

CHAPTER 9

Subcutaneous Allergen Immunotherapy Extract Preparation for Aeroallergens and Venom

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INTRODUCTION

Subcutaneous allergen immunotherapy is an effective treatment for certain atopic conditions. 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 through pollen extract inoculations. These inoculations involved 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. More than 100 years later, these same principles hold true 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 Parameter²⁶ suggests, “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, allergic rhinitis, or conjunctivitis symptoms during treatment, especially during the first few months of therapy.

There is no consensus on when to discontinue allergen 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 continue treatment indefinitely, given the risk of 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 the wrong dose or extract to the wrong patient. Other factors that may contribute to immunotherapy fatalities include symptomatic asthma and delay in administering 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

Because allergen immunotherapy carries the risk of life-threatening anaphylaxis, it is key to ensure specialized and systematic competency training for 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 that deliver allergen immunotherapy. 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 immunotherapy delivery.

Qualifications for doctors and staff who provide immunotherapy to patients vary by role. The prescribing practitioner is a physician who is specially trained in the practice and science of allergy immunotherapy. In some practices, the prescribing practitioner is also compounding and administering immunotherapy. However, having a staff member trained as compounding or extract preparation personnel is more common practice.

The practitioner supervising administration of allergen immunotherapy should be either the prescribing physician or another physician (or advanced degree medical provider) comfortable with recognizing signs and symptoms of and treating anaphylaxis. Personnel administering immunotherapy should be trained health

professionals working in a medical office. They should be specially trained in how to assess a patient’s health readiness before receiving immunotherapy, as well as in the technical aspect of administering an allergy immunotherapy injection.

Suggested qualifications of extract preparation personnel from the Practice Parameter²⁶ include:

- 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
- Be an appropriately trained health professional, including, but not limited to, registered nurse, licensed practical nurse, medical technician, medical assistant, physician assistant, advanced practice nurse, or physician

Compounding personnel requirements²⁹:

Before being allowed to compound allergen extract prescription sets, all personnel must complete requisite training confirming knowledge of the theoretical principles of sterile technique and demonstrating adequate skills for sterile technique. Initially, personnel must:

- 1) Successfully complete the gloved fingertip and thumb sampling at least three times
- 2) Successfully complete media fill test

These competencies must be updated every 12 months. If failed, deficiencies must be addressed

with reevaluation before resuming compounding duties, and if there is a more than 6-month gap in compounding, the competencies must be re-evaluated.

Each patient’s immunotherapy prescription is unique, and the administration schedule (build-up or maintenance) may also vary. Each patient should be evaluated before immunotherapy administration to determine whether any recent health changes might require modifying or withholding the treatment. Risk factors for severe immunotherapy reactions include symptomatic asthma and injections administered during symptom exacerbations. Clinical judgment is required when altering the dose or schedule of administration. State laws may differ regarding personnel who may give injections. Training in recognizing signs and symptoms of anaphylaxis and administering prescribed treatments for anaphylaxis is essential. Personnel administering allergy immunotherapy are often the first to notice when a patient is not tolerating a shot.

TRAINING OPPORTUNITIES

There are various 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 (American Academy of Allergy Asthma and Immunology) and ACAAI (American College of Allergy Asthma and Immunology) workshops and seminars

- Manuals from allergen extract manufacturers
- Journal articles from published recommendations

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 and ACAAI focuses on evidence-based recommendations that 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).

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 annually.
- Compounding personnel must be trained in preparation of allergenic products.
- Compounding personnel must pass a media-fill test annually, as Addendum A described.
- Compounding personnel who fail written or media-fill tests would be reinstructed and re-evaluated.
- Compounding personnel who do not compound for 6 months must have all competencies re-evaluated
- Compounding personnel must demonstrate understanding of antiseptic hand cleaning and disinfection of compounding 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, physician assistants, advanced practice nurses, pharmacists, pharmacy technicians, and physicians.

(Adopted from: Cox, L., Nelson, H., Lockey, R., et al. (2011), Allergen immunotherapy: A practice parameter third update. *J Allergy Clin Immunol*,

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.

Compounding 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 for extract preparation. The site must be either a Dedicated Allergenic Extracts Compounding Area (AECA) or an ISO Class 5 PEC (Primary Engineering Control).
- The extract preparation area must be sanitized with an EPA approved one step disinfectant cleaner.
- Apply 70% isopropanol on AECA horizontal compounding surface and the PEC between each prescription set..
- Extract preparation personnel must perform hand hygiene and thoroughly wash hands to wrists with detergent or soap and potable water. Substitution of hand washing by means 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 compounding 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, type of extract, dilution, storage requirement and the beyond-use date (BUD).

BUD: beyond-use date (BUD) no later than the expiration of the earliest expiration date of any individual extract. Maximum BUD for 1:1 (V:V) extract is one year from the date the prescriptive vial/set is mixed

Compounding log: A compounding log is to be kept with information on the patient's name, extract used for compounding, compounding 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 compounding, 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 for 7 days at 20-25 degrees followed by 7 days at 30-35 degrees. 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 personnel involved in administering allergen immunotherapy is widely recognized as a critical requirement for safety and efficacy. Content should include core cognitive knowledge and demonstration of procedure performance

competency. Appendix 9.1 contains a sample document for assessing and documenting personnel's competency in preparing 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 focus more on sterility by adding a sterile glove test that involves hand preparation, donning sterile gloves, cutting off the tip, and culturing for sterility.

ALLERGEN EXTRACT COMPOUNDING CONDITIONS

In addition to standardization of allergen stock extract manufacturing, there are new requirements for conditions under which allergen extracts should be prepared. Compounding 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 using antiseptics to clean working surfaces and vial tops before transfers.

Two sets of guidelines can be referenced in the preparation of clinic-specific standard operating procedures. The first is the Allergen Immunotherapy Practice Parameter prepared by the Joint Task Force on Practice Parameters, representing the AAAAI and ACAAI (Table 9.1).²⁶ The second set of guidelines is outlined in a 2019 bulletin from the AAAAI,³¹ which summarizes the final requirements for compounding allergy immunotherapy based on USP <797>. It should be noted that these standards are less rigorous than the standards required for typical sterile drug compounding in pharmacies.

Compounders should 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 compounding of allergen extracts involves a 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 chapter 797 only when the following criteria are met:^{31,32}

1. The compounding process involves transfer via sterile needles and syringes of conventionally manufactured sterile allergen products and appropriate conventionally manufactured sterile added substances; and
2. Manipulations are limited to penetrating stoppers on vials with sterile needles and syringes and transferring sterile liquids in sterile syringes to sterile vials.

USP emphasizes that full compliance with the much more stringent “low risk compounding requirements” is indicated unless appropriate measures are taken.

FACILITY REQUIREMENTS

The physician has two options for where extracts can be compounded in the office: a dedicated Allergenic Extracts Compounding Area (AECA) or an ISO Class 5 Primary Engineering Control (PEC) hood. Requirements for the compounding area include:

- Away from unsealed windows and doors that open to the outside

- Restricted traffic with no other activities in the area while compounding
- At least one meter away from the sink
- Good lighting
- Temperature and humidity controlled
- No carpet

In addition to those listed above, the dedicated AECA has extra requirements:

- Located away from restrooms, kitchen-food area, and warehouse storage
- Visible perimeters with 6 feet in each direction
- Compounding surface must be cleanable, resist damage from disinfectants and cleaners, and be smooth with no cracks or shedding. Appropriate surfaces include laminate, granite, composite, glass, or stainless steel.
- Overhangs must be easily cleanable

The extract preparation area (AECA or ISO class 5 PEC hood) must be sanitized with an EPA approved one-step disinfectant cleaner at least once per month (most have a 5-to-10-minute dwell time). The extract preparation area must also be cleaned with 70% isopropanol between patients' prescription sets.

PERSONNEL REQUIREMENTS

Extract preparation personnel must perform hand hygiene and thoroughly wash hands to wrists with detergent or soap and potable water. Substitution of hand washing using treatment with sanitizing agents containing alcohol, 70% isopropanol, or both is acceptable.

Minimal garbing by preparation personnel is required. Personnel should wear:

- Powder free sterile gloves
- Low-lint garment with sleeves that fit snugly around the wrists and enclosed at the neck (may be re-used for additional compounding tasks on the same day)
- Face mask
- Low-lint disposable shoe covers
- Low-lint disposable cover for the head, hair, ears
- Disposable cover for facial hair, if applicable

To further guard against contamination during the compounding process, allergy practices can consider these additional personnel garbing measures as outlined in the broader USP 797 standards:

- No ear buds or headphones
- No electronic devices that are not necessary for compounding or other required tasks
- No nail polish, artificial nails, and nail extenders (natural nails should be clean and neatly trimmed to minimize particle shedding and avoid glove puncture)
- No cosmetics, which can shed or flake
- No hand, wrist, and other exposed jewelry, including piercings that could interfere with the effectiveness of garbing

ALLERGEN EXTRACTS

Allergen extracts used for immunotherapy are made from collections of raw material (i.e., pollens, danders, dust mites, insects, molds, and cockroaches) 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 extracts 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 various purification steps, resulting in a stable liquid solution under normal storage conditions (4°C) without precipitation that can change the concentration of allergens in the mixture.

Each allergen extract can contain multiple 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 raw material components 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 nationwide and are used to mix individual treatment sets and prepare test panels.

Concentrated stock extracts are available in these forms:

- Aqueous
- Glycerinated

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

Glycerinated stock extracts contain 50% glycerin. 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 before 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 a depot when injected, 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 build-up 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, forming the liquid backbone of allergen extracts. Diluents are used to reconstitute lyophilized extracts, dilute extracts for diagnostic use, dilute vials in treatment sets, and fill maintenance vials to final volume after addition of stock allergen quantities.

Commonly used diluents include:

- 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 the preservation of extract potency and sterility. For example, glycerin is both a preservative and a 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.³⁰ Manufacturers then use these reference standards 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 range²⁶):

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 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 the 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
- Reference to administration schedule

All prescriptions should be reviewed for accuracy before preparation. Even though some of these elements may be routine for a clinic, reviewing them for each patient is important.

Optimal compounding 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 pollens³³
 - Do not combine venom extracts with aeroallergen extracts²⁶
- Selection of allergens and adjustment of doses using 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 Parameter.²⁶ Figure 9.2 is an example of a completed allergen immunotherapy prescription. In this example, the desired maintenance dose for cat antigen 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. 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)
- $5\text{mL} \times 4000 \text{ BAU/mL} = V2 \times 10,000 \text{ BAU/mL}$ [$V2 = \text{stock volume} = (5 \times 4\text{K})/10\text{K} = 2 \text{ mL}$]
- Repeat for each antigen in vial
- Total antigen volume = [cat 2 mL + *D. farinae* 0.5 mL + *D. pteronyssinus* 0.5 mL + timothy 0.2 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 µg/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, using these prescribing principles will result in a safe and effective product that can ensure the maintenance of expected potency through the expiration date. Combining 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 many 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 Standardized allergen extract prescription forms will remind clinicians and extract preparers to focus on these elements during the prescribing and extract preparation process.

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; $(V1 \times C1)/C2 = V2$.
 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 wt/vol $1/10 = 0.1 \times 100 = 10\%$ solution
 1:20 wt/vol $1/20 = 0.05 \times 100 = 5\%$ solution
 1:40 wt/vol $1/40 = 0.025 \times 100 = 2.5\%$ 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:
 $V1 \times C1 = V2 \times C2$ $5 \times (1/200) = V2 \times (1/10)$
 $5 \times (0.005) = V2 \times (0.1)$
 $V2 = (V1 \times C1)/C2$ $V2 = 0.025/0.1 = 0.25$
 To determine amount of diluent needed:
 $V1 - V2$ $5 - 0.25 = 4.75 \text{ mL}$

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

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

Example of a Completed Allergen Immunotherapy Prescription

Patient Name: Jerry Cleanex Patient Number: 23456 Birth Date: 05/05/90 Telephone: 645-345-0987	Prescribing Physician: Dr. Ah Choo Address: 665 Rosebud Lane Hollywood, FL 33424 Telephone: 645-123-4444 Fax: 645-123-4567												
Allergen Extract Content: C, R, G, T, W	Maintenance Concentrate Prescription Form												
<table border="1" style="font-size: 8px;"> <tr><th colspan="2">Bottle Name Abbreviations</th></tr> <tr><td>Tree: T</td><td>Mold: M</td></tr> <tr><td>Grass: G</td><td>Cat: C</td></tr> <tr><td>Wheat: W</td><td>Dog: D</td></tr> <tr><td>Ragweed: R</td><td>Codfish: Cr</td></tr> <tr><td>Mite: Mx</td><td>Dust Mite: Dm</td></tr> </table>	Bottle Name Abbreviations		Tree: T	Mold: M	Grass: G	Cat: C	Wheat: W	Dog: D	Ragweed: R	Codfish: Cr	Mite: Mx	Dust Mite: Dm	Prepared by: Mary Lancet Date Prepared: 6/10/09
Bottle Name Abbreviations													
Tree: T	Mold: M												
Grass: G	Cat: C												
Wheat: W	Dog: D												
Ragweed: R	Codfish: Cr												
Mite: Mx	Dust Mite: Dm												

* Components of mixes listed on a separate sheet

Antigen Number	Extract Name Allergen or Diluent (Common name or Genus/species)*	Concentration and Type Manufacturer's Extract (AU, BAU, W/V, PNU) (50% G, Aq, Ly, AP)	Volume of Manufacturer's Extract to Add [†]	Extract Manufacturer	Lot Number	Expiration Date
1	Short ragweed	1:10 w/v G (350 mcg Amb al)	0.25 ml	Greer	12345	1/01/10
2	<i>D. farinae</i>	10,000 AU/ml G	0.5 ml	Allermed	5765	2/06/10
3	<i>D. Pteronyssinus</i>	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	9898	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			6/09/09

[†] Assumes 0.5 ml injection as target maintenance dose

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

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
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Updated on 4/5/2011

COLOR CODING, LABELS, AND EXPIRATION DATES

The Allergen Immunotherapy Practice Parameter and Joint Commission National Patient Safety Goals emphasize the need for clear and consistent labeling.³⁵ Standardizing allergen immunotherapy label contents and vial coding improves communication between care providers and patients and can prevent errors in extract administration.³⁶

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

- Two patient identifiers (e.g., name and date of birth)
- Vial content (extracts in the vial or letter code to this information in the prescription)
- Concentration in vol/vol
- Color code or alphanumeric code ("1" for highest concentration if numbered)
- Expiration or "beyond use" date (BUD)
- Storage conditions

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). Still, this system may be complicated if allergens with different potency units are used (e.g., wt/vol, BAU, AU, or PNU). These differences make it challenging 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**, the next 10-fold dilution (i.e., yellow vial) should 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 (usually corresponding to the patient's red maintenance treatment vial and the 1:10 vol/vol, or yellow, vial) are generally stable for at least 12 months unless a component of the dilution mixture expires earlier. Expiration dates for venom extracts are sometimes shorter; perhaps 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.

The FDA regulates expiration dating periods for allergen extract products. 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 dates in accordance with FDA regulations (21 CFR, Section 610.53), keeping in mind whether products are glycerinated or alum precipitated, as well as other specific manufacturing

characteristics.³⁷ A total of six years from the time of extraction is allotted to 50% or more glycerin bulk extracts. This six-year period is divided into a maximum of three years for manufacturer storage and three years after leaving manufacturer's storage (when stored at 2 to 8°C). Products with less than 50% glycerin are allowed only a total of three years or half the expiration time allotted for 50% or more glycerinated extracts—18 months for manufacturer storage and 18 months after leaving manufacturer's storage.

Expiration dating practices for patient allergen extract treatment sets must be based on the earliest expiration date from all stock allergens used in each prescription. The potency of allergen extracts is specific to the characteristics of each extract and is affected by the contents of other extracts that may be in the mixture. High protease extracts (mold/fungi, cockroach, and imported fire ant)³⁸ and those not containing glycerin or other preservatives are likely to have lower potency as the time from reconstitution of the dilution increases.³⁹ Thus, the manufacturer instructions, storage conditions, vial extract content, and dilution strength (vol/vol) must be considered in determining expiration dates. However, the following sample expiration dates are usually accepted, and patient vials are commonly given 6-12 month dating³¹:

Diagnostic Products	Expiration Date*
Prick test materials	Follow manufacturer’s expiration date
ID test materials	Follow manufacturer’s expiration date; if diluting from prick test extracts, not to exceed 6 months from date of dilution†

Immunotherapy

Treatment Sets†

Stock solution	Follow manufacturer instructions
Dilutions from Stock:	
1:10 – 1:200 vol/vol	Up to 12 months*
< 1:200 vol/vol	3-6 months*

*Unless a component of the mixture has an earlier expiration date. Use earliest of stock extract label expiration date.

†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 into 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

Concentration	Typical Expiration Dates§
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. §During times of venom shortage, longer expiration dates were found to be effective.

COMPOUNDING INDIVIDUAL PATIENT ALLERGEN EXTRACT TREATMENT SETS

Every clinic should develop a standard operating procedure document or manual to ensure standardization and safe compounding practices of allergen extract. 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. These procedures should emphasize the importance of individual treatment vials and vial sets, especially when compounding of allergens is required. Compounding antigens in a syringe is not recommended because of the potential for cross-contamination of extracts. Although detailed resources for recommended facility, extract compounding, and personnel requirements can be found at AAAAI Compounding Corner at <https://education.aaaai.org/compounding-corner/compoundingcorner>, here are a few guiding principles for compounding allergen extracts:

- Optimal dosing should be within defined minimal effective-dose ranges.
- Avoid incompatible mixtures.
 - Stinging insect and aeroallergen extracts should not be combined.
 - High-protease extracts (molds and cockroach) have low compatibility with pollens.
- Selection and dose of allergens used for treatment 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 of personnel.
 - Accurate prescriptions, labels and color coding are required to prevent errors.
 - Use of quality assurance checks throughout the compounding process is highly recommended.
- 4) ISO Class 5 PEC hood:
 - a) External venting not required.
 - b) Hood must be recertified every 6 months.
 - 5) Dedicated AECA:
 - i) Must be away from restrooms, kitchen-food area, warehouse-storage.
 - ii) Should have visible perimeters, 6 feet in each direction and this area should be cleanable and kept clean including walls, doors, ceilings, fixtures, shelves, counters and cabinets, at least once a month using EPA approved one-step disinfectant cleaners (most have 5-10 minutes dwell time).
 - iii) Compounding surface must be cleanable, resist damage from disinfectants and cleaners, smooth with no cracks or shedding (laminated, granite, composite, glass, stainless steel).
 - iv) If dust collecting overhangs, must be easily cleanable.

INITIAL PREPARATION

- 1) Develop clinic-specific standard operating procedures complying with USP 797 requirements.
- 2) Designate an allergen compounding location. Choose either a dedicated allergen extract compounding area (AECA) or International Standards Organization (ISO) Class 5 Primary Engineering Control (PEC) hood.³²
- 3) For both dedicated AECA and ISO class 5 PEC hood:
 - i) The area should be well-lighted with temperature and humidity controls.
 - ii) Location should be an area of the clinic where personnel traffic is restricted and exposure to potential contaminants is minimized. The location should be away from unsealed windows, doors that open to the outside, restrooms, warehouses, or food preparation areas.
 - iii) Should be at least one meter away from sinks.
 - iv) Compounding areas must be cleansed and prepared before every compounding session
- 6) Beyond Use Date (BUD): no later than the expiration of the earliest expiration date of any component (any extract or diluent). Maximum BUD for 1:1 (V:V) is one year from the date the prescriptive vial/set is compounded.
 - a) More dilute vials usually have an earlier expiration date - see notes above.
- 7) Become familiar with stock allergen extract ordering and storage procedures.
- 8) Orient personnel to stock allergen extracts, refrigerator storage recommendations, designated compounding location, compounding equipment, prescriptions, documentation, and packaging.

- 9) Undergo training on standard operation procedures and safety measures. Only authorized personnel with the requisite training should be allowed to compound allergen extracts.

PRE-COMPOUNDING PREPARATION

- 1) Verify that a supervising physician is present in the same building as the compounding location(s), and available if needed.
- 2) Prepare specific compounding location(s) as per the USP 797 guidelines.
- 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:
 - a) Name and second identifier, such as date of birth.
 - b) Vial extract content.
 - c) Concentration.
 - d) Antigens on label match those that are to be added from the prescription.
 - e) Expiration date is consistent with clinic procedures and source antigens.
- 5) Apply label to treatment set vials.
- 6) If using color-coded vials, verify that color and concentration match.

EXAMPLES OF ALLERGEN EXTRACT COMPOUNDING 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 facility’s needs.

COMPOUNDING 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 them in order from most concentrated (maintenance/red) to most dilute.
- 2) Pull the stock extract vial for each antigen on the prescription and stock diluents from refrigerator.
 - a) Check stock antigens for turbidity/particulate matter. If present, consult package insert or manufacturer guidelines, including possible recommendations for re-suspension or filtering.
 - b) For prolonged compounding sessions, return unused stock extracts to refrigerator or cooling tray (2° to -8°C) between prescriptions or during extended breaks.
- 3) Place a new syringe by each stock antigen vial and the diluent.
 - a) A separate syringe is used for each antigen and diluent.
 - b) Label each syringe (i.e., abbreviation for antigen or diluents).
 - c) 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 dates do not exceed earliest stock vial extract.
 - 6) Wear appropriate personal protective equipment.
 - a) Wash hands/nails to elbows for at least 30 seconds with soap and water.
 - b) Don shoe, hair and facial hair covers (low lint disposable covers), gowns (low lint with snug sleeves and enclosed neck), and face masks.
 - c) Use alcohol-based surgical hand scrub prior to gloving.
 - d) 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 prior to use to allow the vial/ampule to dry
 - 9) Maintain aseptic technique by minimizing contact with secretions, skin, glove fingertips, and the like during compounding.
 - 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 that 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.
 - a) The empty syringes should be discarded immediately into an appropriate Sharps disposal container.
 - b) If the sterile maintenance vial is not a vacuum (air-filled), an equal volume of air may need to be withdrawn before injecting stock extract volumes.
 - 13) If there is precipitate present in the stock antigen vials,
 - a) Particulates and precipitates suspended in an extract solution are not uncommon.
 - b) These particulates and precipitates often do not cause any significant loss in potency. Consult manufacturer recommendations in package insert or bulletins for additional information.
 - c) Attempted re-suspension by agitation (shaking or rolling) may be indicated in accordance with the package insert and your clinic operating procedures. For alum-precipitated vials, consider adding a “shake well” label, as the alum-allergen complex is likely to settle.
 - 14) After compounding is complete, conduct a final quality assurance check (preferably by compounder and trained co-worker), including:
 - a) Solution color check.
 - b) Label check.
 - c) Vial color-code check.
 - d) Final volume, liquid turbidity, precipitate, and consistency check.
 - e) Vial physical integrity (closures, leaks, cracks, deformities, etc.) 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 compounding log (see Practice Parameter appendices for sample forms), as follows:
 - a) Name of preparer(s) and date prepared.
 - b) Stock allergen extract manufacturer, concentration, lot number, and beyond-use (BUD) or expiration date for each component.
 - c) Compounding 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, resulting 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**, which involves taking from a parent vial and placing it 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, potentially increasing 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 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 the yellow vial for a final total 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 vials' labeling and order (color coded, label concentration) are correct.
- 2) Ensure the maintenance vial is compounded by inverting or rolling.
- 3) Using a fresh syringe and aseptic technique, remove 0.5 mL from the compounded 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 compounded 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 vial, labeled 10% glycerol-saline prefilled vial):
 - a) 0.5 mL from yellow 1:10 vol/vol into 4.5 mL diluent-filled blue 1:100 vol/vol vial
 - b) 0.5 mL from blue 1:100 vol/vol into 4.5 mL diluent-filled green 1:1000 vol/vol vial
 - c) 0.5 mL from green 1:1000 vol/vol into 4.5 mL diluent-filled silver 1:10,000 vol/vol vial
 - d) And so on for additional more dilute (silver) vials
- 7) Whereas using a fresh syringe for each dilution transfer is often preferred, using 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 it 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 compounding the initial maintenance vial.)

ALLERGEN EXTRACT TREATMENT SET PREPARATION HINTS

- 1) Do not compound 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.

- 4) Regularly review operating procedures for opportunities to make the process safer and more efficient.
- 5) Establish a regular inventory check.
 - a) Identify stock allergen extracts, diluents, and compounding supplies in need of reordering.
 - b) Check for expiring stock allergen extracts, diluents, and compounding 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) Do not use stock refrigerators for food or drink storage.

ADDITIONAL QUALITY ASSURANCE CHECKS

Additional quality assurance checks are ideally done by a co-worker before allergen extract shipping and use. Quality assurance checks include vial inspection for:

- Label accuracy-“five rights” as described below
- Vial serial color dilution that matches label concentration and vial color coding
- Vial integrity (closure, cracks, leaks, and deformities.)
- Vial content (particulate matter, fill volume)

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 Parameter for that color code (see Tables XI and XII from Practice Parameter²⁶). Additionally, the color of the solution should be a shade consistent with 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 particulate or solid materials and cloudiness. If found, vials may be contaminated or contain precipitated raw allergen extract. Contamination may be bacterial or another 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, deformities, or stopper disruptions. Any abnormal finding during these checks should be followed by an investigation for the cause and, in most instances, starting over and recompounding 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 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 the whole insect body as opposed to concentrated venom proved not to be as effective as extracts made from venom.⁴⁰ 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 are 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. Whole body extracts (WBE) for fire ant venom hypersensitivity have been shown to be effective. Fire ant venom extracts are being

investigated for clinical use but require significant 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). Fire ant immunotherapy protocols have been described, including rush protocols.⁴¹ The Practice Parameter 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 recommendations using 0.5 mL of a 1:100 wt/vol maintenance concentrate of *S. invicta* or a combination of *S. richteri* and *S. invicta*.⁴² Maintenance doses at 1:200 wt/vol have also often been used, and growing evidence supports its efficacy.⁴³ Insect venom (and fire ant) extracts generally should not be combined 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

Elevated temperatures, contamination, and protease degradation of key allergenic proteins responsible for the efficacy of immunotherapy can compromise the stability and potency of allergen extracts.^{33,34,44-46} Stock extract manufacturers and healthcare personnel take several measures to minimize the risk of loss of potency of extracts during normal storage and use.

Dilution of extracts alone can also affect the long-term potency of extracts.³⁷ For example, diluted extracts have lower concentrations of essential preservatives and stabilizers. Furthermore, lower concentrations of proteins decrease three-dimensional protein structure stabilization achieved through protein-protein interactions facilitated at higher protein concentrations. Finally, dilutions may also magnify the effect of allergenic protein loss due to binding on glass vials. This level of protein loss would be 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 refrigerators’ stock inventory is routinely rotated so 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 aseptic technique and manufacturer recommendations are followed when compounding extracts, as per the most current guidelines.
- Ensure that personal allergen extract storage trays are stored at recommended temperatures.
- Confirm that individual allergen mixtures promote optimal extract stability

- Ensure that extracts are kept cool during extended periods of compounding.

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** if 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 phenol is that it may denature (unfold or break down) 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.

Disinfectants are antimicrobial agents applied to nonliving objects (e.g., countertops). **Seventy percent isopropanol** is a disinfectant but not a preservative. 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 a preservative, whereas HSA is not a preservative. Glycerin potently stabilizes proteins in solution, inhibits proteases found in some allergen extracts, and is bacteriostatic at concentrations $\geq 20\%$.⁴⁶

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 irritates 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 allergen immunotherapy 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 in 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. Therefore, care

should be taken to determine the final glycerin content in the maintenance treatment extracts. For this same reason, the preferred diluent for preparing extracts for intradermal diagnostic testing is HSA in order to limit skin irritation and the possibility 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 with more dilute extracts may also lead to lower “sensitivity,” resulting in missing the identification of relevant allergens that could have been identified at a higher concentration (higher rates of false negative test results).

Allergen extracts are stored in refrigerators at a **temperature of 4°C** or in accordance with manufacturer recommendations. A temperature range of 2° to -8°C is considered acceptable by most experts. Given the expense and temperature sensitivity of stock allergen extract concentrates and mixed patient treatment sets, conducting some form of temperature monitoring is also reasonable to 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., $>78^{\circ}\text{F}$ 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)⁴⁷. 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. The presence of 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 also increases the observed loss in potency of extracts. Thus, accidentally frozen extracts should be replaced with new extracts before 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 Parameter recommends the separation of extracts with high proteolytic enzyme activities, such as mold, cockroach, and insects, from other extracts, such as pollens. Also of note, although there have been reports to the contrary,⁴⁸ dust mite extracts in the United States do not appear to significantly degrade pollen or animal dander extracts and can be combined 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. Specific guidance on preparation has also been provided by USP 797, and this document adapts that guidance. Using a team approach to develop clinic/facility-specific policies and procedures will ultimately improve the quality and precision of allergen immunotherapy preparation. Ongoing review of these procedures is important and 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 staff training will promote the safety of the patients entrusted to our care and those performing allergen extract preparation.

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

- Contamination is prevented by use of aseptic technique, appropriate compounding environment, and adequate training.
- Advanced preparation, accurate labels, and color coding are highly recommended to prevent errors.
- Use of quality assurance checks throughout the compounding 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 combined.

All personnel involved in allergen extract preparation should be familiar with the contents of the most recent Practice Parameter. 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 AAAAI website.

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APPENDIX 9.1: INITIAL AND ONGOING COMPETENCY ASSESSMENT – ALLERGEN EXTRACT COMPOUNDING

Name: _____ Job Title: _____ Clinic: _____

Allergen Extract Preparation	Date	Validated By	Comments/Notes
Passed 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 compounding surface and washes hands appropriately			
Uses appropriate personnel protective equipment			
Checks stocks and compounded 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 numbers and preparation details per clinic SOPs			
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: _____

<p><u>Self-Assessment</u> 1 = Experienced 2 = Needs Practice/Assistance 3 = Never Done NA = Not Applicable</p>	<p><u>Evaluation/Validation Methodologies</u> T = Tests D = Demonstration V = Verbal I = Interactive Class</p>
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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 COMPOUNDING STOCK EXTRACT VOLUMES FOR 5- OR 10-ML VIALS BASED ON PROBABLE EFFECTIVE MAINTENANCE DOSE RANGES

Allergen	Probable effective 0.5-ml maintenance dose range	Typical volume range for 10-ml vial (stock concentrate)	Typical volume range for 5-ml vial (stock concentrate)
Grass (e.g., Phl p 5)	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 d 1)	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, mold/fungi, cockroach	Highest tolerated dose	Variable (1:10-1:20 w/v)	Variable (1:10-1:20 w/v)
Nonstandardized extracts, pollen	0.5 mL of 1:100 w/v	1 (1:10-1:20 w/v)	0.5 (1:10-1:20 w/v)
Nonstandardized extracts, Dog (Can f 1)	15 µg	2 (1:100 w/v AP Dog) 1 (1:10-1:20 Dog epithelia)	1 (1:100 w/v AP Dog) 0.5 (1:10-1:20 Dog epithelia)
Hymenoptera Venom	50-200 µg of each venom	Stock concentrate- 100 µg single venom or 300 µg of mixed vespid	Stock concentrate- 100 µg single venom or 300 µg of mixed vespid

*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.

w/v: weight/volume