

CHAPTER 9

Allergen Immunotherapy Extract Preparation Manual

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INTRODUCTION

Allergen immunotherapy was first introduced by Leonard Noon in 1911.¹ Dr. Noon originally hypothesized that patients suffering from “hay fever” were sensitive to a “toxin” contained in grass pollen. He proposed that patients would benefit by stimulating the immune system against the toxin by use of inoculations of pollen extract. These inoculations involve giving increasing amounts of allergen extracts to reduce symptoms on re-exposure to those particular allergens. The procedure has been widely used since its inception to treat immediate

hypersensitivity disorders mediated by allergen-specific IgE antibodies. These same principles hold true today, more than 100 years later, for current allergen immunotherapy.

There is good evidence that allergen immunotherapy is effective for the treatment of²⁻²⁵:

- Allergic rhinitis
- Allergic conjunctivitis
- Asthma
- Atopic dermatitis
- Insect allergy (Hymenoptera)

Multiple studies have demonstrated the effectiveness of allergen immunotherapy in these conditions for both children and adults.²⁻²⁵ The degree of effectiveness may vary for the individual patient. Clinical improvement should occur within or soon after the first year of treatment, and this benefit may improve with continued treatment. The Allergen Immunotherapy Practice Parameters²⁶ suggest that, “If clinical improvement is not apparent after 1 year of maintenance therapy, possible reasons for lack of efficacy should be evaluated. If none are found, discontinuation of immunotherapy should be considered, and other treatment options should be pursued.” It has been observed that some patients may experience a worsening of their asthma, atopic dermatitis and allergic rhinitis or conjunctivitis symptoms during treatment, especially during the first few months of therapy.

There is no consensus on when to discontinue aeroallergen immunotherapy, but benefits are often maintained for years after stopping therapy in some individuals, and indefinitely in others. In grass-pollen allergy, a three-year course of subcutaneous immunotherapy gave prolonged relief of symptoms.²⁵ For many patients with stinging insect allergy, 3-5

years of treatment may be sufficient for sustained effectiveness after discontinuing therapy. Patients experiencing more severe reactions to stings may be considered for longer durations of treatment, given the risk of the recurrence of a life-threatening reaction over time.

Subcutaneous allergen immunotherapy is not used for patients with food allergies. Although studies have demonstrated an increased tolerance to peanut challenge in patients who received subcutaneous peanut immunotherapy,^{27, 28} there was an unacceptably high incidence of systemic reactions (e.g., anaphylaxis) in most of the patients during treatment.²⁸

Adverse reactions to allergen immunotherapy do occur, including death from severe systemic allergic reactions. Although very rare, deaths associated with immunotherapy may be due to clerical and medical errors by healthcare personnel. Examples include administering a wrong dose or wrong extract to the wrong patient. Other factors that may contribute to immunotherapy fatalities include symptomatic asthma and delay in the administration of epinephrine during a systemic reaction. Nonetheless, allergen immunotherapy extracts are relatively easy to prepare and administer, and are usually well tolerated. Initial and ongoing training will improve the expertise of healthcare workers responsible for administering immunotherapy and, ultimately, the safety of their patients.

PRACTITIONER QUALIFICATIONS

Allergen immunotherapy is an effective therapy and is indicated for the treatment of patients with

allergic rhinitis, allergic conjunctivitis, asthma and stinging insect allergy (Hymenoptera). Each patient's immunotherapy prescription is unique, and the administration schedule (buildup or maintenance) may also vary. Each patient should be evaluated prior to the immunotherapy administration visit to determine whether any recent health changes might require modifying or withholding the immunotherapy treatment. Risk factors for severe immunotherapy reactions include symptomatic asthma and injections administered during periods of symptom exacerbations. Clinical judgment is required when altering the dose or schedule of administration. State laws may differ with regard to personnel who may give injections.

Allergen immunotherapy carries a significant risk for life-threatening anaphylaxis and therefore requires even more competency training for both nursing personnel and physician supervisors. The responsibility for supervision and competency of the staff preparing and administering allergen immunotherapy falls to the supervising physician. Documentation of training and competency in allergen immunotherapy as well as diagnosis, treatment and prevention of anaphylaxis are critical quality management issues for all clinics involved in the delivery of allergen immunotherapy. Until recent recommendations from specialty societies are uniformly adopted, many facilities that provide immunotherapy are forced to deal with a variety of allergen immunotherapy extracts in a variety of packaging and labeling formats that further increase the risk for incorrect dosing during administration. Although efforts to standardize labeling and documentation are underway, training in what to look for and how to ensure extract prescriptions are

administered safely and effectively is crucial to safe delivery of immunotherapy.

TRAINING OPPORTUNITIES

There are a variety of ways to receive training in allergen immunotherapy preparation and administration. Formats may vary from lectures to hands-on training to meet the needs of each learner and include the following:

- On-the-job training from a qualified co-worker or supervisor
- AAAAI and ACAAI workshops and seminars
- Manuals from allergen extract manufacturers
- Journal articles (i.e., Cox²⁶)
- Online course at www.instanted.com. Click on Project Immune Readiness, password = paper. Two modules (anaphylaxis and allergen immunotherapy overview) are currently available. Allergen extract preparation manual and exam are currently in development.

The most current and widely adopted recommendations in the United States for all aspects of allergen immunotherapy are embodied in “Allergen immunotherapy: A practice parameter

third update.”²⁶ This joint effort by experts from AAAAI, ACAAI, and JCAAI focuses on evidence-based recommendations that will optimize immunotherapy efficacy and safety. All healthcare providers involved in immunotherapy preparation and administration should be oriented to the contents of this practice parameter, which contains practical clinical information and sample forms. The sample forms can be downloaded from www.aaaai.org (members-only section).

Some suggested qualifications of extract preparation personnel from Practice Parameters²⁶:

- Pass a written test on aseptic technique and extract preparation
- Be trained in preparation of allergenic products
- Annually pass a media-fill test, as described in Addendum A in Table 9.1.
- Demonstrate understanding of antiseptic hand cleaning and surface disinfection
- Correctly identify, measure and mix ingredients
- Should be appropriately trained health professionals, including, but not limited to, registered nurses, licensed practical nurses, medical technicians, medical assistants, physicians’ assistants, advanced practice nurses and physicians

TABLE 9.1. ALLERGEN IMMUNOTHERAPY EXTRACT PREPARATION GUIDELINES

Qualifications of extract preparation personnel:

- Compounding personnel must pass a written test on aseptic technique and extract preparation.
- Compounding personnel must be trained in preparation of allergenic products.
- Compounding personnel must annually pass a media-fill test, as described in Addendum A.
- Compounding personnel who fail written or media-fill tests would be reinstructed and re-evaluated.
- Compounding personnel must be able to demonstrate understanding of antiseptic hand cleaning and disinfection of mixing surfaces.
- Compounding personnel must be able to correctly identify, measure and mix ingredients.
- Compounding personnel should be appropriately trained health professionals, including, but not limited to, registered nurses, licensed practical nurses, medical technicians, medical assistants, physicians' assistants, advanced practice nurses and physicians.

Physician responsibility: A physician with training and expertise in allergen immunotherapy is responsible for ensuring that compounding personnel are instructed and trained in preparation of immunotherapy with aseptic techniques as defined below and that they meet the requirements of these guidelines. Evidence of such compliance shall be documented and maintained in personnel files. The physician is responsible for providing general oversight and supervision of compounding.

Bacteriostasis: Allergen extract dilutions must be bacteriostatic, meaning that they must contain phenol concentrations of at least 0.25%, or if the phenol concentration is <0.25%, the extract must have a glycerin concentration of at least 20%.

Dilutions prepared in accordance with manufacturer's instructions: Allergen extracts must be diluted in accordance with the antigen manufacturer's instructions.

Potency: The manufacturer's expiration dates must be followed. Beyond-use dates for allergy extract dilutions should be based on the best available clinical data.

Mixing of extracts with high and low proteolytic enzymes: Cross-reactivity of antigens: Separation of aqueous extracts with high proteolytic enzyme activities from other extracts is recommended.

Storage: Extracts should be stored at 4°C to reduce the rate of potency loss or according to the manufacturer's directions. Extracts beyond the expiration date of the manufacturer are to be discarded. Storage must be in a designated refrigerator for medications and not used for food or specimens.

Subcutaneous injection: Allergen extracts can only be administered intradermally or through subcutaneous injection unless FDA-approved package inserts or accepted standards of clinical practice permit another route of administration.

Aseptic technique: Preparation of allergy immunotherapy shall follow aseptic manipulations defined as follows:

- The physician must designate a specific site, such as a countertop, in an area of the practice facility where personnel traffic is restricted and activities that might contribute to microbial contamination (e.g., eating, food preparation and placement of used diagnostic devices and materials and soiled linens) are prohibited.
- The extract preparation area must be sanitized with 70% isopropanol that does not contain added ingredients, such as dyes and glycerin.
- Extract preparation personnel must thoroughly wash hands to wrists with detergent or soap and potable water. Substitution of hand washing by mean of treatment with sanitizing agents containing alcohol, 70% isopropanol or both is acceptable.
- Necks of ampules to be opened and stoppers of vials to be needle punctured must be sanitized with isopropanol.
- Direct contact contamination of sterile needles, syringes and other drug-administration devices and sites on containers of manufactured sterile drug products from which drugs are administered must be avoided. Sources of direct contact contamination include but are not limited to touch by personnel and nonsterile objects, human secretions and blood, and exposure to other nonsterile materials.
- After mixing is complete, visual inspection is to be performed for physical integrity of the vial.

Labeling: Immunotherapy vials are to be clearly labeled with the patient's name and the beyond-use date of the vial.

Mixing log: A mixing log is to be kept with information on the patient’s name, extract used for mixing, mixing and expiration dates and lot numbers.

Policy and procedure manual: Practices preparing allergy extracts must maintain a policy and procedure manual for the procedures to be followed in mixing, diluting or reconstituting of sterile products, and in the training of personnel in the standards described above.

Addendum A: Example of a media-fill test procedure

This or an equivalent test is performed at least annually by each person authorized to compound allergen immunotherapy extracts under conditions that closely simulate the most challenging or stressful conditions encountered during compounding of allergen immunotherapy extracts. Once begun, this test is completed without interruption. A double-concentrated medium, such as from Valiteq®, is transferred in ten 0.5-mL increments with a sterile syringe to a sterile 10-mL vial. Five milliliters of sterile water (preservative free) is added. This is the “concentrate.” The vial is incubated within a range of 28°C to 35°C for 14 days. Failure is indicated by visible turbidity in the medium on or before the 14th day.

Adapted from “Allergen immunotherapy: A practice parameter third update.”²⁶

COMPETENCY ASSESSMENT AND DOCUMENTATION

Training of personnel involved in the administration of allergen immunotherapy is widely recognized as a critical requirement for safety and efficacy. Content should include core cognitive knowledge as well as demonstration of procedure performance competency. Appendix 9.1 contains a sample document for assessing and documenting competency of personnel in the preparation of allergen immunotherapy treatment sets. It is adapted from competency elements for allergy technicians/nursing personnel at the U.S. Army Centralized Allergen Extract Laboratory. These competency elements are based on recommendations of the Joint Commission on Accreditation of Hospital Organizations, or JCAHO, requirements. As with all sample forms, this form is merely an example. Because different practice settings will have site-specific standard operating procedures, competency standards and forms should be developed to meet the needs of each practice and practitioner. For example, a practice may have an extra focus on sterility by adding a sterile glove test that involves

hand preparation, donning of sterile gloves, cutting off the tip and culturing for sterility.

ALLERGEN EXTRACTS

Allergen extracts used for immunotherapy are made from collections of raw material (i.e., pollens, danders, dust mites, insects, molds, and cockroach) and a complex series of manufacturing steps. These extracts should be clinically relevant for patients undergoing treatment; in other words, allergens selected for treatment should be present locally and cause symptoms when the patient is exposed.

Allergen extract used for treatment and testing are liquid solutions containing dissolved allergenic proteins from pollens, dust mites, animal dander, molds, and insects. The manufacturing process usually includes crushing raw materials and “extracting” allergenic proteins by adding solvents that release them from the solid raw material into the liquid solvent. This is followed by a variety of purification steps, resulting in a liquid solution that is stable under normal storage conditions

(4°C) without precipitation that can change the concentration of allergens in the mixture.

Each allergen extract can contain a number of allergenic proteins that can induce allergic symptoms with exposure. However, it is important to realize that the end product is a complex mixture of the diluents or solvents, additives, preservatives, allergenic proteins and other components of the raw material that survive the manufacturing process. Stock allergen extracts are licensed by the Center for Biologics Evaluation and Research (CBER) within the Food and Drug Administration (FDA) in the United States. Commercially available stock extracts are supplied by a handful of manufacturers throughout the country, and are used to mix individual treatment sets and to prepare test panels.

Concentrated stock extracts are available in only a few forms:

- Aqueous
- Glycerinated
- Lyophilized (freeze dried)
- Acetone precipitated
- Alum precipitated

Glycerinated stock extracts contain 50% glycerin by definition. Other liquid-based extracts (i.e., saline, buffers, liquid diluents) are referred to as aqueous extracts.

Lyophilized extracts are aqueous extracts that have been freeze-dried to increase stability during storage and shipping. When they are reconstituted in accordance with package insert instructions with an appropriate diluent just prior to use, they become

aqueous extracts. Hymenoptera venom extracts are typically available in lyophilized form.

Acetone-precipitated extracts are liquid extracts that include a processing step of acetone precipitation to create a high concentration stock solution. The acetone squeezes proteins of interest out of liquid into a solid form that is then re-dissolved in a diluent to make the final highly concentrated stock solution.

Alum-precipitated extracts are liquid extracts that include a processing step involving the addition of aluminum hydroxide, or alum. Allergenic proteins attach to the alum and form complexes that serve as depot when injected into skin, slowing the release of allergens on injection. Because of this slow release, they are less effective in skin testing and are thus used for treatment only. The slow-release alum-allergen complexes may allow for larger doses of extract to be given at less-frequent intervals and a more rapid buildup to higher maintenance doses with reduced incidence of systemic reactions. Local reactions at the site of alum-precipitated extract injections may be immediate or delayed. Delayed reactions may start several hours later, with local edema, erythema (redness), itching and pain. The cloudy appearance of the extract, which may contain visible precipitate, is normal and significantly different than typical aqueous extracts. These extracts require shaking before use. Furthermore, only certain diluents can be used to dilute these extracts. The package insert from stock antigens must be consulted to identify the appropriate diluents for use with alum-precipitated extracts. For example, one manufacturer requires the use of phenol saline diluent for all 10-fold dilution vials. Ten percent glycerol-saline and human serum albumin (HSA) diluent usually *cannot* be used

for alum-precipitated prescriptions because of interference with the aluminum hydroxide-antigen absorbed complex.

Diluents are solutions used to keep the allergens in suspension, and they form the liquid backbone of allergen extracts. Diluents are used to reconstitute lyophilized extracts, to dilute extracts for diagnostic use, to dilute vials in treatment sets and to fill maintenance vials to final volume after addition of stock allergen quantities.

Commonly used diluents:

- Glycerin (e.g., 50% glycerin ± phenol)
- Phenol saline (e.g., 0.4% phenol, saline)
- HSA (e.g., 0.03% HSA, 0.4% phenol, saline)

Each diluent has advantages and disadvantages related to preservation of extract potency and sterility. For example, glycerin is both a preservative and stabilizer. Meanwhile, HSA is a stabilizer, and phenol is a preservative. These additives are discussed in further detail in this chapter's discussion of extract stability.

STANDARDIZED ALLERGEN EXTRACTS

Several commonly used extracts have been standardized such that allergen content is consistent between manufacturers and between lots made from the same manufacturer. Extracts are standardized based on intradermal skin test responses in allergic individuals. Specifically, reference standards from the FDA CBER are obtained for standardized allergen extracts by identifying concentrations that reproducibly produce erythema with a sum of perpendicular long axes of 50 mm, or ID₅₀EAL.²⁹

These reference standards are then used by manufacturers to ensure that the allergen content of each new lot falls within specified ranges for potency labeling. Blood tests (immunoassays) have been developed that correlate allergenic protein content to skin test reactions and, in some cases, treatment results. These include measurement of major allergen content (cat hair Fel d 1 and ragweed Amb a 1), total protein/hyaluronidase/phospholipase content (Hymenoptera venom) and other assays (pooled sera immunoassay inhibition activity).

Units of potency applied to standardized extracts vary and include bioequivalent allergy unit/mL (BAU/mL), allergy unit/mL (AU/mL), microgram protein/mL (µg/mL) or, in the case of some standardized short ragweed stock extracts, in weight per volume (wt/vol). Some allergen extract labels also include the concentration of major allergenic proteins in µg/mL. Since the standardization is based on allergen content falling within a range, it is possible that actual allergenic protein content can vary several-fold for the same potency label. Only a few allergen extracts have been standardized to date (see Appendix 9.2 for probable effective dose range, as cited in Cox²⁶):

Standardized allergen extracts in the United States are as follows:

- Cat hair and pelt (BAU/mL potency labeling based on Fel d 1 content)
- Dust mite (*Dermatophagoides pteronyssinus* and *D. farinae*; potency in AU/mL)
- Short ragweed (potency in AU/mL or wt/vol with lot-specific Amb a 1 concentration)
- Grass (Bermuda, Kentucky bluegrass, perennial rye, orchard, timothy, meadow)

fescue, red top and sweet vernal; potency in BAU/mL)

- Hymenoptera venoms (yellow jacket, honey bee, wasp, yellow hornet, white-faced hornet, and mixed vespids; potency in µg/mL)

ALLERGEN EXTRACT MIXING CONDITIONS

In addition to standardization of allergen stock extract manufacturing, there are new requirements for conditions under which allergen extracts should be prepared. Mixing condition recommendations are designed to decrease the risk of bacterial contamination during the preparation of allergen extract treatment and diagnostic sets. Recommended measures include good personal hygiene, hand washing and the use of antiseptics to clean working surfaces and vial tops prior to transfers.

Two sets of guidelines can be referenced in the preparation of clinic specific standard operating procedures. The first is the Allergen Immunotherapy Practice Parameters prepared by the Joint Task Force on Practice Parameters, representing the AAAAI, ACAAI and JCAAI (Table 9.1).²⁶ The second set of guidelines is outlined in a 2008 revised bulletin from the U.S. Pharmacopeia,³⁰ with an effective date of June 2008 (USP <797>).^{26, 28} It should be noted that these standards are less rigorous than standards required for typical sterile drug compounding in pharmacies. The bulletin recommends that mixers be aware of the greater potential risk of contamination and adhere to recommendations listed. USP created scaled-back recommendations for allergen extracts under the assumption that mixing of allergen extracts involves

simple transfer of sterile substances in the presence of preservatives. Therefore, allergen extracts as compounded sterile preparations (CSPs) are not subject to the personnel, environmental and storage requirements for all CSP Microbial Contamination Risk Levels in this chapter when all criteria are met (Table 9.2).³⁰ USP emphasizes that unless appropriate measures are taken, full compliance with the much more stringent “low risk compounding requirements” are indicated. This includes laminar flow hood use and testing with buffer area, media-fill testing, ISO class 5 air quality, training, garb, and so on, for clinics/facilities requiring compliance with USP 797.

In addition to these measures, work surfaces should be sanitized with a cleaning solution, hot water or a chemical disinfectant. The surface area for preparing allergen extracts should be sanitized using a water-based disinfectant followed by the application of 70% isopropanol (alcohol). The alcohol should be allowed to dry because alcohol kills organisms by dehydration. Sanitizers are used to prevent bacterial contamination and are less effective against other types of living organisms.

Ideally, sites where allergen extract patient treatment sets are prepared should be compliant with the recommendations contained in both of these allergen immunotherapy extract preparation guidelines. However, these conditions and practices may not be required for all clinics or offices. Some hospital-based clinics may be required to be fully compliant with USP recommendations. Clinic and facility supervisors can help determine the applicability of these two sets of guidelines. Regardless, preparers and supervisors should have in place appropriate measures that

focus on proper mixing technique, minimizing the risk of contamination, and appropriate vial labeling

in accordance with the recommendations cited by Cox.²⁶

TABLE 9.2. USP 797 STERILE COMPOUNDING STANDARDS FOR ALLERGEN EXTRACTS

Allergen extracts as CSPs are single- and multiple-dose intradermal or subcutaneous injections that are prepared by specially trained physicians and personnel under their direct supervision. Allergen extracts as CSPs are not subject to the personnel, environmental and storage requirements for all CSP Microbial Contamination Risk Levels in this chapter only when all of the following criteria are met:

- Before beginning compounding activities, personnel perform a thorough hand-cleansing procedure by removing debris from under fingernails (using a nail cleaner under running warm water), followed by vigorous hand and arm washing to the elbows for at least 30 seconds with either non-antimicrobial or antimicrobial soap and water.
- Compounding personnel wear hair covers, facial hair covers, gowns and face masks.
- Compounding personnel perform antiseptic hand cleansing with an alcohol-based surgical hand scrub with persistent activity.
- Compounding personnel wear powder-free sterile gloves that are compatible with sterile 70% isopropyl alcohol before beginning compounding manipulations.
- Compounding personnel disinfect their gloves intermittently with sterile 70% isopropyl alcohol when preparing multiple allergenic extract as CSPs.
- Ampule necks and vial stoppers on packages of manufactured sterile ingredients are disinfected by careful wiping with sterile 70% isopropyl alcohol swabs to ensure that the critical sites are wet for at least 10 seconds and allowed to dry before they are used to compound allergen extract as CSPs.
- The label of each multidose vial of allergen extract as CSPs lists the name of one specific patient, a beyond-use date and a storage temperature range that is assigned based on the manufacturer's recommendations or peer-reviewed publications.
- Single-dose allergen extract as CSPs shall not be stored for subsequent additional use.

Adapted from "Allergen immunotherapy: A practice parameter third update."²⁶

ALLERGEN IMMUNOTHERAPY PRESCRIPTIONS

Allergen immunotherapy prescriptions specify the precise contents of individual treatment sets for patients receiving immunotherapy. They may be written or electronic, but should contain several essential elements. Standardization of content will promote proper preparation, minimize risk for errors in allergy shot administration and facilitate patient transfers of care.

Each prescription should contain:

- Two patient identifiers (consider a picture for the file or electronic record)
- Patient contact information
- Name of prescriber
- Date of prescription
- Name, concentration and volume for each allergen
- Name and volume of diluents
- Schedule for administration (including adjustments for interruptions and reactions)
- Special measures such as pre-shot peak flow criteria for asthma patients

All prescriptions should be reviewed for accuracy prior to preparation. Even though some of these elements may be routine for a clinic, it is important to review them for each and every patient.

Optimal mixing of allergens to create an individual patient treatment set should be based on:

- Use of relevant allergens for each patient
- Dosing of allergen extracts within minimum effective dose ranges (Appendix 9.2)
- Avoidance of combinations that could affect overall potency or are unproven (Table 9.1)²⁶
 - Separate high protease extracts (mold, cockroach) from pollens³²
 - Do not mix venom extracts with aeroallergen extracts²⁶

FIGURE 9.1. ALLERGEN IMMUNOTHERAPY EXTRACT DILUTION CALCULATIONS²⁶

All dilutions can be calculated by using the following formula:

$$V1 \times C1 = V2 \times C2,$$

where

V1 = Final volume you want to prepare

C1 = Concentration (wt/vol or PNU) of extract you want to prepare

V2 = Volume of extract you will need for dilution

C2 = Concentration of extract you will use.

Example: Solve for V2; (V1 × C1)/C2 = V2.

To determine the concentration of an item in a mixture:

1. determine which formula you need to use;

2. choose the numbers/fractions that will be inserted into the formula for V1, C1, V2, and C2;

3. change all wt/vol fractions to a decimal number and insert into the formula (see below); and

4. multiply first and then divide to get the answer.

To express concentration as a percentage:

$$1:10 \text{ wt/vol } 1/10 = 0.1 \times 100 = 10\% \text{ solution}$$

$$1:20 \text{ wt/vol } 1/20 = 0.05 \times 100 = 5\% \text{ solution}$$

$$1:40 \text{ wt/vol } 1/40 = 0.025 \times 100 = 2.5\% \text{ solution}$$

Example:

V1 = 5 mL Final volume you want to prepare

C1 = 1:200 Concentration you want to prepare

V2 = Unknown Volume of extract you will need for dilution

C2 = 1:10 Concentration of extract you will use

Add values into formula:

$$V1 \times C1 = V2 \times C2 \quad 5 \times (1/200) = V2 \times (1/10)$$

$$5 \times (0.005) = V2 \times (0.1)$$

$$V2 = (V1 \times C1)/C2 \quad V2 = 0.025/0.1 = 0.25$$

To determine amount of diluent needed:

$$V1 - V2 \quad 5 - 0.25 = 4.75 \text{ mL}$$

Adapted from the Greer Allergy Compendium, Lenoir (NC): Greer Laboratories; 2005. p. 71. Permission provided by Robert Esch, PhD.

- Selection of allergens and adjustment of doses using a knowledge of cross-reactivity

Figure 9.1 contains formulas for calculating stock extract volumes to be added to maintenance vials for any desired dose, such as those listed in the probable effective dose range table in the Practice Parameters.²⁶ Figure 9.2 is an example of a completed allergen immunotherapy prescription. In this example, the desired maintenance dose for cat was 2000 BAU for a 0.5-mL injected dose from a 5-mL maintenance vial. Using the formula in Figure 9.1, 2 mL of standardized cat extract (10,000 BAU/mL) is needed to achieve a final maintenance vial concentration of 4000 BAU/mL and an injection dose of 2000 BAU.

FIGURE 9.2. ALLERGEN IMMUNOTHERAPY EXTRACT PRESCRIPTION EXAMPLE (FROM AAAAI WEB SITE)

Example of a Completed Allergen Immunotherapy Prescription

Patient Name: Jerry Cleaves
 Patient Number: 23456
 Birth Date: 05/05/90
 Telephone: 645-345-0987

Prescribing Physician: Dr. Ah Choo
 Address: 665 Sunland Lane
 Hollywood, FL 33424
 Telephone: 645-123-4444
 Fax: 645-123-4567

Allergen Extract Content: C, R, G, T, W

Maintenance Concentrate Prescription Form

Prepared by: Mary Lancelot Date Prepared: 6/18/99

State Name Abbreviations:
 TX = Texas MA = Maine
 CA = California MI = Michigan
 WA = Washington NY = New York
 FL = Florida IL = Illinois
 MO = Missouri AZ = Arizona

* Components of mixes listed on a separate sheet

Antigen Number	Extract Name Allergen or Extract (Common name or Genus/species) ^a	Concentration and Type Manufacturer's Extract (AU, BAU, W/V, PNU) (99% G, 50, 50, 02)	Volume of Manufacturer's Extract to Add ^b	Extract Manufacturer	Lot Number	Expiration Date
1	Sheep ragweed	1:10 w/v G (350 mcg Anib al)	0.25 ml	Greer	12345	1/01/10
2	D. ferox	10,000 AU/ml G	0.5 ml	Allergend	5765	2/06/10
3	D. Pterocarpus	10,000 AU/ml G	0.5 ml	Antigen Labs	4222	3/24/08
4	Cat hair	10,000 BAU/ml G	2 ml	Hollister-Stier	9998	2/27/10
5	Timothy grass	100,000 BAU/ml G	0.4 ml	ALK	56789	7/09/09
6						
7						
8						
9						
10						
Diluent	HSA		1.35 ml	ALK	68597	12/12/11
Total Volume			5 ml			0/00/00

^b Assumes 0.5 ml injection at target maintenance date

Volume to add = $\frac{\text{Maintenance Concentration}}{\text{Conc. Of Manufacturer's}} \times \text{Total Volume}$

Maintenance concentration and subsequent dilutions reported as volume/volume (v/v) dilutions with maintenance concentration: 1:1 v/v

BAU = Bioequivalent Allergy Unit, AU = Allergy Unit
 PNU = Protein Nitrogen Unit
 W/V = Weight per Volume Ratio
 G = 50 % Glycerinated
 Aq = Aqueous, Ly = Lyophilized
 AL = Alum precipitated, AP = Acetone precipitated

Updated on 4/5/2011

Step-by-step calculations are as follows:

- Maintenance vial concentration = injection dose/injection volume = 2000 BAU/0.5mL = 4000 BAU/mL
- $V1 \times C1 = V2 \times C2$ (maintenance vial volume \times maintenance vial concentration = stock volume \times stock concentration)
- $5\text{mL} \times 4000 \text{ BAU/mL} = V2 \times 10,000 \text{ BAU/mL}$ [$V2 = \text{stock volume} = (5 \times 4K)/10K = 2 \text{ mL}$]
- Repeat for each antigen in vial
- Total antigen volume = cat 2 mL + *D. farinae* 0.5 mL + *D. pteronyssinus* 0.5 mL + timothy 0.4 mL + short ragweed 0.2 mL]
- Diluent volume = (maintenance vial volume) - (sum antigen volumes) = 5 mL – 3.6 mL = 1.4 mL
- Final maintenance vial contents:

Antigen	Concentration	0.5-mL injection dose
Cat	4000 BAU/mL	2000 BAU
Short ragweed	17.5 $\mu\text{g/mL}$	8.75 μg Amb a 1
Timothy grass	4000 BAU/mL	2000 BAU
<i>D. farinae</i>	1000 AU/mL	500 AU
<i>D. pteronyssinus</i>	1000 AU/mL	500 AU

In summary, use of these prescribing principles will result in a product that is safe and effective, and that can ensure maintenance of expected potency through the expiration date. Mixing high-protease extracts with most other aeroallergens will result in a loss of potency that can affect immunotherapy efficacy. Aeroallergens with known high cross-reactivity allow prescribers to treat with fewer allergens while providing coverage for a large number of related allergens. For example, treatment with one or two northern pasture grass allergen extracts should be sufficient to provide benefit for the more than 10

cross-reactive northern grass species.³⁴ Finally, the use of standardized allergen extract prescription forms will remind clinicians and extract preparers to focus on these elements during the prescribing and extract preparation process.

COLOR CODING, LABELS AND EXPIRATION DATES

The Allergen Immunotherapy Practice Parameters and Joint Commission National Patient Safety Goals emphasize the need for clear and consistent labeling. Standardizing allergen immunotherapy label contents and vial coding will improve communication between care providers and patients, and likely prevent errors in extract administration.

Each patient’s treatment vial label should contain at a minimum:

- Two patient identifiers (e.g., name and date of birth)
- Concentration in vol/vol
- Color code or alphanumeric code (“1” for highest concentration if numbered)
- Expiration or “beyond use” date

Immunotherapy treatment vial concentrations are now labeled in vol/vol, with 1:1 vol/vol representing the maintenance concentrate.

Alternatively, the vial concentration can be labeled in actual units (e.g., 1000 BAU, 100 BAU), but this system may be complicated if allergens with different potency units are used (e.g., wt/vol, BAU, AU or PNU) and these differences make it difficult to interpret the vial label. All the vials in the treatment set are numbered and/or color coded in the following manner:²⁶

RED	Maintenance concentrate	1:1 vol/vol	#1
YELLOW	10-fold dilution	1:10 vol/vol	#2
BLUE	100-fold dilution	1:100 vol/vol	#3
GREEN	1000-fold dilution	1:1000 vol/vol	#4
SILVER	10,000 fold dilution	1:10,000 vol/vol	#5



If a numbering system is used, the **highest concentration should be labeled #1** and the next 10-fold dilution (i.e., yellow vial) would be labeled #2, and so forth. Variation from patient to patient occurs when labeling vials of higher concentrations with larger numbers. This practice resulted in patients often having a different number on their maintenance vial that was based on the total number of dilutions prepared.

Expiration dates should follow the manufacturer’s recommendations. The rule of thumb is that the expiration date for a treatment or skin testing vial is the earliest expiration date recommended for any extract in the mix. Less-concentrated extracts are more sensitive to temperature and might not maintain potency until the listed expiration date; 1:10 to 1:200 dilutions of stock extracts are generally stable for at least 12 months. This usually includes at least the patient’s red maintenance treatment vial and the 1:10 vol/vol, or yellow, vial. Expiration dates for venom extracts are sometimes shorter; perhaps

this is due to the use of diluents with low levels of glycerin. The venom extract package inserts provide guidelines for expiration dates for the different dilutions.

Expiration dating periods for allergen extract products are regulated by the FDA. Even under ideal refrigerated conditions, some loss of potency occurs over time. The potency and stability of these products are not guaranteed beyond their labeled expiration date. Nonstandard extract products are assigned expiration dating in accordance with FDA regulations (21 CFR, Section 610.53) with regard to whether products are glycerinated or nonglycerinated. A total of six years from the time of extraction is allotted to 50% glycerin bulk extracts. This six-year period is divided into a maximum of three years for manufacturer storage and three years for shipped vial expiration dating. Nonglycerinated products are allowed only a total of three years or half the expiration the time allotted for glycerinated extracts—18 months for manufacturer storage and 18 months for shipped expiration dating.

Sample expiration dates for diagnostic and treatment sets prepared by the U.S. Army Centralized Allergen Extract Laboratory are based on stock concentrate manufacturer recommendations for its suppliers. It is important that expiration dating practices for patient allergen extract treatment sets be based on the earliest expiration date from all stock allergens used in each prescription.

Diagnostic Products	Expiration Date*
Prick test materials	1 Year
ID test materials	6 Months
Immunotherapy Treatment Sets	
1:10 -1:5000 wt/vol	1 Year
1:50,000 wt/vol and weaker	3-6 Months†
1000-20,000 PNU/mL	1 Year
<1000 PNU/mL	3-6 Months†
500 AU/mL and stronger	1 Year
<500 AU/mL	3-6 months†
1000 BAU/mL and stronger	1 Year
<1000 BAU/mL	3-6 months†

*Use earliest of stock extract label expiration date or date below.

†The stability of lower extract concentrations (e.g., 1:1000 and 1:10,000 vol/vol) has not been extensively studied. Loss of potency in these lower concentrations may be due to absorption of the allergenic proteins to the glass wall. HSA may have a more protective effect against this cause of loss of potency than other diluents such as normal saline.

Reconstituted Venom Freeze-Dried Preparations

100 µg/mL	6 or 12 Months*
1-10 µg/mL	1 Month
0.1 µg/mL	14 Days
<0.1 µg/mL	24 Hours

*Varies with manufacturer. Guidelines for dilution expiration dating are in the extract package inserts.

MIXING INDIVIDUAL PATIENT ALLERGEN EXTRACT TREATMENT SETS

Every clinic should develop a specific standard operating procedure document or manual to ensure standardization and safe practices of allergen extract mixing. Responsible providers developing the procedures should consult stock extract manufacturer recommendations and the most recent Allergen Immunotherapy Practice Parameter Update to incorporate the most up-to-date recommendations. USP 797 requirements should also be reviewed if relevant for your clinic. These procedures should emphasize the importance of individual treatment vials and vial sets, especially when mixing of allergens is required. The mixing of antigens in a syringe is not recommended because of the potential for cross-contamination of extracts. Here are a few guiding principles for mixing allergen extracts:

- Optimal dosing should be within defined minimal effective-dose ranges.
- Avoid incompatible mixtures when possible.
 - Stinging insect and aeroallergen extracts should not be mixed.
 - High-protease extracts (molds and cockroach) should not be mixed with pollens.
- Selection of allergens should factor in known cross-reactivity for like antigens.
- Initial treatment sets consist of a maintenance vial and a series of 10-fold dilutions.
- Contamination is prevented by use of aseptic techniques and adequate training.
- Accurate prescriptions, labels and color coding are required to prevent errors.

- Use of quality assurance checks throughout the mixing process is highly recommended.

INITIAL PREPARATION

- 1) Develop clinic specific standard operating procedures.
- 2) Designate an allergen extract mixing location.
 - Location should be an area of the clinic where personnel traffic is restricted and exposure to potential contaminants is minimized.
 - The same location(s) should be used each time extracts are prepared.
 - Location can be used for other purposes outside of mixing, but should be cleansed and prepared before every mixing session.
 - Minimize contamination by limiting high-risk activities during mixing such as eating, food preparation, use or placement of diagnostic devices (used specula, skin test or biopsy devices, endoscopes, used absorbent pads, and so forth) or soiled linen storage in the designated area.
- 3) Identify expiration dating standards for your clinic.
 - More dilute vials usually will have an earlier expiration date.
 - Dates should not exceed expiration date of earliest expiring antigen or diluents used in each prescription.
- 4) Become familiar with stock allergen extract ordering and storage procedures.

- 5) Orient personnel to stock allergen extracts, refrigerator storage designated mixing location, mixing equipment, prescriptions, documentation and packaging.
- 6) Undergo training on standard operation procedures and safety measures.

PRE-MIXING PREPARATION

- 1) Verify that a supervising physician is present in the same building as the mixing location(s).
- 2) Prepare specific mixing location(s).
- 3) Cleanse and maintain an aseptic work environment using an approved disinfectant solution (i.e., 70% isopropanol) without additives like dyes and glycerin.
- 4) Prepare vial labels in accordance with prescription and verify accuracy of
 - Name and second identifier.
 - Concentration.
 - Antigens on label match those that are to be added from the prescription.
 - Expiration date is consistent with clinic procedures and source antigens.
- 5) Apply label to treatment set vials.
- 6) If using color-coded vials, verify color and concentration match.
- 7) Alternatively, labels may be applied after mixing. For example, the label for the empty maintenance concentrate (red) vial (or all vials) can be left off until all contents are injected into the vial to improve visibility during checks for impurities, final volume and color comparison of dilution series.

EXAMPLES OF ALLERGEN EXTRACT MIXING STEP-BY-STEP PROCEDURES

This sample set of procedures does not constitute “recommended” procedures, but can be used as a starting point to develop procedures that best fit a specific clinic/facility needs.

MIXING THE MAINTENANCE (RED) VIAL

- 1) Pull new empty sterile vials (usually 5, 8 or 10 mL) for each vial in the patient’s treatment set, and put in order from strongest (maintenance/red) to most dilute.
- 2) Pull the stock extract vial for each antigen on the prescription, and stock diluents from refrigerator.
 - Check stock antigens for turbidity/particulate matter. If present, consult package insert or manufacturer guidelines, including possible recommendations for re-suspension or filtering.
 - For prolonged mixing sessions, return unused stock extracts to refrigerator or cooling tray (2°-8°C) between prescriptions or during extended breaks.
- 3) Place a new syringe by each stock antigen vial and the diluent.
 - A separate syringe is used for each antigen and diluent.
 - Label each syringe (i.e., abbreviation for antigen or diluents).
 - For immediate use only, stock extracts should not be pre-drawn for extended periods because of the risk of potency loss and misidentification.
- 4) Document lot number and manufacturer for each antigen (preferably one per antigen).
- 5) Note expiration dates of stock extracts and that label expiration date do not exceed earliest stock vial extract.
- 6) Wear appropriate personal protective equipment.
 - Wash hands/nails to elbows for at least 30 seconds with soap and water.
 - Don hair and facial hair covers, gowns and face masks (if following USP 797 guidelines).
 - Use alcohol-based surgical hand scrub prior to gloving.
 - Don powder-free sterile gloves compatible with 70% isopropyl alcohol.
- 7) Disinfect gloves with isopropyl alcohol before mixing (and intermittently for lengthy mixing).
- 8) Wipe vials and/or ampules with 70% isopropyl alcohol for at least 10 seconds.
- 9) Maintain aseptic technique by minimizing contact with secretions, skin, glove fingertips and the like during mixing.
- 10) Draw the correct amount of each antigen and the diluent into the syringe, and place each syringe by the respective stock antigen vial.
- 11) Verify drawn doses are correct volume and antigen. (Quality checkpoint opportunity: have a co-worker verify, if available.)
- 12) Inject contents of all drawn antigens one by one into the maintenance concentrate (red) vial.
 - The empty syringes should be discarded immediately into an appropriate Sharps disposal container.

- If the sterile maintenance vial is not a vacuum (air filled), an equal volume of air may need to be withdrawn prior to injecting stock extract volumes.
- 13) If there is precipitate present in the stock antigen vials,
- Particulates and precipitates suspended in an extract solution are not uncommon.
 - These particulates and precipitates often do not cause any significant loss in potency. Consult manufacturer recommendations in package insert or bulletins for additional information.
 - Attempted re-suspension by agitation (shaking or rolling) may be indicated in accordance with the package insert and your clinic operating procedures.
- 14) After mixing is complete, conduct final quality assurance check (preferably by mixer and trained co-worker), including:
- Solution color check.
 - Label check.
 - Vial color-code check.
 - Liquid turbidity, precipitate and consistency check.
 - Vial physical integrity (leaks, cracks and so on) check.
- 15) If applicable, package treatment set for transport or shipping.
- 16) Document preparation details according to clinic-specific procedures on prescription

or preparation form and in mixing log (see Practice Parameter appendices for sample forms), as follows:

- Name of preparer and date prepared.
- Stock allergen extract manufacturer, lot number and beyond-use or expiration date.
- Mixing log, to be maintained in the unlikely event of a stock antigen recall or for extract or adverse-event troubleshooting.

SPECIAL PROCEDURE NOTES CONCERNING ALUM-PRECIPIATED EXTRACTS

- Diluent: Alum-precipitated extracts generally require phenol saline diluent for all 10-fold dilution vials. Ten percent glycerol-saline or HSA diluent *cannot* be used for alum- precipitated prescriptions as it interferes with the aluminum hydroxide-antigen absorbed complex.
- For alum-precipitated extract treatment vials, consider applying a small “shake well” label, as the alum-precipitated antigens are very viscous in nature. Precipitated alum-antigen complex will settle to the bottom of the vial.
- Unlike aqueous and glycerinated extracts that generally do not lose potency with filtering, large antigen-alum complexes may be lost during the filtering process, with the result being a result in loss of potency. Therefore, **do not filter alum-precipitated extracts.**

PREPARING SERIAL 10-FOLD DILUTIONS OF THE MAINTENANCE (RED) VIAL

Serial 10-fold dilutions are prepared to complete a patient's initial allergen immunotherapy treatment vial set. **Dilutions are made by serial dilution** (taking from a parent vial and placing into a new vial prefilled with diluent to create a 10-fold dilution (1/10 the amount of allergen contained in the parent vial). This newly diluted vial becomes the parent vial, and dilutions are repeated until the desired number of 10-fold dilutions is achieved. **Diluted allergen immunotherapy vials (yellow, blue, green, and the like) should not be made by pulling directly from a manufacturer's concentrated stock vial extract.** The primary reason for this is the potential for error that increases with each dilution. For dilute vials, a very small amount of allergen would need to be pulled from the stock extract vial, and this is virtually impossible to do with the precision needed for the most dilute vials. Thus, a dilution vial prepared by this method may contain less or more than expected and potentially increase the risk of adverse events during vial transitions within the buildup phase.

The volume used to make serial dilutions from parent vials depends on both the desired dilution (10-fold in this case) and the final volume. Typical treatment set vials are 2, 5, 8 or 10 mL. Treatment set vials are now available with original or snap-on colored caps to create sets according to the recommended color scheme. Vials also are available empty or prefilled with diluents suitable for intradermal or subcutaneous administration. Prefilled volumes correspond to the

amount of diluent needed to make a 10-fold dilution. For example, a prefilled 5-mL yellow vial will contain 4.5 mL of diluent and have a yellow cap. To make the yellow 10-fold dilution vial, 0.5 mL would be taken from the parent red maintenance vial (1:1 vol/vol) and added to yellow vial, for a total final volume of 5 mL (0.5 = 1/10 of 5 mL, a 10-fold dilution or 1:10 vol/vol). To make the same 10-fold diluted yellow vial using one that was not prefilled, 0.5 mL is added from the red maintenance vial and 4.5 mL is added from a stock diluent vial.

PREPARING OF 5-ML SERIAL 10-FOLD DILUTION VIALS FOR PATIENT TREATMENT SETS

- 1) Verify that the labeling and order (color coded, label concentration) for vials are correct.
- 2) Ensure the maintenance vial is mixed by inverting or rolling.
- 3) Using a fresh syringe and aseptic technique, remove 0.5 mL from the mixed 5-mL maintenance concentrate red or 1:1 vol/vol vial.
- 4) Using aseptic technique, inject this 0.5 mL from the maintenance vial into the 4.5-mL prefilled (10% glycerol-saline or HSA) yellow or 1:10 vol/vol vial. This vial will be a 10-fold dilution of the maintenance concentration vial.
- 5) Ensure this newly made 10-fold diluted (yellow) vial is mixed by inverting or rolling.
- 6) Subsequent 10-fold dilutions are done in the same manner for the rest of the vials in the treatment set (0.5 mL into 4.5 mL of the 10-fold weaker, labeled 10% glycerol-saline prefilled vial):

- 0.5 mL from yellow 1:10 vol/vol into 4.5 mL diluent-filled blue 1:100 vol/vol vial
 - 0.5 mL from blue 1:100 vol/vol into 4.5 mL diluent-filled green 1:1000 vol/vol vial
 - 0.5 mL from green 1:1000 vol/vol into 4.5 mL diluent-filled silver 1:10,000 vol/vol vial
 - And so on for additional more dilute (silver) vials
- 7) Whereas using a fresh syringe for each dilution transfer is often preferred, use of the same syringe for serial dilution transfers is an alternative if a “mix/rinse” step is included. A mix/rinse step consists of pulling up a full syringe volume (1 mL for a 1-mL syringe) from the vial just injected and re-injecting into the same vial without removing the syringe. This is often repeated (i.e., for a total of three times) prior to pulling up the final volume for the transfer to the next dilute vial. (**Reminder:** Do not reuse syringes or mix/rinse between different stock solutions when mixing the initial maintenance vial.)
- 4) Regularly review operating procedures for opportunities to make the process safer and more efficient.
 - 5) Establish a regular inventory check.
 - Identify stock allergen extracts, diluents and mixing supplies in need of reordering
 - Check for expiring stock allergen extracts, diluents and mixing supplies
 - 6) Return antigen stock trays to the refrigerator when away from the compounding area for an extended period of time.
 - 7) Minimize diversions during extract preparation.
 - 8) Stock refrigerators are **not** to be used for food or drink storage.

ALLERGEN EXTRACT TREATMENT SET PREPARATION HINTS

- 1) Do not mix prescriptions for more than one patient at a time.
- 2) Train multiple qualified personnel in allergen extract preparation in case of absences and for participation in quality checks.
- 3) Avoid putting hand lotion on before the compounding of allergen extract vaccines and skin test antigens. Lotion tends to harbor bacteria.

ADDITIONAL QUALITY ASSURANCE CHECKS

Additional quality assurance checks before allergen extract shipping and use ideally are confirmed by a co-worker. Vials should be inspected for:

- Label accuracy—“five rights” as described below
- Vial serial color dilution that matches label concentration and vial color coding
- Vial integrity (no cracks, leaks, and so on)
- Vial content (particulate matter, fill volume, and so on)

Verify that the label contains the right name, right content (allergens), right concentration, right alphanumeric number in the right order with lowest = 1 (if numbers used), right expiration date (dilute vials may have earlier expiration dates than more concentrated vials). Additionally, a **solution color check** for each vial should be conducted. The solution

in the maintenance concentrate vial should be the darkest in color, and vials should be lighter in color with each 10-fold dilution. The weakest strength vial should contain the lightest-colored solution. When using color-coded vials, a **vial color code check** should be performed. Vials in the treatment set should be arranged in order (red/maintenance, yellow, blue, green and silver). For each color-coded vial, the label concentration, in vol/vol, or number should match what is recommended in the Practice Parameters for that color code (see Tables XI and XII from Practice Parameters²⁶). Additionally, the color of the solution should be a shade consistent for that dilution (lighter if not the red maintenance vial). All vials should also undergo a **content check**. Vials should be filled to the expected volume. Solutions within each vial should be inspected for the presence of particulate or solid materials and cloudiness. If found, vials may be contaminated or contain precipitated raw allergen extract contents. Contamination may be bacterial or other microbial source, but may also be a result of introduced solid materials like the rare occurrence of vial stopper fragments from manufacturing or repeated puncturing. All vials should also undergo a **vial integrity check** by inspecting the vial for any cracks, leaks or stopper disruptions. Any abnormal finding during any of these checks should be followed by an investigation for the cause and, in most instances, starting over and remixing that patient's vial set.

STINGING INSECT ALLERGEN EXTRACT PREPARATION

Extracts are available for five winged Hymenoptera species at a concentration of 100 µg/mL: **honey**

bee, wasp, yellow jacket, yellow hornet and white-faced hornet. The last three (yellow jacket, yellow hornet and white-faced hornet) are closely related members of the Vespidae family and have also been combined in a single “mixed vespid” extract at a reconstituted concentration of 300 µg/mL. Lyophilized or freeze-dried stinging insect venom extracts are available commercially for diagnostic testing and patient treatment. These extracts are composed of venom isolated directly from dissected venom sacs. Previously manufactured extracts using whole insect body as opposed to concentrated venom proved not to be as effective as extracts made from venom.³⁵ Accordingly, handling of these extracts is limited to reconstitution and dilution. The same principles and requirements for labeling apply with the exception of number/color coding and use of vol/vol concentration. The concentration of these extracts and all dilutions is expressed in µg/mL. Reconstitution and dilution of all insect venom extracts is most commonly performed with HSA (HSA/phenol) diluent.

Extracts are also available for **imported fire ant** Hymenoptera species. Two fire ant species, *Solenopsis richteri* and *S. invicta*, are commercially available as individual extracts for testing or treatment, or as a fire ant mix containing both species. Fire ant extracts are made from whole fire ant bodies. Fire ant venom extracts are being investigated for clinical use, but require a significant amount of time and resources for mass production. Fire ant stock concentrate extracts typically are available as glycerinated extracts in wt/vol concentrations (i.e., 1:20 wt/vol). The Practice Parameters for Insect Allergy contains survey data on common fire ant maintenance

doses ranging from 0.5 mL of 1:100 wt/vol to 0.5 mL of 1:10 wt/vol maintenance concentrate, with most using 0.5 mL of a 1:100 wt/vol maintenance concentrate.³⁶

Insect venom (and fire ant) extracts generally should not be mixed with other venom or aeroallergen extracts for either testing or treatment because of the lack of sufficient stability, safety and efficacy studies to support mixing. The only FDA-approved mixture is commercially available mixed vespid extract containing 100 µg/mL of each of the three common vespids.

ALLERGEN EXTRACT STABILITY AND STORAGE

The stability and potency of allergen extracts can be compromised by elevated temperatures, contamination and protease degradation of key allergenic proteins responsible for the efficacy of immunotherapy.^{31-34, 37} Several measures are taken by stock extract manufacturers and healthcare personnel to minimize the risk of loss of potency of extracts during normal storage and use.

Dilution of extracts alone can affect the long-term potency of extracts. For example, diluted extracts have lower concentrations of important preservatives and stabilizers. Furthermore, lower concentrations of proteins decrease three-dimensional protein structure stabilization achieved through protein-protein interactions that are facilitated at higher protein concentrations. Finally, dilutions may also magnify the effect of allergenic protein loss as a result of binding to sites on glass vials that is essentially insignificant at higher protein concentrations.

There are several “routine” operating procedures that when performed consistently should promote extract stability and reduce errors associated with the use of outdated materials:

- Routinely check expiration dates on all products.
- Ensure that the stock inventory in refrigerators is routinely rotated such that expiring products are placed in the front and used first.
- Verify that expiration dates on labels for treatment and diagnostic sets are no later than the stock extract used with the earliest expiration date.
- Immediately discard or separate products that have expired.
- Ensure that personal allergen extract storage trays are stored at recommended temperatures.
- Ensure that extracts are kept cool during extended periods of mixing.

Manufacturer processing steps include **additives** that stabilize the allergenic proteins and preservatives that prevent contamination of the stock extract and individual patient treatment sets derived from them. **Preservatives** are added to allergen extract solutions to **prevent microbial growth** in the event that bacteria or fungi are introduced into the solution during the preparation process or when needles are inserted into vials for administration of immunotherapy. All allergen extracts must contain preservatives that are bacteriostatic. **Bacteriostatic agents** prevent the growth of microbial contaminants like bacteria, but do not necessarily kill microorganisms. Sterilization and pasteurization processes that kill microorganisms are less commonly used.

Phenol is a common bacteriostatic preservative added to allergen extracts and is used at a final concentration of approximately 0.4%. One possible ill effect of using phenol is that it may denature (unfold or breakdown) allergenic proteins even if in 50 % glycerin.^{31, 33} HSA may protect against phenol's adverse effects on allergenic proteins.³¹ Other recognized preservatives such as thimerosal and methylparaben are not generally used in allergen extract preparation. **Seventy percent isopropanol** is a disinfectant but not a preservative.

Disinfectants are antimicrobial agents applied to nonliving objects (e.g., countertops). Thus, they are not "preserving" viability, potency or purity. Disinfectants should also be distinguished from antibiotics that kill microorganisms within the body. Sanitizers are high-level disinfectants that kill more than 99.9% of a target microorganism. Sterilization refers to the complete elimination of all microorganisms.

Stabilizers are added to diluents **to maintain the structure of allergens** in solution and prevent sticking or adherence to the glass vials in which they are contained. Common stabilizers include glycerin and HSA. Fifty percent glycerin is often considered the best stabilizer alternative and is also considered a preservative, whereas HSA is not a preservative. Glycerin potently stabilizes proteins in solution and inhibits proteases found in some allergen extracts, and is bacteriostatic at concentrations $\geq 20\%$.³⁷

It should be noted that these preservative and stabilizing properties are diminished as the concentration of glycerin is decreased. One drawback of glycerin is that it is irritating to the skin

in higher concentrations. Although most extracts used for prick or percutaneous skin testing have 50% glycerin, extracts used for intradermal testing contain considerably less, often 100- to 1000-fold dilutions of those used for percutaneous skin testing.

The manufacturers and practice parameters recommend that care is advised when administering a volume >0.2 mL of an extract in 50% glycerin because of the potential for discomfort and pain. This is equivalent to 0.1 mL of straight 100% glycerin. For example, if a 5-mL maintenance vial contains 5 mL of a mixture of all stock extracts in 50% glycerin with no additional diluent, the final concentration of glycerin this vial is 50%. A typical 0.5-mL maintenance dose would exceed 0.2 mL, providing an explanation for a patient experiencing increased pain during treatment. For this same reason, the preferred diluent for preparing extracts for intradermal diagnostic testing is HSA to limit skin irritation and the possibilities of pain and false positive skin test results. The rule of thumb is that the more dilute the extract, the less likely it will cause an irritant reaction. However, testing more dilute extracts may also result in lower "sensitivity," resulting in missing the identification of relevant allergens that could have been identified at a higher concentration (higher false negative test result).

Allergen extracts are stored in refrigerators **at a temperature of 4°C** or in accordance with manufacturer recommendations. A temperature range of 2°-8°C is considered acceptable by most experts. Given the expense and temperature sensitivity of stock allergen extract concentrates and mixed patient treatment sets, it is also reasonable to conduct some form of temperature monitoring to

TABLE 8.8

Aspirin Induction of Drug Tolerance Scripps Protocol®	
Assessment and premedication (1-7 days before procedure)	FEV ₁ >60% predicted (>1.5L) Start or continue treatment with montelukast, 10 mg daily Start or continue treatment with inhaled corticosteroid and long-acting β-agonist Systemic steroid burst if low FEV ₁ or bronchial instability
Protocol	
Time	Aspirin Dose
Day 1: 0	30 mg
Day 1: 3 hours	60 mg
Day 1: 6 hours	100 mg
Day 2: 0	150 mg
Day 2: 3 hours	325 mg
Day 2: 6 hours	650 mg
<p>Start intravenous catheter with heparin lock (keep in for 2-3 days). FEV₁ and clinical assessment every hour and with symptoms. Reactions typically occur with a provoking dose of 20-101 mg. Treat with medication described below. Chance of reaction to repeated threshold dose is small, but if occurs, repeat dose until reactions cease and then proceed. After patient completely stabilized, provoking dose can be repeated (assuming another 3 hours of observation time), otherwise start with provoking dose on day 2. If nasal, gastrointestinal, or cutaneous reactions occur on day 1, pretreat with histamine₁ and histamine₂ receptor antagonists for remainder of procedure.</p>	
Medications for treatment of aspirin-induced reactions	
Ocular	Topical antihistamines
Nasal	Antihistamine, topical decongestant
Laryngeal	Racemic epinephrine nebulization
Bronchial	β-Agonists
Gastrointestinal	Histamine ₂ -receptor antagonists
Urticaria/angioedema	Antihistamine
Hypotension	Epinephrine
Abbreviation: FEV ₁ , forced expiratory volume in 1 second.	

TABLE 8.9

Aspirin Induction of Drug Tolerance, Aspirin Desensitization Joint Task Force Recommendations*	
Assessment and premedication (within 1 week before procedure)	FEV ₁ >70% predicted Consider starting or continuing leukotriene modifier therapy Start or continue treatment with high-dose inhaled corticosteroid and long-acting β -agonist if poorly controlled asthma Systemic steroid burst if low FEV ₁ or bronchial instability If receiving maintenance systemic steroids, consider doubling daily dose (if on alternate day steroids change to daily dose)
Protocol	
Time	Aspirin Dose
0	20.25 mg
90 min	40.5 mg
180 min	81 mg
270 min	162.5 mg
360 min	325 mg
<p>Document informed consent and advise patient it may take several days to complete (most will take 2 days). Establish intravenous access. FEV₁ and clinical assessment every 90 minutes and with symptoms. Dosing interval may be extended to 3 hours based on individual patient characteristics. Reactions will likely occur with early doses, usually 81 mg. Treat reactions as indicated below. After patient completely stabilized (but not less than 3 hours after the last dose), the provoking dose can be repeated. A persistent >15% decrease in FEV₁ with or without associated symptoms, lasting longer than 3 hours despite therapy, is an indication to discontinue the desensitization process for the day. If nasal, gastrointestinal, or cutaneous reactions occur on day 1, pretreat with histamine₁ and histamine₂ receptor antagonists for remainder of procedure.</p>	
Medications for treatment of aspirin-induced reactions	
Ocular	Oral antihistamines
Nasal	Oral antihistamine, topical decongestant
Laryngeal	Racemic epinephrine nebulization and/or intramuscular epinephrine
Brochial	β -Agonists
Urticaria/angioedema	Oral or intravenous antihistamines
Hypotension	Parenteral epinephrine
<p>Abbreviation: FEV₁, forced expiratory volume in 1 second. *This recommended protocol is intended to be more practical, using doses based on commercially available 81 mg aspirin products and a shorter dosing interval. There are no data on safety and efficacy of this protocol.</p>	

ensure that extracts are not exposed to temperature extremes. For example, a log of daily temperatures can be maintained or an automated continuous temperature monitoring device can be installed. Facilities might also consider installing temperature alarms. Many allergen extracts are heat sensitive. The loss in potency when allergen extracts are exposed to high temperatures (i.e., >78°F or 26°C) may be due to the heat-labile (-sensitive) proteins that unfold or degrade at these temperatures. Loss of potency can also occur at lower temperatures, including room temperature (i.e., 68°-72°F and 22°C). This is possibly due to proteases in the extract that are activated at these temperatures and degrade relevant allergen proteins in the extract. Allergen extracts exposed to room temperature over time may thus lose potency, such as extracts frequently left out of the refrigerator for long periods during testing or treatment. For example, skin testing trays with extracts that are taken out of the refrigerator in the morning every day and not replaced until the clinic closes in the evening may suffer from reductions in potency unless the trays are cooled while out of the refrigerator. Short intervals for testing or treatment rarely result in clinically significant losses of potency. Fifty percent glycerin may help protect against the effects of prolonged exposure to room temperature, possibly due to its effect on proteolytic enzymes. Less is known about the effects of freezing (<0°C) on allergen extract potency, but at least one study found a moderate loss of potency when an extract was stored frozen and thawed for use.¹⁵ An increase in the number of multiple freeze-thaw cycles increases the observed loss in potency of extracts. Thus, extracts that are accidentally frozen should be replaced with new extract prior to use.

Some extracts contain proteolytic enzymes or proteases that can degrade proteins needed for allergen extract effectiveness. Tree, grass and weed pollens and some pet danders are particularly susceptible to these proteases. For this reason, the most recent Practice Parameters recommend the separation of extracts with high proteolytic enzyme activities, such as mold and cockroach, from other extracts, such as pollens. Also of note, dust mite extracts do not appear to significantly degrade pollen or animal dander extracts and can be mixed together with these extracts.

Investigations have shown that extracts stored in vials only partially filled with solution are less stable. In other words, 1 mL of extract in a 10-mL vial will lose potency more rapidly than 10 mL of extract in a 10-mL vial. This volume effect is more pronounced with higher dilutions. For this reason, it is reasonable to consider reordering and preparing treatment and diagnostic materials as the extract volume in current vials diminishes.

SUMMARY

The preparation of allergen immunotherapy extracts is a technical skill that requires training and a high level of attention to detail. Errors may cause life-threatening allergic reactions in patients receiving immunotherapy. Using a team approach to develop clinic/facility-specific policies and procedures and verify ongoing competency will ultimately improve the quality and precision of allergen immunotherapy preparation. Ongoing review of these procedures will lead to increased knowledge of and adherence by individuals preparing allergen extracts. These steps will ensure the end product is accurately

prepared according to the most recent standards and manufacturer recommendations. Thorough knowledge and training will promote the safety of the patients entrusted to our care and of those performing allergen extract preparation.

There are several major themes that new personnel assigned to prepare allergen extracts should become familiar with. These include, but are not limited to, the following:

- Contamination is prevented by use of aseptic techniques and adequate training.
- Accurate labels and color coding are highly recommended to prevent errors.
- Use of quality assurance checks throughout the mixing process is highly recommended.
- Initial treatment sets consist of a maintenance vial and a series of 10-fold dilutions.
- Stinging insect and aeroallergen extracts should not be mixed.

All personnel involved in allergen extract preparation should be familiar with the contents of the most recent Practice Parameters. A companion examination has been developed based on this training document to assist in satisfying competency assessment and documentation requirements. It will be available along with this document on the JCAAI web site (www.jcaai.org) as a joint collaboration of AAAAI, ACAAI and JCAAI.

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APPENDIX 9.1. INITIAL AND ONGOING COMPETENCY ASSESSMENT—ALLERGEN EXTRACT MIXING

Name _____ Job Title: _____ Clinic: _____

Allergen Extract Preparation	Date	Validated by	Comments or Notes
Passed a written test on aseptic technique and extract preparation.			
Passed media-fill test or equivalent verifying aseptic technique			
Reviews prescription(s) for accuracy			
Accurately prepares labels and shipping material (if applicable)			
Checks expiration dating of antigens and diluents			
Cleans mixing surface and washes hands appropriately			
Uses appropriate personal protective equipment			
Checks stocks & mixed extracts for turbidity/particulate matter			
Swabs vials off with antiseptic (e.g. alcohol swabs)			
Draws up appropriate amounts			
Disposes of syringes in an appropriate manner			
Documents lot #'s and preparation details per clinic SOP			
Packages materials and supplies in a neat and efficient manner			

I understand that of all the topics listed, I will be allowed to perform only those for my skill level/scope of practice and only after I have demonstrated competency.

Employee signature _____ Date _____

* Self Assessment:	+ Evaluation/Validation Methodologies:
1 = Experienced	T = Tests
2 = Needs Practice/Assistance	D = Demonstration
3 = Never Done	V = Verbal
NA = Not Applicable	I = Interactive Class

APPENDIX 9.2. PROBABLE EFFECTIVE DOSE RANGE FOR ALLERGEN EXTRACTS, U.S. STANDARDIZED UNITS—
FROM “ALLERGEN IMMUNOTHERAPY: A PRACTICE PARAMETER THIRD UPDATE”²⁶

TABLE IX. Probable effective dose range for standardized and nonstandardized US- licensed allergen extracts

Allergenic extract	Labeled potency or concentration	Probable effective dose range	Range of estimated major allergen content in US-licensed extracts
Dust mites: <i>D farinae</i> and <i>D pteronyssinus</i>	3,000, 5,000, 10,000, and 30,000 AU/mL	500-2,000 AU	10,000 AU/mL 20-160 µg/mL Der p 1, Der f 1* 2-180 µg/mL Der p 2, Der f 2* 78-206 µg/mL Der p 1, Der f 1† 13-147 µg/mL Der p 2, Der f 2‡
Cat hair	5,000 and 10,000 BAU/mL	1,000-4,000 BAU	10,000 BAU/mL 20-50 µg/mL Fel d 1*‡ 30-100 µg/mL cat albumin§
Cat pelt	5,000-10,000 BAU/mL	1,000-4,000 BAU	10,000 BAU/mL 20-50 µg/mL Fel d 1*‡ 400-2,000 µg/mL cat albumin§
Grass, standardized	100,000 BAU/mL	1,000-4,000 BAU	100,000 BAU/mL 425-1,100 µg/mL Phl p 5* 506-2,346 µg/mL group 1
Bermuda	10,000 BAU/mL	300-1,500 BAU	10,000 BAU/mL 141-422 Cyn d 1 µg/mL*
Short ragweed	1:10, 1:20 wt/vol, 100,000 AU/mL	6-12 µg of Amb a 1 or 1,000-4,000 AU	1:10 wt/vol 300 µg/mL Amb a 1‡ Concentration of Amb a 1 is on the label of wt/vol extracts
Nonstandardized AP Dog	1:100 wt/vol	15 µg of Can f 1	80-400 µg/mL Can f 1† 10-20 µg/mL dog albumin¶
Nonstandardized extract, dog	1:10 and 1:20 wt/vol	15 µg of Can f 1	0.5 to 10 µg/mL Can f 1† <12-1,500 µg/mL dog albumin¶
Nonstandardized extracts: pollen	1:10 to 1:40 wt/vol or 10,000-40,000 PNU/mL	0.5 mL of 1:100 or 1:200 wt/vol	NA
Nonstandardized extracts: mold/fungi, cockroach	1:10 to 1:40 wt/vol or 10,000-40,000 PNU/mL	Highest tolerated dose	NA
Hymenoptera venom	100 µg/mL single venom 300 µg/mL in mixed vespid extract	50-200 µg of each venom	100-300 µg/mL of venom protein
Imported fire ant	1:10 to 1:20 wt/vol whole-body extract	0.5 mL of a 1:100 wt/vol to 0.5 mL of a 1:10 wt/vol extract	NA

NA. Information not available.

*ALK-Abelló ELISA.

†Indoor Biotechnology ELISA.

‡FDA radial immunodiffusion assay.

§Greer Radial Immunodiffusion assay.

||Greer ELISA.

¶Hollister-Stier ELISA using Innovative Research, Inc. reagents.

APPENDIX 9.3. SAMPLE MIXING STOCK EXTRACT VOLUMES FOR 5- OR 10-ML VIALS BASED ON PROBABLE EFFECTIVE MAINTENANCE DOSE RANGES

Allergen	Probable effective dose range	Typical volume range for 10-ml vial	Typical volume range for 5-ml vial
Grass (e.g., Phl p5)	7-20 µg (1000-4000 BAU)	0.2-0.8 mL (100K)	0.1-0.4 (100K)
Short ragweed* (Amb a 1, Ag E)	6-12 µg or 1000-4000 AU	1.2-2.4 (1:10,100 µg/mL) or 0.2-0.8 (100K AU/ml)	0.6-1.2 (1:10,100 µg/mL) or 0.1-0.4 (100K AU/ml)
Cat (Fel d1)	11-17 µg (1000-4000 BAU)	2-8 (10KG)	1-4 (10K)
<i>D. pteronyssinus</i> (Der p 1)	7-9 µg (500-2000 AU)	1-4 (10K)	0.5-2 (10K)
<i>D. farinae</i> (Der f 1)	10 µg (500-2000 AU)	1-4 (10K)	0.5-2 (10K)
Nonstandardized extracts	1:10–1:40 wt/vol or 10K–40K pnu/mL	1 (1:10-1:20)	0.5 (1:10-1:20)

*Standardized short ragweed stock available in wt/vol with Amb a 1 (or Ag E; 1 U = 1 µg) concentration on label, or in AU/mL. Thus, volumes to be added will vary based on major antigen concentration in each lot.