American College of Allergy, Asthma & Immunology

Allergen Immunotherapy Extract Preparation

Instructional Guide

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Table of Contents

Acronym Key

AECA	Allergenic Extract Compounding Area
AU	Allergy Unit
BAU	Album
BUD	Beyond Use Date
CBER	Center for Biologics Evaluation and Research
C	Centigrade
CSP	Compounded Sterile Preparations
F	Fahrenheit
FDA	Food and Drug Administration
HEPA	High Efficiency
HSA	Human Serum Albumin
IPA	Isopropyl Alcohol
ISO	International Organization for Standardization
JCAHO	Joint Commission Accreditation Hospital Organization
mcg/mL	Microgram/Milliliter
mL	Milliliter
PEC	Primary Engineering Control
PNU	Protein Nitrogen Unit
SCA	Segregated Compounding Area
SOP	Standard Operating Procedures
USP	United State Pharmacopeia
Vol/Vol	Volume per volume
W/V	Weight per Volume

I. INTRODUCTION

Origins of Allergen Immunotherapy

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 basic principles hold true today, more than 100 years later, for current allergen immunotherapy.

Efficacy of Allergen Immunotherapy

Multiple studies have demonstrated the effectiveness of allergen immunotherapy for the following conditions for both children and adults:[2-29]

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

Each patient's immunotherapy prescription is customized and tailored to their particular allergic triggers, and the administration schedule (buildup or maintenance) may vary. The degree of effectiveness may vary for the individual patient and is in part determined by whether causative symptoms are predominantly allergic vs non-allergic – patients may be more likely to have a limited response if other causes are contributing to impairment. Other symptomatic causes may include vasomotor rhinitis, chronic infectious sinusitis, or anatomical obstruction such as severe adenoidal hypertrophy.

In those whose symptoms are predominantly allergic in nature, symptomatic improvement is noted in most individuals within 12-18 months of initiation of allergy immunotherapy. The Allergen Immunotherapy Practice Parameter third update[30] suggests that, "If clinical improvement is not apparent after one 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.

Duration 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. However, patients who have experienced life-threatening hymenoptera venom reactions should be considered for life-long treatment. Elevated tryptase levels are associated with higher relapse rates after discontinuation of venom immunotherapy.[30]

Adverse Reactions

Allergen immunotherapy is usually well tolerated; however, adverse reactions do occur, ranging in severity from mild to severe. Allergen immunotherapy carries a small but significant risk for life-threatening anaphylaxis and has very rarely been associated with death.

Systemic reactions associated with immunotherapy may have numerous causes.[30] Risk factors include asthma and injections administered during periods of symptom exacerbations. Human error, due to administering an incorrect dose or the wrong extract to a patient is another risk factor. Adverse effects have also been associated with initial doses from new maintenance vials, gaps in scheduled dosing and administration during pollen seasons for highly sensitive patients. Delays in the administration of epinephrine during a systemic reaction may increase the risk of fatalities, which highlights the importance of having trained personnel capable of identifying and managing reactions in a medical setting.

Each patient should be evaluated prior to the immunotherapy administration visit to determine whether any recent health changes such as illness, acute asthma symptoms, or new medication - which might require modifying or withholding the immunotherapy treatment. Clinical judgment is required when altering the dose or schedule of administration. While allergen immunotherapy extracts are relatively easy to prepare and administer, initial and ongoing training will improve the expertise of health care workers responsible for mixing and administering immunotherapy to help ensure patient safety.

Subcutaneous allergen immunotherapy is not indicated for patients with food allergies. Although studies have demonstrated an increased tolerance to peanut in patients who received subcutaneous peanut immunotherapy,[31,32] there was an unacceptably high rate of systemic reactions (e.g., anaphylaxis) in patients during treatment.[32]

II. ALLERGEN EXTRACTS

Manufacturing of allergy treatment extracts typically involves crushing raw materials and "extracting" allergenic proteins by addition of solvents capable of isolating the protein from the solid raw material. This is followed by a purification process, resulting in a liquid solution that is stable under normal storage condition (approximately 4 degrees C or 39 degrees F), consisting of a mixture of the diluents or solvents, additives, stabilizer / preservative, allergenic proteins and other minor components of the raw material. Proteins are derived from pollens, dust mites, animal dander and epithelia, molds, cockroach, and/or venom. Extracts added to treatment vials should be customized and clinically relevant for an individual patient based on perennial and seasonal symptoms, the regional presence of pollens and molds, and causative allergens based on exposure and results of testing. Stock allergen extracts used for immunotherapy are licensed by the Center for Biologics Evaluation and Research (CBER) within the Food and Drug Administration (FDA) in the United States for skin testing and immunotherapy by percutaneous, subcutaneous and intradermal routes. Commercially available stock extracts, supplied by manufacturers are used to mix individual treatment sets and to prepare test panels.

Concentrated stock extracts are available in multiple 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 form 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 is used to precipitate proteins from liquid into a solid form, which 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. 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 injection 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 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 may be referenced 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. The slow release described above makes these less effective in skin testing and are thus used for treatment only.

Diluents are solutions used to keep the allergens in suspension. They 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. These additives are discussed in further detail in this chapter's discussion of extract stability.

Standardized Subcutaneous Allergen Extracts

Standardized subcutaneous allergen extracts in the United States include:

- 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 mcg/mL)

These extracts have been standardized with the intent of ensuring consistency of allergen content and potency between manufacturers and lots made from the same manufacturer. Standardization is 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 $ID_{50}EAL$.[33]

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 a1), 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), and microgram protein/mL (mcg/mL). Some allergen extract labels also include the concentration of major allergenic proteins in mcg/mL. Since the standardization is based on allergen content falling within a specified range, it still remains possible for actual allergenic protein content to vary several-fold for the same potency label. Only a few allergen extracts have been standardized to date (see Appendix 1 for effective dosing ranges).

III. ALLERGEN EXTRACT MIXING STANDARDS, QUALIFICATIONS, AND COMPETENCIES

Multiple guidelines are available detailing recommendations and best practices for allergen extract mixing personnel. The most widely adopted recommendations in the United States for all aspects of allergen immunotherapy are contained in the 2011 publication, "Allergen immunotherapy: A practice parameter third update."[30] This joint effort by experts from the College and AAAAI focuses on evidence-based recommendations to optimize immunotherapy efficacy and safety. All health care 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 task force is in the process of conducting the 4th update which will include updated allergen extract preparation recommendations based on emerging evidence and the latest national guidelines.

A second publication, USP Chapter <797> Section 21, focuses on maintaining sterility in the compounding of allergen extracts, including requirements for training and testing of personnel[34]. USP 797 sets standards for preparing sterile preparations, including allergen extract preparation. The requirements for compounding of allergenic extracts are laid out in a specific paragraph of USP 797 for allergen extract preparation. These standards are extensive and more rigorous than earlier USP 797 requirements, but remain less complex for adherence by mixing personnel than standards required for typical sterile drug compounding in pharmacies.[35]

Practitioner Qualifications

There are many positions within the allergy immunology office that have an impact on the safety of allergen immunotherapy. These include:

1. Supervising physician: responsible for overseeing and ensuring the competency of staff preparing and administering allergen immunotherapy.

2. Designated supervisor: person with training and expertise in allergen immunotherapy who is responsible for ensuring that personnel who will be preparing allergen immunotherapy are trained, evaluated and supervised.

3. Compounding personnel: those who mix allergenic extracts. Before beginning to independently prepare allergenic extracts, all compounding personnel must complete training and demonstrate knowledge of principles and skills for sterile compounding and understand relevant aspects of allergen extract preparation.

4. Shot personnel: those who administer allergy immunotherapy injections. State laws may differ with regard to approved personnel. Knowledge of how to appropriately administer immunotherapy extracts is critical for patient safety, making personnel training a requirement. Recognizing the signs and symptoms of anaphylaxis are also critical for any facility involved in the delivery of allergen immunotherapy. Efforts at standardization, including color coding vials, standardizing labeling and documentation are intended to decrease the risk of dosing errors for any facility administering immunotherapy.

Training, Competency Assessment and Documentation

Training of personnel involved in the mixing of allergen extracts and/or administration of allergen immunotherapy is a critical requirement for safety and efficacy.[28,30] Content of the training should include core cognitive knowledge as well as demonstration of procedure performance competency.

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:

- On-the-job training from a qualified co-worker and supervisor.
- Workshops and seminars offered at dedicated mixing personnel workshops and at the College's Annual Meeting.
- Educational offerings via the College Learning Connection.
- Allergen Extract Preparation Quiz.
- Manuals from allergen extract manufacturers.
- Allergen Immunotherapy: A Practice Parameter Third Update and other journal articles.
- Allergen Extract Mixing Toolkit.

Suggested qualifications of extract preparation personnel:

- Demonstrate understanding of appropriate hand hygiene, garbing, surface disinfection, aseptic technique, achieving and/or maintaining sterility, calculating/measuring/mixing, use of equipment and documentation.
- Pass a written test on aseptic technique and extract preparation.
- Annually pass a media-fill or equivalent test verifying use of aseptic technique.
- Annually pass a gloved fingertip-thumb sampling test verifying hand sterility after passing three initial tests.
- Be reinstructed and reevaluated if failing the written test, media-fill test or gloved fingertip-thumb sampling test.

To be compliant with USP Chapter 797 requirements, allergist offices must keep records of training, assessment results, evaluations and qualifications for all compounding personnel, including any corrective actions following assessments and evaluations. Appendix 2 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 serves as an example – each mixing center should develop site-specific standard operating procedures and competency standards, with forms that meet the needs of their specific practice.*

IV. 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. Employing the prescribing principles that follow will result in a product that is safe and effective, and can ensure maintenance of expected potency through the expiration date. Each prescription / immunotherapy record should contain:

- Two patient identifiers (i.e., Name and Date of Birth or Medical Record Number)
- 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)

All prescriptions should be reviewed for accuracy prior to preparation.

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 1)
- Avoiding combining extracts that may adversely affect overall potency
  - Separate high protease extracts (mold, cockroach) from pollens[32]
  - Avoid mixing venom extracts with aeroallergen extracts.[26] Selection and adjustment of doses using knowledge of cross-reactivity

Mixing high-protease extracts with most other aeroallergens will result in a loss of potency that can affect immunotherapy efficacy.[28,30,36-38] 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.[26,39,40]

Figure 1 is an example of a completed allergen immunotherapy prescription. In this example, the desired maintenance dose for cat was 2,000 BAU for a 0.5-mL injected dose from a 5-mL maintenance vial (a blank form is available in the Appendix).

2 mL of standardized cat extract (10,000 BAU/mL) is needed to achieve a final maintenance vial concentration of 4,000 BAU/mL and an injection dose of 2,000 BAU. To do this use the step-by-step calculations below.

Step-by-step calculations are as follows:

- Maintenance vial concentration of cat = injection dose/injection volume = 2,000 BAU/0.5 mL = 4,000 BAU/mL
- V1 x C1 = V2 x C2 (maintenance vial volume x maintenance vial concentration = stock volume x stock concentration)
- 5mL x 4,000 BAU/mL = V2 x 10,000 BAU/mL [V2 = stock volume = (5 x 4K)/10K = 2 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	Dose
Cat	4,000 BAU/ml	2,000 BAU
Ragweed	17.5 mcg/mL	8.75 mcg Amb A 1
D. farina	1,000 AU/mL	500 AU
D. pteronyssinus	1,000 AU/mL	500 AU

FIGURE 1. EXAMPLE – ALLERGEN IMMUNOTHERAPY EXTRACT PRESCRIPTION *(blank form available in Appendix)*

Patient Name: **Mary Wheeze**	Prescribing Physician: **Dr. Allergist**
Patient Number:	Address:
Birth Date:	Telephone:
Telephone:	Fax:

Allergen Extract Name: **R, Dm, C, G**	
Bottle Name Abbreviations	
Tree: T	Mold: M
Grass: G	Cat: C
Weed: W	Dog: D
Ragweed: R	Cockroach: CR
Mixture: Mx	Dust Mite: Dm

Maintenance Concentrate Prescription Form

Prepared by: **Great Nurse** Date Prepared: **11/01/2019** Time: **10 a.m.**

Dates of subsequent dilutions from maintenance concentration with expirations dates:
Vial _____ From Vial _____ on ____/____/____ Expiration date: ____/____/____
Vial _____ From Vial _____ on ____/____/____ Expiration date: ____/____/____
Vial _____ From Vial _____ on ____/____/____ Expiration date: ____/____/____
Vial _____ From Vial _____ on ____/____/____ Expiration date: ____/____/____

Antigen Number	Extract Name Allergen or Diluent (Common name of *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 a1	0.25 ml	Greer	11111	12/25/2021
2	*D. farina*	10,000 AU/ml G	0.5 ml	Allermed	22222	12/26/2021
3	*D. Pteronyssinus*	10,000 AU/ml G	0.5 ml	Antigen labs	33333	2/7/2022
4	Cat	10,000 BAU/ml G	2 ml	Hollister-Stier	44444	12/5/2021
5	Timothy Grass	100,000 BAU/ml G	0.4 ml	ALK	55555	12/3/2021
6						
7						
8						
9						
10						
Diluent	HAS		1.35 ml	ALK	66666	12/5/2021
Total Volume						

**Components of mixes listed on a separate sheet.

Specific Instructions:	
Prescribing Physician's Signature	Date

Volume to add = $\frac{\text{Maintenance Concentration}}{\text{Conc. of Manufacturer's Extract}}$ x Total Volume

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

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

By Use Date _____

Storage Requirements _____

Results of Quality Control (e.g. visual inspections, second verification of questions) _____

*Adapted from Allergen immunotherapy: A practice parameter second update. *J Allergy Clin Immunol* 2007;120: S77.

V. COLOR CODING, LABELS AND BEYOND USE DATES

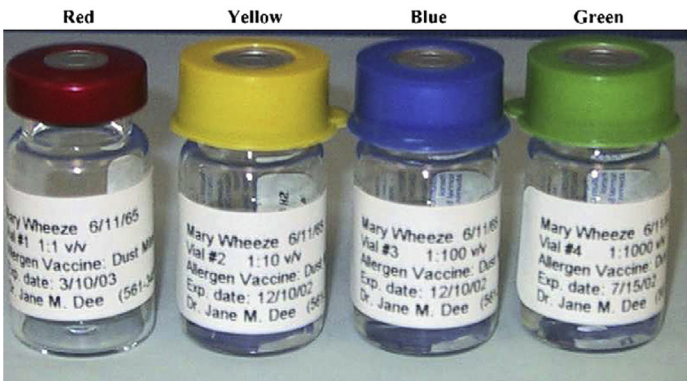
The Allergen Immunotherapy Practice Parameters and Joint Commission National Patient Safety goals emphasize the need for clear and consistent labeling. Standardization of allergen immunotherapy label contents and vial coding helps improve communication between care providers and patients, and likely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

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

- Patient name, plus a second identifier (e.g., date of birth)
- Concentration in vol/vol
- Type and fractional dilution of each vial, with a corresponding vial number.
- Color code and vial number, per the Practice Parameters[26]
- 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., 1,000 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 vials in the treatment set should be numbered and/or color coded in the following manner as outlined in the most recent practice parameters (Figure 2, page S44):[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



The **highest concentration should be labeled #1** and the next 10-fold dilution (i.e., yellow vial) would be labeled #2, and so forth. This system was designed to eliminate variation from patient to patient that 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.

Vial expiration dates should follow the manufacturer's recommendations. The expiration date for a treatment vial is required to be no later than the earliest expiration date recommended for any stock allergen or diluent contained in each prescription vial. The BUD cannot exceed one year from the mix or dilution date of the prescription set. 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.[36,37] 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 may be 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 dates in accordance with FDA regulations (21 CFR, Section 610.53) with regard to whether products are glycerinated or non-glycerinated.

The following sample expiration dates are adapted from U.S. Army Centralized Allergen Extract Laboratory treatment set preparation procedures, and are based on stock concentrate manufacturer and supplier recommendations.

Diagnostic Products	Expiration Date*
Prick Test Materials	1 Year
ID Test Materials	6 Months
Immunotherapy Treatment Sets	
1:1 v/v-1:10 v/v	1 Year
1:100 v/v and weaker	3-6 Months
1:10 w/v-1:5,000 w/v	1 Year
1:50,000 w/v and weaker	3-6 Months**
1,000 PNU/ml – 20,000 PNU/ml	1 Year
<1,000 PNU/ml	3-6 Months**
500 AU/ml and Stronger	1 Year
< 500 AU/ml	3-6 months**
1,000 BAU/ml and Stronger	1 Year
< 1,000 BAU/ml	3-6 months**

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

**The stability of lower extract concentrations (e.g., 1:1,000 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. Human serum albumin 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	
100mcg/ml	6 or 12 months*
1-10mcg/ml	1 month
0.1mcg/ml	14 days
<0.1mcg/ml	24 hours

*Varies with company. Guidelines for dilution expiration dating are in the extract package insert or information provided along with the stock extract.

VI. MIXING INDIVIDUAL PATIENT ALLERGEN EXTRACT TREATMENT SETS

General Principles

Every clinic should develop a specific standard operating procedure (SOP) document or manual to ensure standardization and safe practices of allergen extract mixing in accordance with most recent national guidelines. 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 must also be reviewed and followed (see Appendix 5). The mixing of antigens in a syringe is not recommended because of the potential for cross-contamination of extracts.

Initial Preparation

1) The compounding process may only occur in either a dedicated allergenic extract compounding area (AECA) or an ISO Class 5 Primary Engineering Control (PEC) room. If a PEC is used, PEC certification must occur at least every 6 months (See USP 797 - 5. Certification and Recertification).

   A. If using an AECA, designate an area with a visible perimeter that establishes the boundaries of the AECA, and meets the following conditions (as required by USP 797):

   - Access restricted to authorized personnel.
   - No other activity permitted during compounding.
   - All surfaces are cleanable and kept clean.
   - Carpet is not allowed.
   - Surfaces must be impervious to cleaning and sanitizing agents; must be smooth, non-shedding, and free of cracks or crevices.
   - Overhangs should be avoided or must be easily cleaned.
   - Work areas must be well lit, temperature and humidity controlled for comfort of compounding personnel.
   - Work surface must be cleaned and disinfected before and after each compounding session; disinfected between each new set, as well as at the time of any spill or contamination during compounding.

The following requirements apply to both PECs and AECAs.

2) Before beginning compounding, ensure your personnel performs hand hygiene and garbing procedures (that should include the minimum requirements noted below) and according to your clinic's SOPs.

   - Low-lint garment with sleeves that fit snugly around the wrists and that is enclosed at the neck (e.g., gowns or coveralls);
   - Low-lint, disposable covers for head that cover the hair and ears and, if applicable, disposable cover for facial hair;

- Face mask; and
- Sterile powder-free gloves.
- Ensure compounding personnel rub sterile 70% IPA onto all surfaces of the gloves and allow them to dry thoroughly. Repeat throughout the mixing process for extended periods of use.

3) Become familiar with stock allergen ordering and storage procedures.

4) Orient personnel to stock allergen extracts, refrigerator storage designated mixing location, mixing equipment, prescriptions, documentation and packaging. Clearly identify expiration dating standards for your clinic.

5) Ensure appropriate personnel are trained on SOP and safety measures and have the required competencies to independently mix allergen extracts.

Pre-mixing Preparation

1) Verify that a supervising physician is present in the same building as the mixing location(s).

2) Cleanse and maintain an aseptic work environment using 70% isopropanol as recommended in USP 797 Allergen Extract Preparation

3) Prepare vial labels in accordance with prescription and verify accuracy of:
   - Name and second identifier
   - Concentration
   - Beyond use date (BUD) is consistent with clinic procedures and source antigens.
   - Labels may be applied prior to or 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, color comparison of dilution series, and better aseptic technique.

4) Verify color-coded vials with designated concentration

Example Allergen Extract Mixing Step-By-Step Procedures

This sample set of procedures provides general guidelines that can be used as a starting point to develop procedures that best fit a specific clinic/facility needs.

Mixing the Maintenance (Red) Vial (see Appendix 3)

1) Select new empty sterile vials (usually 5, 8 or 10 mL) for each vial in patient's treatment set, using color codes to line up strongest (maintenance/red) to most dilute.

2) Select stock extract for each antigen contained in the prescription, and stock diluents.
   - Check stock antigens for turbidity/particulate matter. If present, consult package insert or manufacturer guidelines including possible recommendations for resuspension 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).
   - Avoid storing stock extracts in a syringe for extended periods due to risk of potency loss and misidentification.

4) Document lot number and manufacturer for each antigen.

5) Ensure that label expiration date does not exceed earliest stock vial extract or diluent expiration date and does not exceed 1 year from the mix or dilution date of the prescription set.

6) Perform Hand Hygiene per USP 797 standards.
   - Remove visible debris from underneath fingernails under warm running water using a disposable nail cleaner.
   - Wash hands and forearms up to the elbows with soap and water for at least 30 seconds.
   - Dry hands and forearms to the elbows completely with low-lint disposable towels or wipers.

7) Personnel garbing requirements.
   - Don hair and facial hair covers, gowns and face masks.
   - Use alcohol- based surgical hand scrub prior to gloving.
   - Don powder-free sterile gloves compatible with 70% isopropyl alcohol.

8) Disinfect gloves with isopropyl alcohol and allow them to dry before mixing (and intermittently for lengthy mixing).

9) Wipe vials and/or ampules with 70% isopropyl alcohol and allow to dry.

10) Maintain aseptic technique by minimizing contact with secretions, skin, glove fingertips and other potential sources of contamination during the mixing process.

11) Draw the correct amount of each antigen and the diluent into the syringe, and place each syringe by the respective stock antigen vial.

12) Verify drawn doses are correct volume and antigen. (Quality checkpoint opportunity: have a co-worker verify, if available. This is a best practice, but this is not required by USP 797.)

13) Sequentially inject contents of antigens into the maintenance concentrate (red) vial.
- Empty syringes should be discarded immediately into an appropriate sharps disposal container.
- If the sterile maintenance vial is not a vacuum vial, an equal volume of air may need to be withdrawn prior to injecting stock extract volumes.

14) After mixing is complete, conduct final quality assurance check (best practice would include mixer and trained co-worker), including:
- Solution and color dilution check.
- Label check.
- Vial color-code and numbering 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 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 allergen recall or for extract or adverse-event troubleshooting.

Notes Regarding Alum-Precipitated 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

- 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 then becomes the "new" parent vial, and dilutions are repeated until the desired number of 10-fold dilutions is achieved.
- 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.
- To determine how much should be taken from the parent vial for a 10-fold dilution for final volume X, divide X by ten (i.e., for a 10 ml vial: 10ml/10 = 1 ml). Then calculate the amount of diluent needed by subtracting this X/10 volume from the final volume X (i.e. 10 ml – 1 ml = 9 ml). The final concentration of the diluted vial is 1/10th that of the parent vial.

Sample Step by Step Preparation of 5 mL Vial Serial 10-fold Dilutions

Verify that the labeling and order (color coded, label concentration) for vials are correct.

1) Ensure the maintenance vial is mixed by inverting or rolling.

2) 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.

3) Using aseptic technique, inject this 0.5 mL form the maintenance vial into the 4.5-mL prefilled (10% glycerol-saline or HSA) yellow or 1:10 vol/vol vial. This vial will consist of 5mL of a 10-fold dilution of the maintenance concentration vial.

4) Ensure the newly made 10-fold diluted (yellow) vial is mixed by inverting or rolling.

5) Subsequent 10-fold dilutions are done in the same manner for the remainder of the vials in the treatment set (0.5 mL into 4.5 mL of the 10-fold, labeled 10% glycerol-saline prefilled vial):
   1. 0.5 mL from yellow 1:10 vol/vol into a 4.5 mL diluent-filled blue 1:100 vol/vol vial
   2. 0.5 mL from blue 1:100 vol/vol into 4.5 mL diluent-filled green 1:1000 vol/vol vial
   3. 0.5 mL from green 1:1000 vol/vol into 4.5 mL diluent-filled silver 1:10,000 vol/vol vial
   4. And so on for additional more dilute (silver) vials

6) 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 syringe volume (1mL for a 1-mL syringe) from the vial just injected and re-injected 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**: This is for making dilutions only. Do not reuse syringes or mix/rinse between different stock solutions when mixing the initial maintenance vial.)

Table 1. Calculations for making Extract Dilutions

Table II. Calculations for making Extract Dilutions* (from Practice Parameters page S10)

All dilutions can be calculated by using the following formula:	
V1 X C1 = V2 X 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 X 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 X 100 = 10% solution	
1:20 wt/vol 1/20 = 0.05 X 100 = 5% solution	
1:40 wt/vol 1/40 = 0.025 X 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 X C1 = V2 X C2	5 X (1/200) = V2 X (1/10)
	5 X (0.005) = V2 X (0.1)
V2 = (V1 X C1)/C2	V2 = 0.025/0.1 = 0.25
To determine amount of diluent needed:	
V1 – V2	5 – 0.25 = 4.75 mL

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

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 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.

- 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 distractions during extract preparation.

8) Stock refrigerators are not to be used for food or drink storage.

9) 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.

10) **Diluted allergen immunotherapy vials (yellow, blue, green, and silver) should not made by pulling directly from a manufacturer's concentrated stock vial extract.**

- The primary reason for this is the potential for error that is increased progressively with each dilution. For dilute vials, a very small amount of allergen would need to be pulled from the stock extract vial, and it is virtually impossible to achieve the precision needed for the most dilute vials.
- Thus, a dilution vial prepared by this method may contain inaccurate amounts of extract and potentially increase the risk of adverse events during vial transitions within the buildup phase.

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:

- 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)
- Label accuracy – "five rights" as described below

Verify that the label contains the 5 rights

- **Right name,**
- **Right content (allergens),**
- **Right concentration and right color coded vial and vial number if used,**
- **Right expiration date (dilute vials may have earlier expiration dates than more concentrated vials).**
- **Right color, 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, label concentration, in vol/vol, or number should match what is recommended in the Practice Parameters for the color code (see Tables XI and XII from Practice Parameters[26]).

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. 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.

Table 2. Preparing Allergen Extract Treatment Sets (2 antigens, 0.5 ml stock of each)

Dilution label/ color	Label Conc vol/vol	Extract added	Diluent added	Ragweed w/v	Grass BAU/ml
stock extract				1:10	100,000
1 (red/maint)	1:1	0.5 mL of each stock	4.0 mL	1:100	10,000
2 (yellow)	1:10	0.5 mL red (1) vial	4.5 mL	1:1,000	1,000
3 (blue)	1:100	0.5 mL yellow (2) vial	4.5 mL	1:10.000	100
4 (green)	1:1,000	0.5 mL blue (3) vial	4.5 mL	1:100,000	10
5 (silver)	1:10,000	0.5 mL green (4) vial	4.5 mL	1:1,000,000	1

*Treatment set for maintenance concentrate with 0.5 ml stock conc. grass (100,000 BAU/ml) & ragweed (1:10 w/v) in 5ml

VII. STINGING INSECT ALLERGEN EXTRACT PREPARATION

Extracts are available for five winged Hymenoptera species at a concentration of 100 mcg/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 mcg/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.[41] 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 mcg/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 non-standardized 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.[42]

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 mcg/mL of each of the three common vespids.

VIII. 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.[35-37,43-45] Several measures are taken by stock extract manufacturers and healthcare personnel to minimize the risk of contamination and loss of potency of extracts during normal storage and use.[46,47]

Dilution of extracts alone can affect the long-term potency of extracts. Less concentrated allergen immunotherapy extracts are more sensitive to the effects of temperature and might not maintain their potency until the listed expiration date.

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 the expiration dates on labels for treatment and diagnostic sets are no later than the stock extract used with the earliest expiration date and does not exceed 1 year from the mix or dilution date of the prescription set.
- Discard products that have expired.
- Ensure that allergen extract storage trays are stored at recommended temperatures.
- Ensure that extracts are kept cool during extended periods of mixing.

Manufacturer processing steps include the use of **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) allergen extract proteins even if in a 50% glycerin.[43,44] HSA may protect against phenol's adverse effects on allergenic proteins.[36,37,43] Other recognized preservatives such as thimerosal and methylparaben are not generally used in allergen extract preparation.

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 ≥20%.[36,37,45]

Preservative and stabilizing properties decrease as the concentration of glycerin is decreased. Glycerin, when injected subcutaneously, can cause local irritation and result is a burning sensation.

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 ensure that extracts are not exposed to temperature extremes. For example, a log of daily temperatures (See Refrigerator Temperature Log Form) 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., over 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°F – 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.

Less concentrated allergen immunotherapy extracts are more sensitive to the effects of temperature and might not maintain their potency until the listed expiration date. 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. Dust mite extracts do not appear to cause significant degradation of pollen or animal dander extracts and thus can be mixed together.

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.

IX. 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.

Regular review of these procedures and both initial and ongoing competency assessments is expected by regulatory bodies and promotes high quality allergen extract preparation. Following these steps and best practices will ensure that the end product is prepared in accordance with the most recent standards optimizing extract efficacy and the safety of patients entrusted to our care.

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

- Contamination is prevented by adequate training and the proper use of aseptic technique
- 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 Immunotherapy Practice Parameter and the USP Chapter 797 Standards effective Nov. 1, 2023 for allergen extract mixing. A companion examination has been developed based on this training document to assist in satisfying competency assessment and documentation requirements. It is currently available via the College Learning Connection.

X. ACKNOWLEDGMENTS

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XII. APPENDICES

Appendix 1. Effective Dose Range for Allergen Extracts, US Standardized Units

Table IX. Probable effective dose range for standardized and non-standardized 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: *D farinae* and *D pteronyssinus*	3,000, 5,000, 10,000, and 30,000 AU/mL	500-2,000 AU	10,000 AU/mL 20-160 mcg/mL Der p 1, Der f 1* 2-180 mcg/mL Der p 2, Der f 2* 78-206 mcg/mL Der p 1, Der f 1† 13-147 mcg/mL Der p 2, Der f 2†
Cat hair	5,000 and 10,000 BAU/mL	1,000-4,000 BAU	10,000 AU/mL 20-50 mcg/mL Fel d 1*‡ 30-100 mcg/mL cat albumin§
Cat pelt	5,000-10,000 BAU/mL	1,000-4,000 BAU	10,000 BAU/mL 20-50 mcg/mL Fel d 1 *‡ 400-2,000 mcg/mL cat albumin§
Grass, standardized	100,000 BAU/mL	1,000-4,000 BAU	100,000 BAU/mL 425-1,100 mcg/mL Phl p 5* 506-2,346 mcg/mL group 1‖
Bermuda	10,000 BAU/mL	300-1,500 BAU	10,000 BAU/mL 141-422 Cyn d 1 mcg/mL*
Short ragweed	1:10, 1:20 wt/vol, 100,000 AU/mL	6-12 mcg of Amb a 1 or 1,000-4,000 AU	1:10 wt/vol 300 mcg/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 mcg of Can f 1	80-400 mcg/mL Can f 1† 10-20 mcg/mL dog albumin¶
Nonstandardized extract, dog	1:10 and 1:20 wt/vol	15 mcg of Can f 1	0.5 to 10 mcg/mL Can f 1† <12-1,500 mcg/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 mcg/mL single venom 300 mcg/mL in mixed vespid extract	50-200 mcg of each venom	100-300 mcg/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 2. Initial and Ongoing Competency Assessment: Allergen Extract Mixing

Extract Preparation Task	Date	Validated by:	Comments, Notes, Additional Testing
Personnel Training documented			
Passed initial and annual written test on aseptic technique & extract preparation			
Passed initial and annual Media-fill test verifying aseptic technique			
Successfully completed initial gloved fingertip and thumb sampling 3 separate times and then at least annually thereafter			
Understands & demonstrates appropriate hand hygiene			
Understands and appropriately dons required garb			
Reviews prescriptions for accuracy			
Checks expiration dating of antigens and diluents			
Cleans mixing surface appropriately			
Checks stocks & mixed extracts for turbidity/ particulate matter			
Swabs vial stoppers with 70% IPA			
Draws up appropriate amounts of extract			
Disposes of syringes in an appropriate manner			
Documents lot # and preparation details per clinic SOP			
Creates appropriate labels			
Stores extracts at appropriate temperatures			
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_____

Appendix 3. Extract Volumes Needed for 5.0 ml Maintenance Vials with 0.5 ml injection volume (From Grier TJ. "How's my dosing? A one-step, math free guide for comparing your clinic's maintenance immunotherapy doses to current practice parameter recommendations" *Ann Allergy Asthma Immunol* 108 (2012) 201-205.

Extract/concentrate strength		Volume of concentrate needed per vial (mL)		
Category	**Concentrate**	**Min**	**Mid**	**Max**
Pollens, fungi, insects	1:10 w/v	0.25	0.375	0.50
	1:20 w/v	0.50	0.75	1.00
	1:40 w/v	1.00	1.50	2.00
Short ragweed[a]	200 AgE U/mL	0.30	0.45	0.60
Cat	10,000 BAU/mL	1.00	2.50	4.00
Dog AP	1:100 w/v	NA	NA	1.00
Dog epithelia	1:10 w/v	0.25	0.375	0.50
	1:20 w/v	0.50	0.75	1.00
Dust mites[b]	30,000 AU/mL	0.17	0.42	0.67
	10,000 AU/mL	0.50	1.25	2.00
Pasture grasses[b]	100,000 BAU/mL	0.10	0.25	0.40
	10,000 BAU/mL	1.00	2.50	4.00
Bermuda grass	10,000 BAU/mL	0.30	0.90	1.50

Abbreviations: w/v, weight-to-volume ratio; AgE. Antigen E or Amb a 1; U Unit; AU, Allergy Unit; BAU. Bioequivalent Allergy Unit; NA. Not applicable.

Minimum recommended dose was not specified for this extract category.

[a]Also applies to ragweed mix (short + giant) products at 1:20 w/v and approx. 100AgE U/mL.

[b]Also applies to dust mite mix and pasture grass mix (eg, KORT,7 grass) products at the same AU/mL or BAU/mL strengths.

Appendix 4. Recommended Documentation for Allergen Immunotherapy Extract Prescription Forms

The purpose of the allergen immunotherapy prescription form is to define the contents of the allergen immunotherapy extract in enough detail that it could be precisely duplicated. The following information should be on an immunotherapy prescription form:

Patient information:

- Patient name, patient number (if applicable), birth date, telephone number, and picture (if available) should be included.

Preparation information:

- Name of person and signature preparing the allergen immunotherapy extract should be included.
- Date of preparation should be recorded.
- Bottle name should be included (eg, trees and grass). If abbreviations are used, a legend should be included to describe the meaning of the abbreviations.

Allergen immunotherapy extract content information:

The following information for each allergen should be included on the form in a separate column:

- Content of the allergen immunotherapy extract, including common name or genus and species of individual antigens and detail of all mixes, should be included..
- Concentration of available manufacturer's extract.
- Volume of manufacturer's extract add added to achieve the projected effective concentration. This can be calculated by dividing the projected effective concentration by the concentration of available manufacturer's extract times the total volume.
- The type of diluent (if used).
- Extract manufacturer.
- Lot number should be included.
- Expiration date should be recorded and not exceed the expiration date of any of the individual components.

Appendix 4. Allergen Immunology Extract Prescription Form*

Patient Name:	Prescribing Physician:
Patient Number:	Address:
Birth Date:	Telephone:
Telephone:	Fax:

Allergen Extract Name:	
Bottle Name Abbreviations	
Tree: T	Mold: M
Grass: G	Cat: C
Weed: W	Dog: D
Ragweed: R	Cockroach: CR
Mixture: Mx	Dust Mite: Dm

Maintenance Concentrate Prescription Form

Prepared by: _____ Date Prepared: ___/___/___ Time: _____

Dates of subsequent dilutions from maintenance concentration with expirations dates:
Vial _____ From Vial _____ on ____/____/____ Expiration date: ____/____/____
Vial _____ From Vial _____ on ____/____/____ Expiration date: ____/____/____
Vial _____ From Vial _____ on ____/____/____ Expiration date: ____/____/____
Vial _____ From Vial _____ on ____/____/____ Expiration date: ____/____/____

Antigen Number	Extract Name Allergen or Diluent (Common name of *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						
2						
3						
4						
5						
6						
7						
8						
9						
10						
Diluent						
Total Volume						

**Components of mixes listed on a separate sheet.

Specific Instructions:	
Prescribing Physician's Signature	Date

Volume to add = $\frac{\text{Maintenance Concentration}}{\text{Conc. of Manufacturer's Extract}}$ x Total Volume

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

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

By Use Date _____

Storage Requirements _____

Results of Quality Control (e.g. visual inspections, second verification of questions) _____

Appendix 5. USP Section 21 – Compounding Allergenic Extracts

Compounding Allergenic Extracts Exception

Final standards for allergen extract compounding under USP Chapter 797, effective November 1, 2023.

An allergenic extract prescription set refers to a vial(s) of premixed licensed allergenic extracts that are diluted with an appropriate diluent to provide **subcutaneous immunotherapy** to an individual patient. Individuals who prepare allergenic extract prescription sets are not required to comply with all of the requirements set forth at USP <797> PHARMACEUTICAL COMPOUNDING – STERILE PREPARATIONS. Instead, Section 21. Compounding Allergenic Extracts describes the standards for compounding allergenic extracts and applies when:

1. The process for compounding encompasses the transfer of conventionally manufactured sterile allergen products and appropriate conventionally manufactured sterile added substances through the use of sterile needles and syringes; and
2. The manipulation process is limited to the use of sterile needles and syringes to penetrate stoppers on vials and the use of sterile syringes to transfer sterile liquids to sterile vials.

USP Section 21 – Compounding Allergenic Extracts

Allergenic extract prescription sets must comply with the following standards established by USP:

Personnel Qualifications

- Personnel charged with preparing allergen extract prescription sets must be supervised, trained, and evaluated. A designated person(s) with training and expertise in allergen immunotherapy must ensure that such personnel meet the aforementioned qualifications.
- All compounding personnel must finish training and demonstrate an understanding of sterile compounding skills and principles before beginning to prepare allergenic extracts independently.
- Before being allowed to compound allergenic extract prescription sets, personnel must demonstrate the knowledge and competency in applicable procedures through written or electronic exams. Records of annual personnel training and competency must be retained.
- Compounders are required to successfully complete gloved fingertip and thumb sampling on both hands, on at least three separate times, before compounding independently (see USP Chapter <797>; Box 1 and Table 1).With respect to each fingertip and thumb evaluation, the evaluation must only happen after the performance of separate and complete hand hygiene and garbing procedures. Compounding personnel are required to complete gloved fingertip and thumb sampling on both hands every 12 months after the initial competency evaluation.
- Evaluation of sterile techniques and related practices must occur at least every 12 months through the successful completion of a media-fill test (see USP Chapter <797>, Box 2). **There is no requirement of a post-media-fill sample if compounding occurs outside of a primary engineering control (PEC).**
- If a person fails competency evaluations, they must first successfully pass reevaluations in deficient areas before continuing to compound allergenic extract prescription sets. Designated persons are responsible for identifying the reasons for evaluation failures and determining the necessary retraining requirements.
- If more than six months have passed since a person compounded an allergenic extract prescription set, that person must be evaluated in all core competencies prior to compounding.

Personnel Hygiene and Garbing

- Personnel are required to perform garbing procedure and hand hygiene according to facility SOPs prior to beginning compounding of allergenic extract prescription sets (see USP Chapter <797>, Box 3.
- Garb requirements include, at a minimum:
  - Face mask
  - Sterile powder-free gloves
  - A low-lint garment (ex. gown) with an enclosed neck and snugly-fit sleeves around the wrists.
  - A head cover that is low-lint, disposable, and covers the hair and ears (a disposable cover for facial hair is required, if applicable).

- All surfaces of gloves must have sterile 70% IPA applied throughout the compounding. Personnel must allow gloves to dry thoroughly after application.

Facilities

- The compounding process may only occur in either a dedicated allergenic extract compounding area (AECA) or an ISO Class 5 PEC.
  - PECs and AECAs cannot be located near doors connecting to the outdoors, unsealed windows, or traffic flow as these conditions can negatively affect the air quality necessary for compounding.
  - PECs and AECAs cannot be located where environmental control challenges could adversely impact the air quality. This includes restrooms, warehouses and food preparation areas.
  - An AECA work surface or PEC must be at least 1 meter away from a sink.
  - When designing a PEC or AECA, the effect of other activities occurring around or adjacent to the area must be carefully considered.
- If PEC is used, PEC certification must occur at least every 6 months (see 5. Certification and Recertification).
- If an AECA is used, a visible and defined perimeter is required.
  - › Only authorized personnel are allowed access to the AECA during compounding.
  - › No other activity is permitted in the AECA when compounding activities occur.
  - › All AECA floors, walls, shelves, fixtures, counters, and cabinets must be cleanable.
  - › An AECA cannot have any carpet.
  - › Surfaces in the AECA should be resistant to damage caused by cleaning and disinfecting agents.
  - › To ensure efficient cleaning and disinfecting, surfaces used for allergenic extract prescription sets preparation are required to be smooth, free from cracks and crevices, impervious, and may not shed.
  - › Overhangs that can collect dust should be minimized. All overhangs or ledges are required to be easily cleanable.
  - › The AECA is required to be well-lit and have temperature and humidity controls.

Cleaning and Disinfecting

- Before use for compounding, vial stoppers on packages of conventionally manufactured sterile ingredients are required to be cleaned with sterile 70% IPA. Critical sites must be wet and allowed to dry before compounding.
- If a PEC is used:
  - All interior surfaces are required to be cleaned and disinfected each day before compounding begins and if surface contamination is suspected or known. Between each prescription set, personnel must apply sterile 70% IPA to the horizontal work surface.
- If an AECA is used:
  - In addition to when surface contamination is suspected or known, all work surfaces for direct compounding must be cleaned and disinfected each day prior to compounding. Between each prescription set, personnel must apply sterile 70% IPA to the horizontal work surface.
  - If the AECA has walls, doors, or door frames, they must be cleaned and disinfected on a monthly basis. They must also be cleaned and disinfected if surface contamination is known or suspected.
  - AECA ceilings must be cleaned and disinfected if visibly soiled. They must also be cleaned and disinfected if surface contamination is known or suspected.

Establishing BUDs

- The by-use-date (BUD) is required to "be no later than the earliest expiration date of any allergenic extract or any diluent that is part of the prescription set."
- The BUD cannot exceed 1 year from the mix or dilution date of the prescription set.

Labeling

- The following information must be prominently and legibly displayed on the label of each vial of a prescription set:
  - Name of the patient
  - Type and fractional dilution of each vial, with a corresponding vial number
  - BUD
  - Storage conditions

Shipping and Transport

- The quality of allergenic extract prescription sets may be negatively affected if inappropriately transported.
- When allergenic extract prescription sets are shipped or transported, compounding personnel are required to use transportation methods that are expected to deliver properly packaged sets that are sterile, damage free, and stable.
- Specific handling instructions must be included on the outside of the container when shipping or transporting prescription sets requiring special handling.

Documentation

- Any facility preparing allergenic extract prescription sets must record and retain written or electronic documentation, including, but not limited to:
  - SOPs detailing all aspects of the compounding process.
  - Personnel records relating to training, competency assessments, qualification records, and corrective actions for qualification failures.
  - If a PEC is used, PEC certification reports and any corrective actions.
  - Refrigerator temperature logs.
  - Compounding Records (CR) for individual allergenic extract prescription sets (see USP Chapter <797>, Box 10).
  - Information pertinent to complaints/adverse events.
  - Investigations and corrective actions.

Box 1. Procedures for Gloved Fingertip & Thumb Sampling

- For each hand, use one sampling media device (e.g., slides or plates) containing general microbial growth agar supplemented with neutralizing additives (e.g., polysorbate 80 and lecithin).
- Each media device must be labeled with a personal identifier, the date and time the sample was taken, and whether the sample was from the left or right hand.
- Do not apply sterile 70% IPA to gloves just before touching the media device. Doing so may cause a false-negative result.
- Roll fingertip pads and thumb pad over the agar surface to collect samples from all gloved fingers and thumbs from each hand. Use a separate media device for each hand.
- Using an incubator, incubate the media device at the following temperatures: 1) 30°–35° C for at least 48 hours then 2) 20°–25° C for at least 5 additional days. Maintain media devices to avoid contamination/condensation from getting onto the agar. (e.g., invert plates)
- For each hand, record the number of colony-forming units (cfus).
- Add the total number of cfus from both hands to determine if cfu action level is exceeded.

Return to Allergy Standards

Table 1. Gloved Fingertip and Thumb Sampling Action Levels

Gloved Fingertip and Thumb Sampling	**Action Levels** (total number of cfu from both hands)
Initial sampling after garbing	>0
Subsequent sampling after media-fill testing	>3

Return to Allergy Standards

Box 2. Media-Fill Testing Procedures

- If the starting components are sterile, manipulate them to simulate sterile-to-sterile compounding activities, and transfer the sterile soybean–casein digest media into the same types of container closure systems commonly used at the facility. Unless indicated by the manufacturer, refrain from further diluting the media.
- If the starting components are nonsterile, "dissolve a commercially available nonsterile soybean–casein digest powder in nonbacteriostatic water to make a 3% nonsterile solution." Personnel must manipulate it to simulate nonsterile-to-sterile compounding activities and prepare no less than 1 container as the positive control to demonstrate growth promotion, which is indicated by visible turbidity upon incubation.

Return to Allergy Standards

Box 2. Media-Fill Testing Procedures (continued)

- Perform a gloved fingertip and thumb sample on each hand and surface sample of the PEC direct compounding area after the compounding simulation is done and the final containers are filled with the test media. Samples must be taken before disinfecting gloves and PEC. Maintain samples to avoid contamination and prevent condensate from dropping onto the agar during incubation (e.g., invert containers).
- Using an incubator, incubate the final containers as follows: 1) 20°–25° C for a minimum of 7 days then 2) 30°–35° C for an additional 7 days. "The order of the incubation temperatures must be described in the facility's SOPs."
- Failure has occurred if there is visible turbidity or other visual manifestations of growth in the media in at least one container closure unit on or before 14 days.

Return to Allergy Standards

Box 3. Procedure for Hand Washing

- Using warm running water and a disposable nail cleaner, clean underneath fingernails.
- For a minimum of 30 seconds, using soap and water, wash hands/forearms up to the elbows.
- Using low-lint disposable towels or wipers, thoroughly dry hands and forearms up to the elbows

Return to Allergy Standards

Box 10. Compounding Records

The following information must be included in the Compounding Records (CRs):

- Each component's name, weight or volume, and strength or activity.
- The compounded sterile preparation's (CSP's) name, strength or activity, and dosage form.
- The date and time of the CSP's preparation.
- Internal identification numbers, including the prescription, order, or lot number.
- Total quantity that was compounded.
- Final yield, including quantity, containers, and number of units.
- Requirements governing the BUD and storage.
- Results of the quality control procedures (e.g., visual inspection, filter integrity testing, and pH testing).
- Each component's vendor, lot number, and expiration date for CSPs that are prepared from a nonsterile ingredient and for CSPs that are prepared for more than one patient.
- A manner to determine the individuals involved in the compounding process and the individuals who verify the final CSP.
- If applicable, the CSP's master formulation record reference and the calculations employed to determine and verify the components' quantities and/or concentrations.

Return to Allergy Standards

Appendix 6. Forms

Competency Assessment – *Initial* Personnel Training: Allergen Extract Mixing

Name:		Job Title:	

Extract Preparation Task	Date	Validated by	Comments, Notes, Additional Testing
1. Passed **initial** written test on aseptic technique and extract preparation. ***See Form A1.***			
2. Passed **initial** Media-fill test verifying aseptic technique. *Attach lab report.*			
3. Successfully completed **initial** gloved fingertip and thumb sampling three separate times. *Attach lab report.* **TOTAL R cfu + L cfu = 0.**			**Test 1:** R cfu + L cfu = **Test 2:** R cfu + L cfu = **Test 3:** R cfu + L cfu =
4. Understands & demonstrates appropriate hand hygiene. ***See Form A2.***			
5. Understands and appropriately dons required garb. ***See Form A2.***			
6. Reviews prescriptions for accuracy.			
7. Checks expiration dates of antigens and diluents.			
8. Cleans mixing surface appropriately. ***See Form A3.***			
9. Checks stocks & mixed extracts for turbidity/particulate matter.			
10. Swabs vial stoppers with 70% IPA.			
11. Draws up appropriate amounts of extract.			
12. Disposes of syringes in an appropriate manner.			
13. Documents lot # and preparation details per clinic SOP.			
14. Creates appropriate labels.			
15. Stores extracts at appropriate temperatures.			
16. 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. Furthermore, I must successfully pass reevaluations in *deficient area(s) before I can resume compounding allergenic extract.

American College of Allergy, Asthma & Immunology

college.acaai.org/extract

_____

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

**Corrective actions on separate page.*

Initial Competency Assessment – Corrective Action

Name:		Job Title:	

Extract Preparation Task	Date	Validated by	Actions Taken
1. Passed written test on aseptic technique and extract preparation.			
2. Passed Media-fill test verifying aseptic technique.			
3. Successfully completed gloved fingertip and thumb sampling three separate times.			
4. Understands & demonstrates appropriate hand hygiene.			
5. Understands and appropriately dons required garb.			
6. Reviews prescriptions for accuracy.			
7. Checks expiration dates of antigens and diluents.			
8. Cleans mixing surface appropriately.			
9. Checks stocks & mixed extracts for turbidity/particulate matter.			
10. Swabs vial stoppers with 70% IPA.			
11. Draws up appropriate amounts of extract.			
12. Disposes of syringes in an appropriate manner.			
13. Documents lot # and preparation details per clinic SOP.			
14. Creates appropriate labels.			
15. Stores extracts at appropriate temperatures.			
16. Packages materials and supplies in a neat and efficient manner.			

*As described beginning on page 10 of tis guide.

Competency Assessment – *Annual* Personnel Training: Allergen Extract Mixing

Name:		Job Title:	

Extract Preparation Task	Date	Validated by	Comments, Notes, Additional Testing
1. Passed **annual** written test on aseptic technique and extract preparation. ***See Form A1.***			
2. Passed **annual** Media-fill test verifying aseptic technique. *Attach lab report.*			
3. Successfully completed **annual** gloved fingertip and thumb sampling three separate times. *Attach lab report.* **TOTAL R cfu + L cfu = 0.**			**Test 1:** R cfu + L cfu =
4. Understands & demonstrates appropriate hand hygiene. ***See Form A2.***			
5. Understands and appropriately dons required garb. ***See Form A2.***			
6. Reviews prescriptions for accuracy.			
7. Checks expiration dates of antigens and diluents.			
8. Cleans mixing surface appropriately. ***See Form A3.***			
9. Checks stocks & mixed extracts for turbidity/particulate matter.			
10. Swabs vial stoppers with 70% IPA.			
11. Draws up appropriate amounts of extract.			
12. Disposes of syringes in an appropriate manner.			
13. Documents lot # and preparation details per clinic SOP.			
14. Creates appropriate labels.			
15. Stores extracts at appropriate temperatures.			
16. 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. Furthermore, I must successfully pass reevaluations in *deficient area(s) before I can resume compounding allergenic extract.

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_____

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

**Corrective actions on separate page.*

Annual Competency Assessment – Corrective Action

Name:		Job Title:	

Task	Date	Validated by	Actions Taken
1. Passed **annual** written test on aseptic technique and extract preparation. ***See Form A1.***			
2. Passed **annual** Media-fill test verifying aseptic technique. *Attach lab report.*			
3. Successfully completed **annual** gloved fingertip and thumb sampling three separate times. *Attach lab report.* **TOTAL R cfu + L cfu = 0.**			
4. Understands & demonstrates appropriate hand hygiene. ***See Form A2.***			
5. Understands and appropriately dons required garb. ***See Form A2.***			
6. Reviews prescriptions for accuracy.			
7. Checks expiration dates of antigens and diluents.			
8. Cleans mixing surface appropriately. ***See Form A3.***			
9. Checks stocks & mixed extracts for turbidity/particulate matter.			
10. Swabs vial stoppers with 70% IPA.			
11. Draws up appropriate amounts of extract.			
12. Disposes of syringes in an appropriate manner.			
13. Documents lot # and preparation details per clinic SOP.			
14. Creates appropriate labels.			
15. Stores extracts at appropriate temperatures.			
16. Packages materials and supplies in a neat and efficient manner.			

Form A1: Competency Assessment – Aseptic Technique and Related Practices of Mixing Personnel

Name:		**Job Title:**	
Facility:		**Location:**	

All mixing personnel must perform media-fill testing to assess their sterile technique and related practices initially and every 12 months thereafter.

TASK (X = acceptable practice; N/A = not applicable; N/O = not observed)	X	N/A	N/O
1. Completed hand hygiene and garbing competency assessment.	☐	☐	☐
2. Performs proper hand hygiene, garbing and gloving procedures according to SOPs.	☐	☐	☐
3. Disinfects work surfaces with sterile 70% IPA.	☐	☐	☐
4. Disinfects components/vials with sterile 70% IPA.	☐	☐	☐
5. Only introduces essential materials in a proper arrangement in work area.	☐	☐	☐
6. Ensures syringes and needles are only opened in work area.	☐	☐	☐
7. Does not expose critical sites to contact contamination.	☐	☐	☐
8. Disinfects stoppers, injection ports and ampul necks by wiping with sterile 70% IPA and allows time to dry.	☐	☐	☐
9. Affixes needles to syringes without contact contamination.	☐	☐	☐
10. Punctures vial stoppers and spikes infusion ports without contact contamination.	☐	☐	☐
11. Correctly prepares labels.	☐	☐	☐
12. Disinfects sterile gloves routinely by wiping with sterile 70% IPA during prolonged mixing.	☐	☐	☐
13. Disposes of sharps and waste according to institutional policy.	☐	☐	☐

The person assessed is immediately informed of all unacceptable activities and informed of corrective actions.

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_____	_____	_____
Signature of person assessed	Printed name	Date
_____	_____	_____
Signature of Qualified Evaluator	Printed name	Date

Form A1: Competency Assessment – Corrective Action

Name:		Job Title:	

Task	Date	Validated by	Actions Taken
1. Completed hand hygiene and garbing competency assessment.			
2. Performs proper hand hygiene, garbing and gloving procedures according to SOPs.			
3. Disinfects work surfaces with sterile 70% IPA.			
4. Disinfects components/vials with sterile 70% IPA.			
5. Only introduces essential materials in a proper arrangement in work area.			
6. Ensures syringes and needles are only opened in work area.			
7. Does not expose critical sites to contact contamination.			
8. Disinfects stoppers, injection ports and ampul necks by wiping with sterile 70% IPA and allows time to dry.			
9. Affixes needles to syringes without contact contamination.			
10. Punctures vial stoppers and spikes infusion ports without contact contamination.			
11. Correctly prepares labels.			
12. Disinfects sterile gloves routinely by wiping with sterile 70% IPA during prolonged mixing.			
13. Disposes of sharps and waste according to institutional policy.			

Form A2: Competency Assessment – Hand Hygiene and Garbing Practices

Name:		**Job Title:**	
Facility:		**Location:**	

Individuals entering a compounding area must take appropriate steps to minimize microbial contamination including hand hygiene, garbing and consideration of needed materials brought into the compounding area. Before entering a compounding area, individuals must remove any items that are not easily cleanable or that are not necessary for compounding.

TASK (X = acceptable practice; N/A = not applicable; N/O = not observed)	X	N/A	N/O
Remove debris from underneath fingernails under running water using a disposable nail cleaner.	☐	☐	☐
Wash hands and forearms up to the elbows with soap and water for at least 30 seconds.	☐	☐	☐
Dry hands and forearms to the elbows completely with low-lint disposable towels or wipers.	☐	☐	☐
The order of hand washing and garbing depends on the placement of the sink. The order of garbing must be determined by the facility and documented in the facility's SOP. Hands must be sanitized with alcohol-based hand rub before donning sterile gloves. Sterile gloves must be donned in a classified room or Segregated Compounding Area (SCA).			
Apply an alcohol-based hand rub to dry skin following the manufacturer's instructions for the volume of product to use.	☐	☐	☐
Apply product to one hand and rub hands together, covering all surfaces of hands and fingers, until hands are dry.	☐	☐	☐
Allow hands to dry thoroughly before donning sterile gloves.	☐	☐	☐
Dons appropriate size gloves; inspects to ensure a tight fit and that there are no defects, holes or tears – apply sterile 70% IPA to gloves – allow to dry before compounding.	☐	☐	☐
While compounding, routinely disinfects gloves with sterile 70% IPA after touching items or surfaces that may contaminate gloves .	☐	☐	☐
Any person entering a compounding area must be properly garbed in accordance with the facility's SOPs. Garb must be replaced immediately if it becomes visibly soiled or if its integrity is compromised.			
Low-lint garment with sleeves that fit snugly around the wrists and that is enclosed at the neck (e.g., gowns or coveralls).	☐	☐	☐
Low-lint, disposable covers for head that cover the hair and ears, and if applicable, disposable cover for facial hair.	☐	☐	☐
Face mask.	☐	☐	☐
Sterile powder-free gloves.	☐	☐	☐
Compounding personnel must rub sterile 70% IPA onto all surfaces of the gloves and allow them to dry thoroughly throughout the compounding process.			
Removes PPE on the clean side of the AECA.	☐	☐	☐
Removes gloves and performs hand hygiene.	☐	☐	☐
Removes and discards gown, mask, head cover and beard cover (if used).	☐	☐	☐
The person assessed is immediately informed of all unacceptable activities and informed of corrective actions.			

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_____	_____	_____
Signature of person assessed	Printed name	Date
_____	_____	_____
Signature of Qualified Evaluator	Printed name	Date

Form A2: Competency Assessment – Corrective Action

Name:		Job Title:	

Task	Date	Validated by	Actions Taken
Remove debris from underneath fingernails under running water using a disposable nail cleaner.			
Wash hands and forearms up to the elbows with soap and water for at least 30 seconds.			
Dry hands and forearms to the elbows completely with low-lint disposable towels or wipers.			
Apply an alcohol-based hand rub to dry skin following the manufacturer's instructions for the volume of product to use.			
Apply product to one hand and rub hands together, covering all surfaces of hands and fingers, until hands are dry.			
Allow hands to dry thoroughly before donning sterile gloves.			
Dons appropriate size gloves; inspects to ensure a tight fit and that there are no defects, holes or tears – apply sterile 70% IPA to gloves – allow to dry before compounding.			
While compounding, routinely disinfects gloves with sterile 70% IPA after touching items or surfaces that may contaminate gloves .			
Low-lint garment with sleeves that fit snugly around the wrists and that is enclosed at the neck (e.g., gowns or coveralls).			
Low-lint, disposable covers for head that cover the hair and ears, and if applicable, disposable cover for facial hair.			
Face mask.			
Sterile powder-free gloves.			
Removes PPE on the clean side of the AECA.			
Removes gloves and performs hand hygiene.			
Removes and discards gown, mask, head cover and beard cover (if used).			

Form A3: Competency Assessment – Cleaning and Disinfecting Procedures

Name:		**Job Title:**	
Facility:		**Location:**	

DAILY TASKS (X = acceptable practice; N/A = not applicable; N/O = not observed)	X	N/A	N/O
Follows garbing procedures when performing any cleaning activities.	☐	☐	☐
In a primary engineering control (PEC) hood area, all interior surfaces of the PEC must be cleaned and disinfected daily and when surface contamination is known or suspected. Apply sterile 70% IPA to the horizontal work surface between each prescription set.	☐	☐	☐
In an AECA, all work surfaces in the AECA where direct mixing is occurring must be cleaned and disinfected daily and when surface contamination is known or suspected. Apply sterile 70% IPA to the horizontal work surface between each prescription set.	☐	☐	☐
Mops floors, starting at wall opposite the entrance; moves carts as needed to clean entire floor surface. Use of a microfiber cleaning system is an acceptable alternative to mops.	☐	☐	☐
MONTHLY TASKS	☐	☐	☐
Follows garbing procedures when performing any cleaning activities	☐	☐	☐
Performs monthly cleaning on a designated day.	☐	☐	☐
If present, walls, doors and door frames within the perimeter of the AECA must be cleaned and disinfected monthly and when surface contamination is known or suspected	☐	☐	☐
Ceilings within the perimeter of the AECA must be cleaned and disinfected when visibly soiled and when surface contamination is known or suspected.	☐	☐	☐
Documents all cleaning activities as to who performed such activities with date and time noted.	☐	☐	☐
The person assessed is immediately informed of all unacceptable activities and informed of corrective actions.			

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_____	_____	_____
Signature of person assessed	Printed name	Date
_____	_____	_____
Signature of Qualified Evaluator	Printed name	Date

Form A3: Competency Assessment – Corrective Action

Name:		Job Title:	

Task	Date	Validated by	Actions Taken
Follows garbing procedures when performing any cleaning activities.			
In a PEC, all interior surfaces of the PEC must be cleaned and disinfected daily and when surface contamination is known or suspected. Apply sterile 70% IPA to the horizontal work surface between each prescription set.			
In an AECA, all work surfaces in the AECA where direct mixing is occurring must be cleaned and disinfected daily and when surface contamination is known or suspected. Apply sterile 70% IPA to the horizontal work surface between each prescription set.			
Mops floors, starting at wall opposite the entrance; moves carts as needed to clean entire floor surface. Use of a microfiber cleaning system is an acceptable alternative to mops.			
Follows garbing procedures when performing any cleaning activities			
Performs monthly cleaning on a designated day.			
If present, walls, doors and door frames within the perimeter of the AECA must be cleaned and disinfected monthly and when surface contamination is known or suspected			
Ceilings within the perimeter of the AECA must be cleaned and disinfected when visibly soiled and when surface contamination is known or suspected.			
Documents all cleaning activities as to who performed such activities with date and time noted.			

Complaints/Adverse Events

Complainant/Adverse Event:		**Date:**	

Complaint received: ☐ Orally ☐ Written *(attach)*

Describe complaint/adverse event and any additional information:

Describe action taken and results:

Describe action taken to avoid same issue in the future:

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_____	_____	_____
Signature	Printed name	Date

Gloved Fingertip and Thumb Sampling

Name:				**Job Title:**				
Date:		**Time:**		**Check**	**Notes**			
Gather and prepare sampling plates – one plate per hand.				☐				
Label each sampling plate with a personnel identifier (i.e., initials); **R**ight or **L**eft hand.				☐				
DO NOT APPLY STERILE 70% ISOPROPYL ALCOHOL TO GLOVES IMMEDIATELY BEFORE TOUCHING THE SAMPLING DEVICE, WHICH COULD CAUSE A FALSE-NEGATIVE RESULT.								
Wipe the work surface with sterile 70% isopropyl alcohol; allow to dry.				☐				
Don garbing, including sterile gloves.				☐				
Using a separate sampling plate per hand, roll all finger pads & thumb pad over agar surface.				☐				
Cover and invert plates to prevent condensation from forming on agar; incubate at **30-35°C for no less than 48 hours and then at 20-25°C for no less than five additional days**.				☐				
					Thumb	Index	Middle	Ring	Pinky
Record the number of cfu per **R**ight hand.				☐					
Record the number of cfu per **L**eft hand.				☐					
Attach report. **TOTAL cfu**				☐					

Action Levels for Gloved Fingertip and Thumb Sampling[a]	
Gloved Fingertip & Thumb Sampling.	Action Levels (total cfu count from both hands)
Initial sampling after garbing.	0
Subsequent sampling after media-fill testing (every 6 months).	≤3

Successful completion of initial gloved fingertip and thumb sampling is defined as zero colony-forming units (cfu). Successful completion of subsequent gloved fingertip and thumb sampling after media-fill testing is defined as ≤3 cfu (total from both hands). [a]Action levels are based on the total cfu count from both hands.

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Signature _____ Printed name _____ Date _____

Media-fill Testing Checklist

Name:			Media:	
Date:	**Time:**	**Manufacturer:**	**Exp. Date:**	**Lot #:**

TASK	Check	Notes
Don garbing, including sterile gloves.	☐	
Wipe the work surface with sterile 70% isopropyl alcohol; allow to dry.	☐	
For sterile components (i.e., commercially sold media-fill kits) do not dilute the media further, unless directed by manufacturer. Obtain certificate of analysis (COA). Using a sterile syringe, transfer media to vial to simulate *sterile-to-sterile* compounding activities.	☐	
If non-sterile components are used, dissolve nonsterile soybean-casein digest powder in non-bacteriostatic water to make a 3% nonsterile solution. (prepare one vial as a control to demonstrate growth promotion) Using a sterile syringe, transfer media to vial and simulate *nonsterile to sterile* compounding activities.	☐	
Once the compounding simulation is completed, incubate vials.	☐	
First incubate at 20-25°C for 7 days.	☐	**Next incubate at 30-35°C for 7 additional days.**
Starting Temp.: Date:		Starting Temp.: Date:

Failure is indicated by visible turbidity or other visual growth in the media in one or more vials on or before 14 days.

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_____	_____	_____
Observer Signature	Printed name	Date
_____	_____	_____
Person who Reads/Documents Results	Printed name	Date

Primary Engineering Control (PEC) Hood

Name of Practice:

Location of PEC:

	Date		
Initial Certification		Report Attached	
6-Month recertification		Report Attached	
6-Month recertification		Report Attached	
6-Month recertification		Report Attached	
6-Month recertification		Report Attached	
6-Month recertification		Report Attached	
6-Month recertification		Report Attached	

_____ _____ _____

Signature Printed name Date

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Refrigerator Temperature Log

Refrigerator Name/Location:

Acceptable Temperature Range: 2-8°C

Date	Time	Temperature	Initials

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Signature _____ Printed name _____ Date _____