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How Allergen Extracts Are Made—From Source Materials to Allergen Extracts

Food allergen extracts to diagnose food-induced allergic diseases
How they are made

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ABSTRACT

Objective: To review the manufacturing procedures of food allergen extracts and applicable regulatory requirements from government agencies, potential approaches to standardization, and clinical application of these products. The effects of thermal processing on allergenicity of common food allergens are also considered.

Data Sources: A broad literature review was conducted on the natural history of food allergy, the manufacture of allergen extracts, and the allergenicity of heated food. Regulations, guidance documents, and pharmacopoeias related to food allergen extracts from the United States and Europe were also reviewed.

Study Selections: Authoritative and peer-reviewed research articles relevant to the topic were chosen for review. Selected regulations and guidance documents are current and relevant to food allergen extracts.

Results: Preparation of a food allergen extract may require careful selection and identification of source materials, grinding, defatting, extraction, clarification, sterilization, and product testing. Although extractions for all products licensed in the United States are performed using raw source materials, many foods are not consumed in their raw form. Heating foods may change their allergenicity, and doing so before extraction may change their allergenicity and the composition of the final product.

Conclusion: The manufacture of food allergen extracts requires many considerations to achieve the maximal quality of the final product. Allergen extracts for a select number of foods may be inconsistent between manufacturers or unreliable in a clinical setting, indicating a potential area for future improvement.

Introduction

Humans consume a wide variety of foods in their daily diet, and virtually any of these foods can cause an allergic reaction. Food allergy is a nonprotective immune response induced by exposure to certain foods or food additives.[1] In the United States, the most common allergenic foods are milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans (Table 1).[2–6] Worldwide, differences in food allergies exist based on factors that include, but are not limited to, geographic location, age, genetic variation, and dietary habits. For example, sesame is a common food allergy in Israel, whereas buckwheat and the edible nest of swiftlets, called bird's nest, are common allergens in Japan and Singapore, respectively.[7–9] In addition, some studies have found that the incidence of food allergy is higher in infants and toddlers when compared with adults and adolescents, indicating that the prevalence of food allergy slightly decreases with age.[2,10] Despite this general trend, allergies to certain foods, fish and shellfish in particular, become more common during adolescence and adulthood.[11] Age may also predict the allergen(s) to which an individual might become sensitized. For example, although allergy to cow's milk, egg, soy, and wheat is more prevalent in infants and children, peanut, tree nut, fish, and shellfish allergies typically persist in adolescents and adults.[2,12,13]

Although treatments are available for food-induced allergic reactions, the only way to prevent adverse reactions is to avoid the problematic food(s). The offending food(s) can be identified using skin prick tests (SPTs) with allergen extracts, specific IgE testing, or double-blind, placebo-controlled food challenges (DBPCFCs). Allergen extracts have been used since the early 20th century for the diagnosis and treatment of allergic diseases. Unlike with some allergies, subcutaneous immunotherapy using allergen extracts is not licensed for the treatment of food allergy.[2,14] We describe the selection of source materials, manufacturing procedures, relevant

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Table 1
Estimated Prevalence of Select Food Allergies

Food	Estimated Prevalence, %
Big 8	
Milk	0.2–2.5
Egg	0.5–5
Fish	0.4
Crustacean shellfish	2.0
Peanut	1.4
Tree nut	1.4
Wheat	<1
Soybean	<1
Other allergens	
Fruit	0.1–4.3
Vegetable	0.1–1.4
Any food allergy	3.5–4.0

regulations, and standardization efforts for food allergen extracts and challenges associated with developing allergen extracts for particular foods.

Methods of Collecting, Identifying, and Processing Food Source Materials

The appropriate selection of the starting source material and all subsequent manufacturing steps contribute to the final quality of an allergen extract. Once the optimal conditions are established, deviations in any of these procedures may result in increased heterogeneity of allergen extracts between manufacturers or even between production lots of a single manufacturer.

Some allergen extract manufacturers purchase source materials from source material suppliers who have already processed foods from vendors into a form ready to use in manufacturing, whereas others maintain divisions dedicated to acquiring source materials directly from food vendors and processing the materials themselves.[15–17] Food source material may be obtained in powdered, liquid, or freeze-dried forms, but processing should be minimal. Ideally, food sources should be fresh or frozen and should be of a quality suitable for human consumption.[18,19] Careful consideration of the origin of source materials may be important to preserve lot-to-lot consistency of the final product.[20] Controlled collection, storage, and processing of the raw materials also enhances consistency.[21] Changes in cultivars, climate, timing of source material collection, geography, or environmental conditions may produce inconsistent levels of specific allergens in source materials.[15,22] Manufacturers may align their production schedules with the harvest season of a particular food or may freeze or freeze-dry it for storage on receipt of the fresh food.[23]

Special attention should also be paid to identification and purity of source materials to minimize heterogeneity.[20] For food allergies, misidentification of source material may result in the production of an improperly labeled allergen extract and incorrect diagnoses for patients. Inaccurate diagnosis of food allergy, based on skin testing alone, may cause patients to avoid foods they are able to tolerate while inadvertently failing to avoid truly problematic foods. Surveys have found that certain foods, particularly fish, may be mislabeled by US wholesalers, restaurants, and grocery stores; a 2016 review of studies published since 2014 revealed a normalized mean rate of seafood fraud of 28% in the United States.[24] In a 2014 study conducted by the Center for Food Safety and Applied Nutrition at the US Food and Drug Administration (FDA), 15% of tested fish samples ($n = 174$) across 14 states were not labeled in accordance with the FDA Seafood List.[25]

Considering these findings, it is important that source materials be properly identified. Although definitive chemical or biochemical procedures are not used at this time in the United States,[15,26] most foods can be identified definitively by gross appearance. In particular, fish and other seafood may be identified with reasonable certainty by visual appearance alone if the whole organism is purchased rather than fillets or fragments. When whole organisms are unavailable, manufacturers can consult the FDA's Regulatory Fish Encyclopedia, which includes high-resolution photographs of fish fillets and isoelectric focusing electrophoresis tissue protein patterns and mitochondrial DNA sequencing information. By sequencing a 600-base pair segment of the highly polymorphic mitochondrial gene cytochrome oxidase I, an organism can be taxonomically identified, an approach called DNA barcoding.[27] In addition, databases such as the Fish Barcode of Life initiative, Catalog of Fishes, and FishBase may assist in seafood identification.

Regulatory Considerations for Source Materials

Regulation of source materials is the responsibility of FDA in the United States and the European Medicines Agency (EMA) and European Pharmacopoeia (EP) in Europe. Requirements for source materials used in the production of allergen extracts licensed in the United States are specifically addressed in 21 CFR §680. The FDA requires that licensed extract manufacturers provide a listing of the manufacturer's source material suppliers.[28] Manufacturers are responsible for ensuring that suppliers of source materials are qualified and that procedures for collection and identification of source materials are appropriate. In addition, animals used as food source materials must have been in good health,[29] and source materials must be used fresh or appropriately stored.[30]

The 1999 FDA guidance document for allergenic product manufacturers provides additional information recommended for products licensed in the United States.[18] Manufacturers should identify source materials by genus, species, common name, and microscopic and macroscopic characteristics.[18,26] For foods, the guidance specifies that canned and processed foods are not to be used as source materials. In addition, the batch production record includes the packaging label from the store where the food is purchased. If no packaging label is available, the location and identity of the supplying store are identified.

Regulation of source materials in Europe is similar: control methods, acceptance criteria for identity and purity, and controlled storage conditions are particularly important. In addition, information detailing source materials suppliers, specifications, quality control methods, storage conditions, and identification are provided. The origin of food source materials is maintained constant to provide uniformity of the licensed product.[31] The procedure for any source materials that have been pretreated (eg, flour, spices) is described. For meat, fish, and seafood, any veterinary and microbiological controls to which the animal or source material was subjected are indicated. If certain part(s) of the animal are used, the procedure for its isolation and treatment is included.[32]

Manufacturing Allergen Extracts

Although the scale and available technologies for extract preparation have changed markedly since the process was first described, many of the manufacturing steps remain unchanged.[33,34] In general, the following procedures apply to the preparation of food extracts: grinding, defatting, extraction, clarification, sterilization, and product testing (Table 2).[23,33] After careful selection and preservation of the allergen source material, foods may undergo preliminary processing, such as grinding or blending, which increases the surface area of the material before extraction. Defatting, the removal of fats, oils, and or waxes using solvents, may be performed. Manufacturers remove these substances to improve exposure of allergenic proteins and extraction efficiency and to remove components insoluble in water.[23] Foods with high water content (such as fruits and vegetables) are usually not defatted; meats, fish, and nuts are often defatted.[34]

Table 2
Manufacturing Workflow for Food Allergen Extracts

Selection of source materials[a]
Foods for use as source materials should be fresh or frozen. Consideration of cultivar, climate, geography, and environmental conditions may be made.
Collection of source materials[a]
Controlled collection conditions, including timing, may increase consistency of the final product.
Identification of source materials[a]
Foods are usually definitively identified by gross appearance alone. Biochemical methods may be used to complement this approach.
Storage
Foods are stored under controlled conditions. Harvested food source materials are frozen or freeze-dried if not immediately proceeding to extraction.
Grinding
Grinding increases surface area of the source material prior to extraction.
Defatting
Defatting source materials with organic solvents removes fats, oils, and/or waxes and improves extraction efficiency.
Extraction
Extraction solubilizes the allergens from the source material in a buffered aqueous solution.
Clarification
Clarification removes solid source materials through a series of filtration steps.
Sterilization
Allergen extracts are sterilized using a 0.2-μm filter.
Product evaluation
Manufacturers test the final allergen extract for sterility, general safety, pH, and preservative content.

[a]Some allergen extract manufacturers purchase ready-to-use source materials from suppliers and do not directly participate in these steps.

Grinding and defatting are followed by extraction, which solubilizes the allergens in the source material into an extracting fluid. Extraction procedures can vary considerably: the extraction ratio (weight of raw material to volume of extracting fluid), extraction time, extraction temperature, and composition of the extraction solution all determine the yield of the allergen in solution. Extraction generally takes place in a buffered, slightly alkaline solution[23]; optimal allergen extraction is buffer dependent, and extraction buffers should ideally be individually optimized for each allergenic food.[35] The solution used for extraction is determined by the desired final product formulation. For aqueous extracts, a buffered saline solution with preservatives, such as phenol or glycerin, is used for extraction[23,36]; in some protocols, glycerin is added after extraction. Allergen extracts may also be distributed as lyophilized products, but no lyophilized food allergen extracts are licensed for distribution in the United States.

After extraction, the solid source materials are removed through the process of clarification. The extract is clarified using a succession of increasingly fine filters.[23] The selected filters must be compatible with the extract, not leach chemicals into the product, and not adsorb the extracted allergens.[23] Some manufacturers may use additional steps, such as dialysis or ultrafiltration, to further clarify the extract or remove low-molecular-weight compounds. The final manufacturing steps for allergen extract are sterilization and product testing. Allergenic extracts are thermolabile and must be sterilized using aseptic filtration, using a 0.2-μm-pore sterilizing filter.[23,33] Final product evaluation by manufacturers includes testing for sterility (for both anaerobic and aerobic microorganisms), general safety, pH, and preservative content.[23]

Because no food allergen extracts have been standardized, manufacturers label the final product with either protein nitrogen units, a measure of total protein content, or extraction ratio (wt/vol) to reflect how the product is manufactured. In Europe, qualitative profiling for allergen content may be performed using electrophoretic methods.[36] In addition, consistency and composition of manufactured food allergen extracts may be assessed through use of in-house references.

Regulatory Considerations for Manufacturing Procedures

In the United States, manufacturing requirements for allergen extracts are described in 21 CFR. §600, §610, and §680. A specific emphasis is placed on manufacturing procedures for product consistency, particularly because no potency or composition standards exist for food allergen extracts. Manufacturers provide written standard operating procedures in their biologic product license file. These procedures include information regarding extraction solutions and their components; complete details of the production; all quality control tests and release limits; procedures for packaging and labeling; storage temperatures and systems for controlling them; expiration dating information; product release procedures; shipping procedures; and records preparation, verification, and retention. Manufacturers also test products for identity, potency, and sterility.[37] Product labeling is consistent with FDA regulations to ensure that the labeling contains the essential scientific information for the safe and effective use of the product.[38] Correct and consistent nomenclature must be used for licensed allergenic extracts.[39]

The EMA and EP maintain similar standards for allergen extract manufacturers.[31,40] The EP requires manufacturers to report the extraction ratio, use manufacturing conditions designed to minimize enzymatic degradation, design purification procedures to remove irritants and nonallergenic components, and justify the addition of any antimicrobial preservatives. It also requires that extract identity be confirmed using an in-house reference preparation. The finished product is tested for water content (lyophilized products), sterility, microbial contamination, protein content, and protein profile. Some additional tests may be applied, including aluminum and calcium content, allergen profile, total allergenic activity, and individual allergen content.

EMA Directive 81/852/EEC, amended by EMA Directive 92/18/EEC, applies to allergen extracts. The EMA also provides additional guidance information for manufacturers submitting marketing authorization applications for these products. Like the FDA, the EMA states that manufacturers should describe the production process, step by step, using a diagram. Each manufacturing step should be clearly explained, and the point at which aseptic precautions are introduced should be identified. In-process controls, purification methods, and fractionation methods should also be reported. Production, characterization, and use of an in-house reference preparation should be described. Appropriate use of an in-house reference is essential for batch-to-batch consistency. In these applications, manufacturers include safety, efficacy, and stability data as well. Total allergenic activity of the finished allergen extract is measured and reported. Sterility testing is performed in accordance with the EP. In addition, the EMA's Committee on Medicinal Products for Human Use has issued a document for allergen products that provides further elaboration on this guidance.[32]

Cooked vs Uncooked Food

In the United States, all food allergen extracts are manufactured from raw food source materials based on the frequent observation that processing reduces the allergenicity of foods. However, there is evidence that for some foods certain thermal or nonthermal processes may enhance allergenicity (Table 3).[41] To the degree that processing might enhance allergenicity of a food, processing of the food source material before extraction could be expected to increase the likelihood that certain relevant allergens will appear in the allergen extract.

It is challenging to generalize the results of published studies regarding the effects of cooking on allergenicity because of the varying cooking methods used and the different time-temperature combinations used. The IgE-binding capacity of an epitope may be unchanged after heating. Heating of food can cause substantial

Table 3
The Effects of Heating on the Allergenicity of Certain Foods

Food	Thermostable allergens	Thermolabile allergens	Tolerance to ingestion of heated food
Egg		OVM, OVT, OVA, α-levitin	Some patients can tolerate baked or heated egg
Milk	Caseins	Whey proteins (α-lactalbumin, β-lactogloblin)	Some patients can tolerate baked milk
Peanut	All		Roasting peanuts increases allergenicity, but boiling or frying reduces allergenicity
Tree nuts	Almond (amandin), walnut, hazelnut, Brazil nut, pecan, pistachio (dry roasted)	Almond (lower MW allergens), cashew, pistachio (steam roasted)	No
Wheat	Most		No
Shellfish	Tropomyosin		Boiling increases the allergenicity of shrimp
Fish	Parvalbumins		Canned fish demonstrates reduced allergenicity

Abbreviations: MW, molecular weight; OVA, ovalbumin; OVM, ovomucoid; and OVT, ovotransferrin.

changes in the allergenic nature of the food by altering the protein and/or glycoprotein structure of relevant epitopes, thereby modulating the IgE-binding capacity.[11,42] In addition to the more predictable changes in protein structure after heating, proteins in processed food may also interact with other components, such as other proteins, fats, and sugars, resulting in a matrix effect. This effect results in the decrease in the availability of allergenic proteins to interact with the immune system and a subsequent decrease in allergenicity. For example, heating of the milk allergen β-lactoglobulin decreases its allergenicity because of the formation of intermolecular disulfide bonds and its complex formation with other food proteins.[43] Below is a brief overview on the effects of thermal processing on the allergenic nature of the most common food allergens.

Egg

The primary chicken egg allergens, ovomucoid (OVM or Gal d 1), ovalbumin (OVA or Gal d 2), ovotransferrin (OVT or Gal d 3), and lysozyme (LYS or Gal d 4), are present in egg white.[44] Egg yolk contains the allergen α-levitin (Gal d 5), also known as chicken serum albumin, and has low levels of allergenicity.[44,45] In vitro studies have found that heating egg decreases the IgE-binding capacity of OVM, OVT, and OVA, thus reducing its allergenicity.[46] The allergenicity of α-levitin was reported to be significantly decreased but not completely eliminated by heating.[47] In some cases of egg allergy, children tolerate baked or heated forms of egg better than its raw form.[48] Furthermore, introduction of baked egg in the diet of allergic children may accelerate the development of tolerance to regular egg compared with strict avoidance.[49]

Milk

Milk allergy is one of the most common food allergies in infants but is usually outgrown by adulthood.[2] The known allergens of cow milk are caseins and globular whey proteins (α-lactalbumin and β-lactoglobulin).[50] Caseins lack a 3-dimensional structure, instead forming micelles in solution, and are heat stable. IgE from milk allergic patients preferentially binds to the linear epitopes of caseins, indicating that IgE binding is not sensitive to denaturation.[51] Unlike in caseins, the binding of human IgE to α-lactalbumin and β-lactoglobulin is reduced when milk is heated.[52,53] Pasteurized and homogenized milk, which is readily commercially available and commonly consumed, is potentially more allergenic than raw milk in allergic individuals.[54] In clinical studies, baked milk products that contain wheat are tolerated by 75% of children with milk allergy.[55] Furthermore, the introduction of baked milk in the diet of children allergic to milk appears to accelerate the development of tolerance to raw milk when compared with strict avoidance.[56] The IgE-binding capacity and stability of milk proteins largely depend on temperature and duration of thermal processing, pH, and food matrix effect and can also be greatly variable among patients. Further studies are therefore necessary before baked milk can be safely considered for introduction into the diet of allergic children.

Peanuts

The major allergens in peanut are vicilin seed storage protein (Ara h 1) and conglutin (Ara h 2). The minor peanut allergens include glycinin (Ara h 3, previously Ara h 4), profilin (Ara h 5), other conglutin family members (Ara h 6, 7), and peanut agglutinin (Ara h agglutinin). Roasting peanuts decreases the solubility of the major peanut allergens, whereas IgE binding remains unchanged[57] or increases.[58] Maleki et al[58] found that roasted peanuts have increased allergenicity, approximately 90-fold higher than raw peanuts, and that the protein modifications caused by the Maillard reaction contribute to this effect. The Maillard reaction is a form of nonenzymatic browning in which amino acids react with reducing sugars. In addition, boiling or frying peanuts significantly decreases the IgE binding of Ara h 1, Ara h 2, and Ara h 3 when compared with raw or roasted peanuts.[59] A 2015 study found that the SPT wheal sizes and IgE-binding properties of peanut protein extracts are significantly lower when extracts were made from boiled compared with raw peanuts and when extracts from raw, fried, and roasted peanuts are subjected to specific conditions of heat and pressure compared with their untreated forms.[60]

Tree Nuts

Tree nuts include almond, cashew, walnut, hazelnut, Brazil nut, pecan, and pistachio. Allergens in most tree nuts, including almond (amandin, Pru du 6),[61] walnut,[62] hazelnut,[63] Brazil nut,[64] pecan,[65] and pistachio (dry roasted)[66] nuts, have antigenic stability after heating in in vitro studies, but binding to human IgE is reduced after heating for almond (lower-molecular-weight allergens),[61] cashew,[67] and pistachio (steam roasted)[66] nuts. In a DBPCFC study, the allergenicity of roasted hazelnut is considerably reduced compared with raw hazelnut, but clinical symptoms are not reduced in all patients. Thus, roasted hazelnut cannot reliably be consumed by hazelnut allergic patients.[68] To further evaluate the effects of heating on the allergenicity of other tree nuts, future studies using DBPCFCs are warranted.

Wheat

Although some allergenic wheat proteins are destroyed by baking, others remain stable. In addition, some proteins become more resistant to digestion with pepsin after heat treatment.[69] This decreased protein digestibility is the result of protein modifications that involve not only protein breakdown but also aggregation, cross-linking, and Maillard-type reactions. Because the crust is subjected to higher temperatures than the crumb during baking, protein aggregation is also greater in the crust, and the solubility and allergenicity of these protein aggregates are different from those found in the crumb.[69]

Fish and Shellfish

The allergens in fish are the calcium-binding parvalbumins (eg, Gal c 1). The allergenicity of fish proteins is not affected by heat treatment, such as boiling or frying.[70] However, the IgE-binding activity is significantly reduced in canned fish because of the extreme temperature and pressure used during canning.[71] Tropomyosin, the major allergen present in shellfish, is thermostable,[72] although boiling increases the allergenicity of shrimp.[73]

Relevance of Panallergens and Cross-reactivity Considerations

Each food contains several glycoproteins that are potential allergens. These glycoproteins have specific physicochemical properties and are broadly divided into 2 classes: class 1 and class 2 allergens.[74] Class 1 (complete) food allergens range from 10 to 70 kDa in size, are water soluble, are resistant to heat and gastric digestion, and are not affected by food processing or preparation. They are capable of inducing IgE sensitization after absorption through the gastrointestinal mucosa[74] and are typically responsible for systemic allergic reactions.[75,76] In contrast, class 2 (incomplete) food allergens (also known as cross-reacting allergens) are sensitive to heat and gastric digestion and do not cause gastrointestinal sensitization but are capable of producing allergic reactions in patients already sensitized through the respiratory route to cross-reactive aeroallergens.[76,77] Cross-reactive ubiquitous allergens belonging to widely different protein families, well preserved throughout various species and able to trigger IgE antibody binding, are known as panallergens.[78] Because of protein homologies, cross-reactivity among the different tree nuts and between tree nuts and pollens is common. For example, the birch pollen allergen Bet v 1, a PR-10 family member, is cross-reactive with Cor a 1 in hazelnut.[79] A 2009 study in mice found a high level of cross-reactivity between cashew and walnut but a weaker cross-reactivity between cashew and peanut.[80] Although such cross-reactivity may be found in vitro or with SPTs, it may not always be evident clinically.[81] Other reports on tree nut cross-reactivity indicate that patients allergic to one nut should avoid all nuts because they are likely to react to others.[82] Finally, consistent clinical reports of cosensitization to latex, banana, avocado, chestnut, and kiwi appear to be due to cross-reactivity of specific latex and fruit allergens, a so-called latex-fruit allergy syndrome; primary sensitization is usually to latex protein.[83]

Fruit and Vegetable Allergy Associated With Pollenosis

Allergies to uncooked fruits and vegetables usually arise from prior sensitization to pollen aeroallergens through the respiratory exposure. This phenomenon is known as oral allergy syndrome or pollen-associated food allergy syndrome.[77] The high degree of structural similarity of allergenic molecules derived from closely related or functionally similar molecules within the same protein family may lead to IgE cross-reactivity.[84] For example, patients allergic to the birch pollen allergen Bet v 1 may develop allergies to fruits in the Rosaceae family (eg, apple, strawberry, peach, plum, pear) and vegetables in the Apiaceae family (eg, carrot, celery).[85] Other examples of pollen-associated food allergy syndromes include sensitization to profilins, associated with grass pollens, leading to reactions to tomato and peach,[86] and sensitization to cross-reacting carbohydrate determinants, leading to reactions to vegetables in the Cucurbitaceae family (eg, pumpkin, melon, squash, cucumber).[87] Other instances of cross-reactivity between aeroallergens and fruit and vegetable allergens are documented.[77] This knowledge is of great clinical importance to individualize treatment and instruct patients to avoid foods that have potentially cross-reactive proteins.

Standardization Considerations

The inherent variability of complicated biologic products, such as allergen extracts, presents a considerable challenge for standardization efforts. Variations in source material selection and extraction procedure, among other variables, contribute to the potential for substantial heterogeneity among products from different manufacturers and even among production lots of a single manufacturer. In most cases, the identity of the active ingredients is uncertain. This uncertainty presents a particular problem to regulatory agencies tasked with ensuring the efficacy, safety, and consistency of these products. Historically, a number of approaches have been used to address such concerns; an international scientific consensus has not been achieved.

In the United States, nonstandardized allergen extracts, including all food extracts, are labeled with either the extraction ratio or with protein nitrogen units per milliliter, using the Kjeldahl method.[21] Neither designation is particularly informative or strongly correlates with the overall biological potency of allergen products. The Center for Biologics Evaluation and Research at the FDA maintains a reference standard for 19 standardized allergen extracts (Table 4) and designates procedures to compare a manufactured product to the reference. The reference extract is assigned a particular unitage, and the manufactured extract is therefore assigned a relative potency in relation to the standard. Bioequivalent allergy units are assigned based on a quantitative intradermal skin test titration in highly allergic individuals; other standardized units include allergen-specific unitage (Amb a 1 or Fel d 1 units), allergy units per milliliter, and mass units, as appropriate.[21,88] Release limits are set such that the manufactured extract must be statistically equivalent to the reference extract at a specified confidence level.[21] Proteomic profile comparison of the manufactured extract to the reference standard may also be part of the assessment of standardized extracts.

The approach to standardization is considerably different outside the United States. Rather than complying with a single reference standard, the European method is based on comparing manufactured products to in-house reference preparations, which are unique to each manufacturer. Another challenge is that Europe lacks a common label for allergenicity. Most manufacturers follow one skin test–based protocol (the Nordic system) for biological standardization, but other manufacturers apply FDA's $ID_{50}EAL$ (intradermal dilution for 50 mm sum of

Table 4
Standardized Allergen Extracts Licensed for Use in the United States

Epidermal extracts
Cat hair (*Felis domesticus*)
Cat pelt (*Felis domesticus*)
Insect extracts
Mite D.f. (*Dermatophagoides farinae*)
Mite D.p. (*Dermatophagoides pteronyssinus*)
Pollen extracts
Bermuda grass (*Cynodon dactylon*)
Kentucky (June) bluegrass (*Poa pratensis*)
Meadow fescue grass (*Festuca elatior*)
Orchard grass (*Dactylis glomerata*)
Redtop grass (*Agrostis alba*)
Perennial ryegrass (*Lolium perenne*)
Sweet vernal grass (*Anthoxanthum odoratum*)
Timothy grass (*Phleum pratense*)
Short ragweed (*Ambrosia artemisiifolia*)
Venom or venom protein extracts
Honeybee venom (*Apis mellifera*)
Wasp venom protein (*Polistes spp*)
White faced hornet venom protein (*Dolichovespula maculate*)
Yellow hornet venom protein (*Dolichovespula arenaria*)
Yellow jacket venom protein (*Vespula* spp)
Mixed vespid (mixed yellow jacket, yellow hornet, and white faced hornet)

erythema diameters determines the allergy unit) protocol.[88,89] Applying these 2 approaches can lead to very different results.

There are several novel approaches on the horizon for standardization of allergen extracts. The European Union Certified Reference Materials for Allergenic Products and Validation of Methods for their Quantification (CREATE) project worked to develop certified recombinant reference materials and validated monoclonal antibody–based immunoassays for measurement of specific allergens.[89] Other researchers have developed additional assays for quantification of major allergens; examples include assays for Ara h 1 and Ara h 2.[90] Another potential method for standardization would use tandem mass spectrometry (MS/MS). MS/MS-based approaches have the advantage of simultaneous detection of many allergens and their unique isoforms.[22] The multiplex allergen extract potency assay has only been applied to aeroallergens so far but may be applied to complex food allergens as well.[91]

Ultimately, regulatory authorities must balance 2 competing priorities. They must consider the need for increased regulatory requirements for product safety and efficacy and public health. However, they must not make regulatory demands so arduous to manufacturers that these companies become limited in their ability to offer a wide range of food allergen extracts.[92] Maintaining a diverse portfolio of these products is important for accurate diagnosis of food allergy in clinical practice.

Relevant Extracts for Clinical Practices

Allergen extracts are used for the diagnosis and sometimes the treatment of allergic diseases. For food allergies, allergen extracts are used to identify the offending food(s). Because there is no cure for food allergy, strict avoidance of allergenic foods is used to prevent food allergy–induced adverse reactions. Unlike some allergies, subcutaneous immunotherapy using allergen extracts is not licensed for the treatment of food allergy, although other routes of immunotherapy administration (eg, oral or sublingual and epicutaneous) are under investigation. In addition, experienced practitioners and investigators have long been aware of the limitations in using food allergen extracts, all of which are nonstandardized, for accurate diagnosis and management. Variability among manufacturers and lots of a given manufacturer raise the possibility that specific allergens within a food allergen extract will be underrepresented or missing in the extract used for testing, rendering the test results unreliable.[93] In the case of fruit and vegetable allergy diagnosis, commercially prepared extracts may lack specificity and sensitivity in SPTs.[94] Direct use of the unprocessed fresh fruit or vegetable in skin tests (prick and prick method) has been proposed as an alternative to the use of licensed extracts.[95] Investigational attempts at molecular-based diagnostics combined with recombinant allergens or hypoallergens represent efforts to confront these issues.[96,97]

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