antibody binding. The radius of the precipitin ring can then be measured. Because the antibody concentration in the agar is constant, the antigen concentration decreases with increasing distance from the well and is proportional to the log of the concentration of the applied test allergen in comparison to the reference extract.

The potency of those standardized allergen vaccines for which the immunodominant components have not been identified with certainty may be estimated using assays for IgE-antigen binding that compare the overall IgE-binding properties of test and reference extracts, using pooled allergic sera. Initially, a RAST inhibition assay was used for this purpose; CBER adopted the competition enzyme-linked immunosorbent assay (ELISA) as its standard assay because of its greater precision and convenience. After coating the wells of the polystyrene microtiter plate with the reference allergen and blocking the wells with bovine albumin, a mixture of the allergen vaccine to be tested and a reference serum pool is added to the wells. The greater the amount of immunoreactive allergen in the mix, the less free IgE antibody will be available from the serum pool to bind to the immobilized allergen on the plate. Once again, the concentration of the allergens in the allergen vaccine is determined by comparison to the reference allergen vaccine. However, because this assay does not explicitly measure a specific allergen, the allergen concentration is expressed as RP, with the reference vaccine assigned an arbitrary RP of 1.0. Early studies showed good an excellent correlation between RP assigned by titration skin testing and RP determined by RAST inhibition (8); subsequent studies showed the competitive ELISA to be equivalent as well (19).

Hymenoptera venoms contain multiple glycoproteins enzymes, the most important of which are hyaluronidase and phospholipases A1 and A2. Venom allergen extracts are standardized using enzymatic assays, which estimate hyaluronidase and phospholipase content based on their enzymatic activity. In these assays, an agar solution is prepared

with the appropriate enzymatic substrate and test samples are then added to cut wells. As the enzyme present in the sample diffuses into the agar, it digests the substrate, forming clearing zones around the wells. The radius of the clear zones is then measured and calculated as the log of the concentration of the enzyme present in the sample.

In addition to determining the potency of these allergen extracts, manufacturers are expected to confirm the identity of certain standardized extracts (*see* Table 1) by IEF. This technique separates the proteins in the test extract based on their isoelectric points. The profile obtained in this technique is compared to the CBER standard to confirm the stated identity of the allergen extract (18).

In the past, manufacturers were required to perform ninhydrin protein assays on most standardized allergen vaccines. CBER developed and adopted a modification of the more cumbersome ninhydrin technique for protein determination (20) in response to concerns about the inaccuracy of the more standard protein-estimation techniques. However, release limits were not established for the total protein content of standardized allergen vaccines. Rather, the results of the ninhydrin assay were required for information only. When the results were checked as part of CBER's lot-release program, CBER required that the results of the CBER assay be within 40% of the manufacturer's result.

In effect, the protein-assay requirement was a quality-control test; in this phase of the allergen standardization program, CBER did not have data on the protein content of the standardized allergens, or the effect of the protein content on potency assays. Recently, we re-examined the requirement that manufacturers perform the ninhydrin assay on their standardized allergen vaccines (21). As a result of these considerations, CBER no longer requires the use of the ninhydrin assay for standardized mite- and grass-allergen vaccines. However, manufacturers should continue to perform a validated protein assay on each lot of material, and CBER continues to require this information as part of its lot release program. The choice of protein assay is left to the manufacturer. Currently approved protein assay methods for other allergens (standardized cat, short ragweed, and hymenoptera venoms) are unchanged.

How Should Release Limits Be Chosen?

Fundamental to the standardization process is establishing an acceptable range of comparability or equivalence. Limits that are too broad lead to unacceptable risk to patients (anaphylaxis when the physician changes from one bottle to another, or changes to a different manufacturer), whereas limits that are too narrow lead to unacceptable risk for manufacturers (the rejection of a large percentage of safe and effective lots of product). In the competition ELISA, potency limits have

been set according to the precision of the test; the candidate vaccines are expected to be statistically equivalent to the reference extract, at a specified level of confidence with a specified test. Mite and grass pollen vaccines are currently expected to be identical to reference at the 98% confidence level, using three replicates of a validated competition ELISA; the standard deviation σ in log (RP) for a single replicate is 0.1375 (19). The 98% confidence interval is given by $10^{\pm 2.326\sigma/\sqrt{3}}$. Consequently, a lot whose RP falls in the range 0.654–1.530 is within the 98% confidence interval and is approved for release. This criterion also implies that, on average, 2% of lots submitted to CBER will fall outside of the release limits even if they are identical to the reference extract. Lots that are not identical to the reference would fail at predictably higher rates, while a small fraction of lots whose RP is outside the limits (as could be established by more exhaustive testing) will pass release testing.

An alternative approach would be to base the potency limits on acceptable ranges established in clinical studies. Three criteria would appear to be important. The first, therapeutic equivalence, addresses the efficacy of allergen vaccines for immunotherapy. Thus, an RP range will have the property of therapeutic equivalence if, for the allergen vaccine in question, lots with RPs anywhere in that range have an equal likelihood of effecting clinical improvement in an immunotherapy trial. Likewise, diagnostic equivalence addresses the efficacy of allergen vaccines for *in vivo* diagnostics. Finally, safety equivalence reflects the likelihood of the safe administration of the vaccine for either diagnostic or therapeutic indications. The acceptable limits should fall within the narrowest of the equivalence ranges established by these criteria.

The aggregate consistency of manufactured lots might also be taken into account when developing testing methods and limits. For example, if typical lot-to-lot consistency is very high and well within clinical limits, then testing protocols could be adjusted to eliminate outliers while rarely failing lots whose RP is close to 1. On the other extreme, if the distribution of lots is broad, equivalence to the reference would be imposed. This would narrow the distribution, but at a cost: at 95% equivalence, 5% of lots whose RP = 1 would fail release.

In a recent analysis of studies using ragweed and dust mite allergens (22), we found that the range of therapeutic equivalence was at least 10-fold, and the ranges of diagnostic equivalence and safety equivalence were approx fourfold. In the same study, we analyzed the lot-to-lot consistency of 412 lots of grass pollen extracts and 91 lots of dust mite extracts. The variability of the samples was comparable to the assay variability. Furthermore, we calculated that the mean ratio (in RP) of two randomly selected lots of allergen would be 1.12 (for mites) and 1.18 (for grass pollen). The calculated 95th percentile ratio

was 1.48 and 1.8. Thus, the equivalence ranges appear to be considerably broader than the current lot-release limits (twofold) and the expected variations in product potency using current manufacturing and quality-control practices. Based on these estimates, CBER has proposed to broaden the release limits for standardized dust-mite and grass-pollen allergen vaccines to 0.5 to 2.0 (23).

Future Standardization Efforts

The effort to standardize allergens in the US has resulted in the development of a core group of highly used allergen vaccines that are better characterized and more consistent than their nonstandardized predecessors. Standardized allergens also facilitate accurate and informative scientific studies of the efficacy, safety, and mechanisms of allergen immunotherapy, and will be essential for the study of novel immunotherapeutic products in the future. In spite of these clear advantages, most allergens marketed in the US remain unstandardized. At a minimum, all allergen vaccines should be subject to potency testing and compared to a reference extract, whether manufacturer-specific, industry-wide, national, or international. CBER will continue to work with the allergen-vaccine industry to establish and maintain US standards of potency for an increasing number of allergen vaccines, and to improve the consistency of those products that are not standardized. In this section, we discuss the criteria that will be used to choose standardization targets, and the ways that allergen standardization will be implemented in the future.

Standardization targets will be selected to maximize the public health benefit of greater allergen consistency. Criteria for allergen selection include:

1. Availability of stable, preferably lyophilized material for use as long-term reference extracts.
2. Consistency of currently marketed product.
3. Widespread use as a diagnostic and/or therapeutic reagent in the US.
4. Number of manufacturers producing the product.
5. Potential use in immunotherapy or diagnostics.
6. Public health impact of correct diagnosis and/or adequate treatment.

These impact criteria are meant to help establish priorities and are not intended to be exclusionary. Thus, for example, an extract produced by only one manufacturer might still be a standardization target if other impact criteria are met. Likewise, CBER might decide to move forward with a little-used product of great public-health importance, the stan-

dardization of which might enhance its availability and quality in the US.

Once an allergen standardization target is selected, the marketed products that contain the allergen will be examined and compared with the best products available worldwide. Biological potency will be established using the ID₅₀EAL method, and a surrogate test will be identified for lot-release purposes. CBER intends to pursue these goals with the full knowledge and, ideally, active participation of the allergen vaccine industry and scientific investigators. When a test for a standard of potency exists, FDA will notify manufacturers (under 21 CFR 680.3(e)). The regulation requires that manufacturers comply with the standard and test each lot of the specified extract prior to release for sale.

CBER has considered the standardization of several products beyond those listed in Table 1, including latex, cockroach, tree pollens, molds, and peanut (24). Based on the above impact criteria, this laboratory is currently working on the standardization of cockroach allergens.

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