

The Manufacture of Allergenic Extracts in North America

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Introduction

The disease state defined as allergy has been reported since the 1800s, beginning with the description of hay fever by Bostock in 1819 (1). In 1873, Dr. Charles Blackley of Manchester proved grass pollen to be the cause of hay fever by performing nasal challenges and skin tests on himself (2). The first published study of allergenic extracts being used for immunotherapy was in 1911, when Noon and Freeman showed that hay fever could be effectively treated by injections of pollen extract (3). Based on those early studies, treatment of allergic individuals with injections of extracts of the offending material evolved to the practices that are used today. As the practices became accepted and expanded in their uses, the industry dedicated to the manufacture of the extracts needed for diagnosis and treatment was established.

Although the overall process of extracting the soluble material from the allergy-causing source materials into a liquid medium is seemingly quite simple, there are actually many aspects to be considered, from the selection and processing of the raw material through formulating treatment mixtures specific for a given patient.

Although the basic concepts remain the same, each manufacturer has developed variations in the equipment, reagents, and processes that are used to produce these extracts. As a result, the extracts from the different manufactures may exhibit subtle differences that the routine testing technologies are unable to detect. Thus, it is important to know what these equipment/reagent/processing differences may be in order to better understand the possible effects they may have on patient reactivity and treatment.

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This chapter will describe some of the parameters that may be used by manufacturers of allergenic extracts. The advantages/disadvantages and possible effects on treatment of patients must be evaluated and assessed by the practitioner using these products.

Types of Allergenic Extracts

In general, allergenic extracts are available in four types, described in the following sections.

Aqueous

Aqueous allergenic extracts are perhaps the most commonly prescribed dosage form of allergenic extract in North America for immunotherapy. Aqueous extracts are water-based, usually a buffered saline solution with a phenol preservative and contain either no glycerin or glycerin in insignificant amounts. Being less viscous than their glycerinated counterparts, aqueous extracts are easier to process (particularly through the filtration steps) and as such may be available in higher concentrations than the 50% glycerinated extracts. Because of the lack of glycerin (glycerin > 2% [w/v] can cause false positive intracutaneous skin tests)(4) these extracts are suitable for intracutaneous skin testing and because they do not sting on subcutaneous injection, they cause less discomfort than glycerinated extracts. The major concern with aqueous extracts is their limited stability. Many allergens such as some grass pollen extracts are simply not stable for more than a few months even at higher concentrations (5,6). To minimize the problem, stabilizers such as human serum albumin (HSA) 0.03% or glycerin at 10% (v/v) may be added to extend the shelf life of these extracts. Formation of precipitates can also be an issue. Without glycerin many aqueous extracts, particularly those in high concentrations, can develop sediments over time. Decanting and or filtration in the clinic to overcome this add to the overall cost of the product.

Glycerinated

This type of extract generally contains glycerin at a concentration of 50%. Some manufacturers prepare the extraction fluid with saline (generally 0.5% sodium chloride) and a buffering agent (generally 0.275% sodium bicarbonate), and include the glycerin in the extraction fluid. Other manufacturers include phenol at 0.4% as a preservative in the extraction fluid, and do not include the glycerin in the extraction fluid. When this process is used, the glycerin is added to achieve the indicated concentration (50%) after the material has been extracted and clarified. The advantage to using this type of product is a longer expira-

tion date than that of the aqueous extracts because the glycerin is an effective protein stabilizer (7–10). The disadvantage is the patient discomfort (generally a stinging sensation at the injection site) and increased viscosity owing to the glycerin.

Lyophilized (Freeze-Dried)

This type of product is manufactured by extracting the soluble components into a liquid medium, which may consist of water or water that contains an excipient, such as mannitol, that will provide bulk to the final product. After the material is extracted, the solution is dispensed into vials, and the product is then frozen and the water removed from the product through sublimation under vacuum. The stoppers are then seated on the vials either while the chamber is still under vacuum, or after a filtered, nonreactive gas (such as nitrogen) has been introduced. The advantage of this type of product is greatly enhanced stability (without the discomfort associated with glycerin) when the product remains in the freeze-dried state. The disadvantage includes the extra handling and manipulation for reconstitution of the product prior to use.

Alum-Precipitated

Alum-precipitated extracts are prepared from aqueous extracts. Aluminum Potassium Sulfate (Potassium Alum) is slowly added to an aqueous extract. At a neutral pH, the Alum will form a gel in the extract. Protein, and presumably the allergens, will adsorb to the gel (11,12). Nonadsorbed extract is washed off and the final precipitate is suspended in a saline solution. The principle advantage of this dosage form is the reduced frequency of systemic reactions (11,13,14). Alum precipitates are widely used in Europe but are less common in North America, where they tend to be used only on highly sensitive patients that are unlikely to tolerate aqueous extracts. Cost, limited product line, and potential for injection granulomas (15,16) are the disadvantages of these extracts.

Classes of Products

Because there are literally thousands of materials that people have claimed cause their allergies, there is quite a range of different products for which extracts are, or have been, available. In general, however, the products will be in one of the following classes: pollens, fungi, animal-derived, foods, insects/arthropods, and miscellaneous. Some special considerations relevant to each of these classes are discussed in more detail in the following section.

Pollen Allergenic Extracts

Pollen is the male gamete of flowering plants. Allergenic extracts of pollens are the most common extracts used for diagnosis and immunotherapy for Type I allergy in the United States. The vast majority of these pollens are from plants whose pollen is shed and carried by the wind (anemophilous) such as Birch, Ragweed, and grasses. To a lesser extent, those plants whose flowers may be pollinated by insects (entomophilous) but release pollen into the atmosphere at least some time during the flowering cycle such as Maple, Willow, and Mesquite can also be a factor in allergic disease.

The challenge is to collect a sufficient quantity of pure unadulterated material suitable as a source material for a sterile injectable drug. As an example of the magnitude of the challenge, several hundred kilograms of grass pollen of high purity and potency are required to supply the annual needs for US extract manufacturers.

Through experience, pollen collection companies have developed several strategies and techniques to fulfill these needs. One common method is harvesting the plant flower and stems and placing these in water in a greenhouse shortly before pollination. The pollen will be shed naturally over a period of a few days onto paper surrounding the plants. It can then be collected off the paper by gentle suction. A second type of collection is to gather the flowering heads of the plant just prior to pollination and then allow the flower to dry on paper. A third method is to vacuum pollen from the flowering heads of a pure stand of allergenic plant during pollination. Lastly, some plants are fickle in their pollination and the collector must mill the flower and then separate the pollen by mechanical or chemical methods described later.

Regardless of the collection method, additional purification is necessary usually by sieving. The collector will pass the pollen through a sieve of known size (depending on species pollens range from 15–100 μ in diameter) and larger plant parts will be captured and discarded. Organic solvents like perchloroethelene and acetone in separatory funnels may also be used. Plant parts and miscellaneous debris are dense and will sink in the solvent and pollen will float on top. Finally the pollen is vacuum dried, weighed, labeled, and stored in airtight containers away from heat and light usually refrigerated or frozen.

Scrupulous housekeeping and record-keeping during this process are critical to maintain purity and freedom from cross-contamination of the source material. These strategies help insure the supplier meeting the manufacturers' 99% purity specification (17).

Fungal Allergenic Extracts

Allergenic extracts of fungal origin are derived from pure seed cultures (usually lyophilized) from reputable biomaterial suppliers such as the American Type Culture Collection (ATCC). Manufacturers and or allergenic source-material suppliers that grow these cultures must have a separate facility suitable for processing spore-bearing organisms (18).

The cultures are grown out and inspected for identity and purity by a qualified microbiologist. The culture medium varies from manufacturer to manufacturer but commonly is a liquid medium free of allergenic protein (19,20). Some species of allergenic fungi may require slight variation in media requirements, such as pH, for optimal growth. Growth periods are specific to species but are usually 2–4 wk when growth is optimal. Most of these organisms produce spores as well as lush vegetative growth at time of harvest. With *Alternaria* at least, it seems that the same allergens appear in both spores and mycelia (21).

The two most common ways to harvest the organism are to separate the pellicle from the media, after which it rinsed and dried with acetone, or to homogenize the pellicle with the media and inactivate growth by the addition of phenol. In the former case, the dried and ground-up pellicle is extracted in aqueous or glycerinated Coca's fluid (bicarbonate-buffered saline). In the latter case, the media becomes the extracting fluid for the pellicle. The media components therefore must be removed later on in the process by dialysis. There have been no systematic studies examining compositional differences, if any, between the two methods with any particular species.

Animal-Derived Allergenic Extract

In most instances of allergy associated with animals, the offending allergens result from the hair or epithelium of the animal, or feathers in the case of birds. Therefore, it is the hair or epithelium or feathers that are used for the source materials. In some instances, the entire skin (pelt) of the particular animal species may be used. Although it is desirable to use "clean" animals, a washing or rinsing process of the animal prior to collection of the hair/dander/feathers would potentially result in the loss of the relevant allergenic materials such as urinary allergens. Caution must be exercised such that only healthy animals are used for such collections. It is also very important that the animals are not infected with external parasites. Animals are inspected by a qualified individual for overt health prior to harvesting hair and epithelium. Horses must have been vaccinated against tetanus (17). Animal-derived source material must be traceable to a Bovine Spongiform Encephalopathy (BSE)-free environment. Once collected, the source material

must be stored under conditions that will prevent the possibility of microbial growth. This is generally refrigerated, or frozen.

For many of the animal-sourced materials, the concentration of the allergens is quite low, and thus the manufacturer may use different techniques to concentrate the allergens. One technique is to initially freeze-dry an intermediate extract, which can then be reconstituted with a lesser volume to achieve a more concentrated final product. Another technique is to precipitate certain proteins through the addition of acetone, and subsequent drying of the precipitated material, which is then extracted at specific weight: volume ratios.

Food Allergenic Extract

Although many foods are quite easy to store and extract, this is not true for foods that are perishable. Some manufacturers will plan their production schedules such that when a particular food is "in season," they will obtain the fresh material and complete the extraction process shortly after receiving the material and before the food begins to degrade. Other manufacturers will obtain the fresh food when it is in season, and will process the food, then dry or freeze-dry the food and keep it stored in that form until used to manufacture the extract when needed. Other aspects in the selection of the food source material that manufacturers may consider are what portion of the food to use (with or without the skin, with or without seeds), whether the food source is cooked or uncooked, and if the food source undergoes processing such as grinding or blending prior to extraction.

Insect and Arthropod Allergenic Extract

This class of products includes flying insects, stinging insects, and house-dust mites. The body exteriors of most insects are designed to withstand potentially adverse environmental conditions, and as such, very little protein is extracted without some type of process to macerate the insects. This is generally done with the use of equipment that will grind the insect bodies and result in increased extraction efficiency.

For the stinging hymenoptera insects, the source of the allergen is the venom, and the venom products are available for diagnosis and treatment. The venom is obtained from honey bees by collecting the venom as the insects sting through a diaphragm following electro-stimulation. The venom from the other vespids is collected following removal of the venom sac from the insects. The venom products that are currently manufactured are available only in the freeze-dried form.

Although many of the insects are collected in their natural habitat, house-dust mites and cockroaches are cultivated and harvested for use in the manufacture of allergenic extracts. It is critical that the cultures are maintained to preclude infestation by molds, bacteria, or other

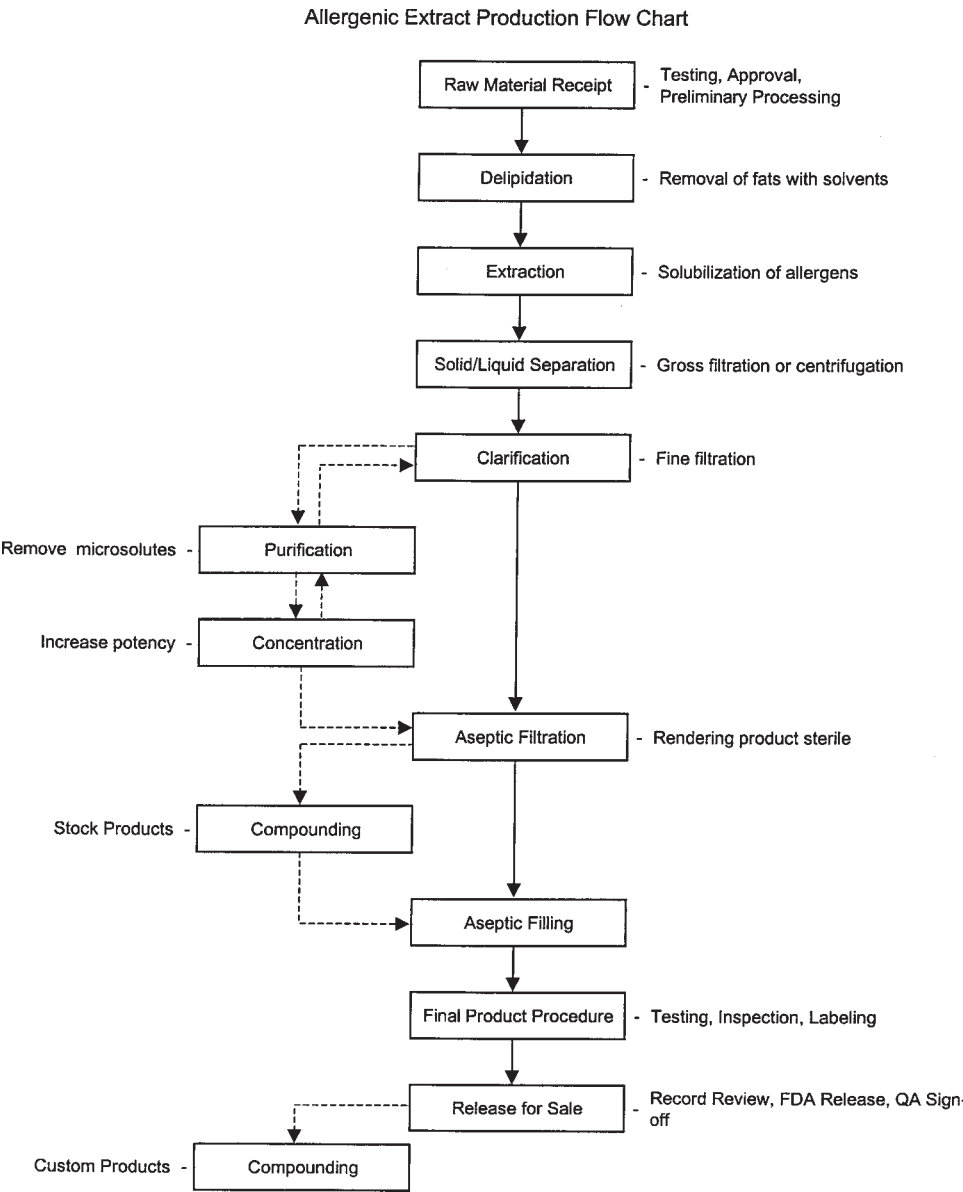


Fig. 1. General flow chart for the manufacture of allergenic extracts.

insects. Each collection that is harvested must be visually examined to ensure that the cultures have not become contaminated.

Miscellaneous Allergenic Extract

As the name implies, this is a wide range of inhalant and ingested allergens. Kapok (an organic upholstery stuffing), silk (raw cocoons), Orris root (a natural ingredient in some cosmetics), Pyrethrum (a natu-

ral insecticide from crushed chrysanthemum), and vegetable gums are some examples of extracts in this group.

Commercial Manufacture of Allergenic Extracts

The manufacture is a series of well-defined processes that convert a natural biological source material into a sterile injectable drug (*see* Fig. 1). There are variations from manufacturer to manufacturer but, in general, the following processes are the most common.

Delipidation (Defatting)

Delipidation is the process of removing fats, oils, and or waxes from allergen source materials. Most source materials of allergens, particularly pollens, animal hair, and epithelia, and foods contain a substantial amount of animal or vegetable fats. For example, 10–15% of the dry weight of Ragweed pollen consists of oleoresin found on the surface of the pollen grain (22).

There are several reasons why it is appropriate for the manufacturer to remove these substances. The most important reason is that some of these oleoresins, particularly Ragweed and related weeds of the compositeae family, can sensitize and cause a Type IV contact dermatitis (23–25). Another reason is to assure that the allergenic proteins are more exposed during the extraction procedure to make extraction more efficient. Finally, these fats are not soluble in water and therefore would render an extract either opalescent or create a waxy film on the surface of the extract.

The most critical consideration for the manufacturer is to remove the fats, oils, and waxes without damage to the allergenic proteins. Three organic solvents are widely used in the extract industry that fit this criterion; Petroleum Ether (N-hexane), Ether (diethyl ether), and Acetone (diethyl ketone). The procedure is to wash the source material gently in the solvent until the fats are removed. Manufacturers will use a number of different types of equipment to accomplish this, most commonly a distillation or reflux apparatus. Aerosols of these source materials can be created during processing and because these solvents are hazardous materials and quite volatile, the procedure is carried out in a dedicated area isolated from the main production areas. After defatting is complete, the material is allowed to dry until all traces of solvent have dissipated.

Extraction

Extraction is one of the most critical steps in the manufacture of an Allergenic Extract (26). Extraction is the process of transferring the active ingredients (allergens) from the source material and into solu-

tion in the extracting fluid. This is accomplished by the gentle mixing of the raw material with an appropriate extracting fluid at a specified temperature and time. Although the process sounds simple, and for the most part it is, it is one of the most variable procedures between different allergenic products and allergen manufacturers. The goal for the manufacturer is to get as much of the allergens into solution in roughly the same ratio (major to minor allergens) as they occur in nature while minimizing the amount of microorganisms, extraneous solutes, or other undesirable material in the resultant extract. What follows are some strategies and techniques that different manufacturers use to accomplish this.

1. Reduction in size. If not done prior to the defatting step, many source materials are reduced in particle size to increase surface area and therefore increase exposure of allergens to extracting fluid. This is not required routinely with pollens but may be done with whole-body insects such as cockroach, fungal mats, many foods such as meats, nuts, legumes, and cereal grains. Several devices are used such as ball mills, food processors, blenders, grinders, and homogenizers.
2. Extraction ratio. A manufacturer must decide what ratio of raw material to blend with the extracting fluid. This ratio is most commonly expressed as weight to volume (w/v). For example a 1:10 (w/v) on a label represents an extract that was prepared with one gram of source material for every 10 milliliters of extracting fluid. Higher source material weights to volume of extracting fluids usually results in higher gross yield of allergen per milliliter. On the other hand, less extract will be recovered because of holdup volumes and general difficulties in processing viscous extracts, particularly those containing 50% glycerin. This is of particular concern with expensive source materials such as some pollens and dust mites. Common extraction ratios for pollens are 1:10 and 1:20 (w/v). Mites usually are extracted at 1:50 to 1:100 (w/v).
3. Extraction time. In general, the longer the extraction time, the greater the yield of allergen per gram dry weight of raw material. Nonetheless, long extraction periods may allow for growth of microorganisms in the extract and an increase in extraneous marginally soluble materials that may precipitate out later on. Clearly there is a point of diminishing returns on extraction time. Extraction time should be tied to the kinetics of allergen release of the allergens from the source material into solution.

For example, Marsh found with short ragweed pollen that the major allergen, Antigen E (Amb a 1) was slowly eluted over a few days, whereas nearly all of a minor allergen, Ra 5 (Amb a 5) was released within minutes (27). Geissler and colleagues examined the

kinetics of allergen release from house-dust mites and found in their lab that almost all of the relevant allergens are released after only four hours (28). All extraction parameters are established based on internal validation work by the extract manufacturers. Extraction periods vary from product to product but are generally in the range of overnight up to 4 d.

4. Temperature of extraction. Extraction is typically conducted at either refrigerated (1–5°C) temperature or controlled room temperature (20–25°C). In general, the higher the temperature the more rapidly these allergens will be solubilized. There must be balance with the possibility of build up of microbiological flora. Manufacturers that use higher temperatures limit themselves to shorter extraction times and or use effective antimicrobial agents in the extracting fluid such as glycerin and phenol.
5. Extraction fluids. Proteins are generally more soluble in saline solutions than in distilled water. Accordingly, most manufacturers use a saline solution for extraction. The most common is a slightly alkaline (pH 8.0–8.5) bicarbonate-buffered saline (NaCl 0.5%, NaHCO₃ 0.275% in water for injection) also known as Coca's fluid (29). To this manufacturers may add glycerin and or phenol as bacteriostatic agents. Many manufacturers will use a combination of extracting solutions including a physiologic saline (0.9% NaCl) and or a phosphate-buffered saline (PBS) also with or without preservative (29).

Solid/Liquid Separation

Once extraction is complete, the insoluble portion of the source material must be removed. The manufacturer physically separates the spent material not in solution from the liquid extract. If the volume is small, the manufacturer may centrifuge the batch. Larger lots are usually passed through a coarse filter paper. The crude extract is now ready for additional filtration.

Clarification

Clarification is the process of passing the extract through a series of filters of decreasing pore size. After a gross filtration or centrifugation a manufacturer will begin at around a 20–30 μ pore size and sequentially work down to 0.2 μ porosity. Crude extracts contain much high molecular-weight material held in suspension that will render the product murky. The manufacturer must use filters that are compatible with the particular extract, and that have been shown not to shed residual chemicals or extractables into the product (22,30). Further these filters cannot adsorb any appreciable amount of allergen onto their surface. Because this property is critical to not only allergens but other

injectable biologicals as well, filter companies have developed relatively inert filter substrates such as Nylon[®] or Teflon[®].

Purification

A manufacturer always tries to use the purest source material possible. However, when a source material by its very nature or collection or propagation method may theoretically possess substances that could either be potentially toxic, uncomfortable, or obscure skin-test results (for skin-test reagents), a manufacturer is obliged to remove or at least minimize their existence in allergenic extracts. This is particularly true of fungal extracts, some food extracts, some epithelia, hair, or feather extracts, and some dust extracts. Manufacturers have developed various strategies to deal with this on a commercial level. The following are some examples.

1. Acetone washing and acetone fractionation. Washing fungal mats with acetone can physically remove media components, metabolites, and fungal toxins. Cold acetone when mixed with extracts at an acid pH will precipitate allergens but not low molecular-weight contaminants. These precipitated allergens can then be separated from the crude extract and redissolved in a saline solution.
2. Dialysis. Low molecular-weight substances may be removed by dialysis. Generally this is accomplished by the use of dialysis tubing of a molecular-weight cutoff such as 3–5 Kd to allow low molecular-weight material such as vasoactive amines, mycotoxins, herbicides, and pesticides, to pass through the tubing and be replaced with purified water over time. A variation of this is diafiltration. In diafiltration the product is passed continually into a hollow fiber cartridge with about the same porosity (~3 Kd). Low molecular-weight material is removed and, as in the first example, replaced with purified water.

The end-point of dialysis may be set as a specific number of exchanges of water that would calculate to a 2 log or greater reduction in microsolutes. Another method is to test for the absence of a low molecular-weight ion that was present in a predetermined amount in the crude extract such as the chloride ion.

Concentration

Many allergenic extracts, even when extracted at a low ratio of source material to extracting fluid, may not be potent enough as a skin-test reagent for prick/puncture testing or contain sufficient allergen for effective immunotherapy. Over the years, allergen manufacturers have developed methods to concentrate these allergens without denaturing

the active ingredients. Acetone precipitation was discussed in the purification section. This method not only purifies but also can concentrate by redissolving the precipitate in a lesser volume of solution.

Another traditional method for concentration is freeze-drying (lyophilization). Briefly described, a volume of liquid extract is placed in flat pans on shelves in a freeze-drier. The product is frozen and then vacuum is applied to the chamber. The ice in the extract under vacuum will sublime (be converted to water vapor). The temperature of the product is slowly increased and more water is driven off. Fine powder is left at the end of the cycle. This dried material is then dissolved in water or saline at a fraction of the original volume. For example, 10 liters of an extract may be freeze-dried and the residual powder can be dissolved in 2 L of saline, resulting in a fivefold concentration.

Another technique also discussed previously under purification is the use of hollow-fiber membranes. It is unlike dialysis, however, in that water and low molecular-weight material are pumped through the membranes and not replaced with purified water. The volume of liquid is reduced while the allergen content remains the same; concentration is thus achieved.

Aseptic Filtration

Although great care is taken in the manufacture of these products, all of the preceding steps take place with nonsterile product. The manufacturer must establish in-process controls during this period to assure they are not adding appreciably to the particulate or microbial load. Because these products can contain thermolabile allergens, products cannot be sterilized by heat, and are therefore filtered aseptically.

Aseptic filtration is the sterilizing process where the purified clarified extract is passed through a sterilizing grade filter (usually a 0.2 μ porosity) into a sterile collecting vessel. Manufacturers must study the bio-burden (number of microorganisms/mL) in their extracts and validate that their filtering process can remove these under a "worst case" scenario, e.g., several orders of magnitude higher bio-burden than what is typically found. Microorganisms are trapped on the filter while the allergen in solution passes through. At this point the extract is commonly referred to as a "sterile bulk."

Additional Aseptic Processing

The sterile bulk is quarantined and tested for sterility, potency, pH, preservative (phenol or glycerin), stabilizer (glycerin or HSA), and safety. If the extract meets the specifications set by the manufacturer, the sterile bulk will either be compounded with other bulks and or diluted for stock individual extracts, stock mixtures, or custom mixtures. Otherwise the product will be filled as a "stock concentrate" for

preparation of individual prescriptions and or finished product for sale. For example, Standardized Timothy Pollen Extract 100,000 BAU/mL may be compounded into a stock grass mixture, used to prepare a custom grass mixture, diluted to a Standardized Timothy 10,000 BAU/mL, filled into containers for eventual use in the production of patient treatment sets, or finally filled into sterile multiple dose vials for sale.

Aseptic filling is the sterile transfer of extract from the bulk container into sterile vials. Because this must be done in an open system, the maneuver is performed in a clean-room environment. A clean room is defined as an area that classifies the maximum amount of particulates and microorganisms permitted. Operators must be specially gowned, the area is constructed with nonparticulating materials that are easily washed and disinfected, and the air-handling system is H.E.P.A. (High Efficiency Particulate-Free Air)-filtered directed away from the operators (either vertical or horizontal laminar flow). The filling, capping, and sealing of the vials is done by manual manipulation or by machine. The vials and stoppers used must be sterile and nonpyrogenic and previously demonstrated by the manufacturer to be compatible with the extract. Until all manufacturing steps and associated tests are complete, the manufacturer quarantines these vials.

Final Product Procedures

Each vial is visually examined for fill volume, seal integrity, particulates, or any other defects. Labels are generated and checked for count and accuracy.

Final product testing includes sterility, potency, toxicity (animal safety testing), pH, preservative, and stabilizer. In addition, all standardized extracts, i.e., winged hymenoptera venom and venom protein, short ragweed and mixed short/giant ragweed pollen, eight species of grass pollens, cat hair and pelt extracts, and two species of house-dust mites must be sampled and sent to FDA along with a protocol that describes the manufacture and test results. These are reviewed and lot released by FDA. The lot release procedure usually takes 4–6 wk.

The extract production and test records are reviewed, retention samples are sequestered and the lot is released into inventory for sale.

The entire production cycle from receipt of source material to having product in inventory for sale usually takes from 5– 10 wk.

Product Forms

After the manufacturer has completed the production and testing of each lot of extract, the products may be filled in several different forms that are available to the market. In general, these are available as follows:

1. **Prick/Puncture Diagnostic:** This form is generally available at the most concentrated ratio available, and may be provided in vials containing a dropper assembly for ease of administration. Most extracts available for this type of diagnostic testing are glycerinated, and are intended for percutaneous administration only.
2. **Intradermal Diagnostic:** This form is generally diluted 100–10,000-fold from the concentrate based either upon a volumetric dilution, or to a specific concentration such as PNU/mL, AU/mL, or BAU/mL. These products are provided in stoppered/sealed vials, and they are generally aqueous since the glycerin forms may cause non-specific irritant reactions (4).
3. **Stock:** These products are those that the manufacturer has determined are ordered most frequently and thus warrant having product available for immediate shipment. Products in this form may be available in a variety of fill volumes, concentrations, and mixtures, depending on what a given manufacturer has opted to stock.
4. **Custom:** These products are those made for a specific order. Generally, these products differ from what the manufacturer keeps in stock, and thus become a custom order. Because the product will not be manufactured until after the order has been received, time must be allowed for the production, testing, and release of the product before it can be shipped.
5. **Treatment Sets, Refills, Custom Patient Prescriptions:** These therapeutic extracts are made with specific products (may be single product or a mix of multiple products) at specific concentrations for a given patient. Manufacturers may fill these orders by using closed systems (withdrawing and dispensing the extracts through the vial stopper using a syringe/needle) or open systems (withdrawing and dispensing the extracts with pipets prior to stoppering/oversealing the vials). Regardless of the method used, each product must be tested for sterility prior to being released. Although many physicians opt to have the specific patient formulations prepared in their office/clinic using the stock product, the advantage of using treatment sets filled by the manufacturer is the added assurance of sterility based on the manufacturer's equipment, aseptic filling conditions, and the sterility test completed on each product prior to release.

Precautions in Handling Allergenic Extracts in the Clinic

What follows is a series of practical considerations when using these products.

1. **Occupational safety.**
These extracts are water-based, and represent a minimal health risk to the medical professional handling the products. Prescription

drugs do not require Material Safety Data Sheets (MSDS). However, in the past US Occupational Health and Safety Administration (OSHA) has asked for these if the practice prepares patient treatment vials from stock concentrates. MSDSs are available from the manufacturer.

The greatest concern with these products is obviously their ability to aggravate allergic symptoms and sensitize. It is important to stress that extract in syringes should never be aspirated into the air and that all spills should be wiped up immediately.

2. Protecting the product.

A. Prescription Compounding.

These are sterile injectable drugs and as such must be handled with aseptic precautions. If the clinic is compounding prescriptions, i.e., preparing patient treatment vials from stock concentrates, it would be wise to check with the local State Board of Pharmacy to obtain the regulations and or guidelines to be followed when compounding sterile injectables. Being in compliance with these guidelines can help avoid any quality assurance issues with the practice.

B. Sterility.

These products are dispensed in multiple-dose vials. As such they will contain a bacteriostatic agent such as phenol and or glycerin. Every entry into these vials theoretically raises the risk of contamination so the number of entries should be limited. Most manufacturers have done studies on the resealability of the stoppers with multiple punctures. They can provide some specific guidance.

C. Maintenance of potency.

There are numerous variables that can effect the stability of the allergens in an extract. These include: structure of the allergen itself, proteolytic enzymes, temperature of storage, agitation, dilution, use of stabilizers, phenol, container size to extract ratio, and so on. It is not possible to study all allergens and combinations thereof. Until more studies are done to investigate some of these variables, one can make some conservative generalizations from the current data in the literature.

1. In patient treatment vials, because of the proteolytic enzymes found in some fungal and whole-body insect extracts, it is best to keep these extracts separate from pollen extracts, particularly grass-pollen extracts (9,31–34).
2. 50% Glycerin is an excellent stabilizer of allergens (7–10, 35).
3. Extracts should be kept refrigerated whenever possible (7, 35–37).
4. Weaker concentration extracts (intradermal strength and below) appear to lose potency more rapidly than concentrates and should be replaced more frequently than concentrates (31, 38, 39).
5. Aqueous (non-glycerinated) and alum-precipitated extracts lose a

significant amount of potency when frozen. If frozen and thawed, they should be discarded regardless of appearance (7, 8).

6. Allergenic extracts should never be vigorously shaken.
 7. The diluent formulation suggested by the manufacturer for their product should be used.
 8. An extract should never be used beyond its expiration date.
3. Miscellaneous concerns regarding the suitability of the product for use.

Allergenic extract in high concentrations, e.g., 1:10 (w/v), 40,000 PNU/mL, 100,000 BAU/mL, particularly nonglycerinated pollen extracts, may develop some sediment over time. In glycerinated extracts this may appear as a haze. This is not owing to bacterial contamination but rather the formulation of precipitates coming out of solution. Although this sediment also appears not to contain a significant amount of allergen as demonstrated by no significant change in potency of the supernate (22), there is the theoretical consideration that injection of large quantities of these precipitates could lead to injection granulomas in some patients.

Another common change in extracts is color. It is common for some pollens, e.g., ragweed, Johnson grass, poplars and cottonwood and food extracts, e.g., cereal grains, mushroom, and fruits, to oxidize and darken over time. This is particularly true of vials that have been exposed to atmospheric oxygen frequently such as percutaneous test vials.

If for any reason an individual questions the suitability for use of an extract, that individual should contact the manufacturer.

Future of Allergenic Extracts

A noble goal for specific diagnosis of IgE-mediated allergic disease would be genetically engineered recombinant allergens of known potency with an array of major, intermediate, and minor allergens to mimic native allergens but without the problems of potency variation, stability, and impurities. The ideal for specific immunotherapy would be the manipulation of these proteins to render them less allergenic but retain their immunotherapeutic efficacy analogous to some of the modified native allergenic extracts currently distributed in Europe.

Until such a vision is a reality, allergists must deal with native allergens. In the near term, extracts will continue to be converted piecemeal from nonstandardized to standardized. It is likely that the menu of allergens as well as concentration and vial size will continue to shrink. Today extract producers must meet the same level of compliance with FDA regulations as any drug company manufacturing injectables. It becomes cost prohibitive for the manufacturer to produce

and quality control small quantities of lesser-used extracts. FDA also inspects raw material suppliers, and they too are consolidating their numbers and offerings to manufacturers.

We also see a consolidation in number of manufacturers. It is expected that this trend will continue. In the 1980s there were 16 FDA-licensed allergenic extract manufacturers. In 2000 there were nine. There is now only one manufacturer in Canada.

Finally there appears to be a trend toward the extract manufacturer compounding individual patient immunotherapy treatments. That is, the allergist is doing the diagnostic work up and forwarding an immunotherapy treatment prescription to the manufacturer.

This is how immunotherapy is practiced in Europe. This system allows for the product to be prepared under current Good Manufacturing Practices (cGMP) conditions while removing the physician from any investment in therapeutic extracts as well as any appearance of conflict of interest in compounding and dispensing the extracts while limiting the physicians' liability for possible compounding or labeling errors.

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