

Physician's Instruction Guide for the Preparation of Allergen Extract

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INTRODUCTION

Allergen immunotherapy was first introduced by Leonard Noon in 1911.¹ Dr. Noon originally hypothesized that patients suffering from 'hay fever' were sensitive to a 'toxin' contained in grass pollen. He proposed that patients would benefit by stimulating the immune system against the toxin by inoculations of pollen extract. These inoculations involve giving increasing amounts of allergen extracts to reduce symptoms upon re-exposure to those particular allergens. The procedure has been widely used since its inception to treat immediate hypersensitivity disorders mediated by allergen-specific Immunoglobulin-E antibodies (IgE). These same principles hold true today, almost 100 years later, for current allergen immunotherapy.

There is good evidence that allergen immunotherapy is effective for the treatment of:

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

Multiple studies have demonstrated the effectiveness of allergen immunotherapy in these conditions for both children and adults.²⁻⁶ The degree of effectiveness may vary for the individual patient. Clinical improvement should occur within or soon after the first year of treatment, and this benefit may improve with continued treatment. The Allergen Immunotherapy Practice Parameters suggest that "If clinical improvement is not apparent after 1 year of maintenance therapy, possible reasons for lack of efficacy should be evaluated. If none are found, discontinuation of immunotherapy should be considered, and other treatment options should be pursued."⁷ It has been observed that some patients may experience a worsening of their asthma, allergic rhinitis or conjunctivitis symptoms during treatment, especially during the first few months of therapy. There is no consensus on when to discontinue aeroallergen immunotherapy, but benefits are often maintained for years after stopping therapy in some individuals, and indefinitely in others. In grass-pollen allergy, a three year course of subcutaneous immunotherapy gave prolonged relief of symptoms.⁸ In many patients with stinging insect allergy, 3-5 years of treatment may be sufficient for sustained effectiveness after discontinuing therapy. Patients experiencing more severe reactions to stings may be considered for longer durations of treatment given the small risk of the recurrence of a life-threatening reaction over time.

Subcutaneous allergen immunotherapy is not used for patients with food allergies. Although studies have demonstrated an increased tolerance to peanut challenge in patients who received subcutaneous peanut immunotherapy^{9, 10}, there was an

unacceptably high incidence of systemic reactions (e.g., anaphylaxis) in most of the patients during treatment.¹⁰

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

PRACTITIONER QUALIFICATIONS

Allergen immunotherapy is an effective therapy and indicated for the treatment of patients with allergic rhinitis, allergic conjunctivitis, asthma and stinging insect allergy (Hymenoptera). Each patient's immunotherapy prescription is unique and the administration schedule (build-up or maintenance) may also vary. Each patient should be evaluated prior to the immunotherapy administration visit to determine whether there have been any recent health changes that might require modifying or withholding the immunotherapy treatment. Risk factors for severe immunotherapy reactions include symptomatic asthma and injections administered during periods of symptom exacerbations. Clinical judgment is required when altering the dose or schedule of administration. State laws may differ in regard to personnel who may give injections. Allergen immunotherapy carries a significant risk for life threatening anaphylaxis and therefore requires even more competency training for both nursing personnel and physician supervisors. The responsibility for supervision and competency of the staff preparing and administering allergen immunotherapy falls to the supervising physician. Documentation of training and competency in allergen immunotherapy as well as diagnosis, treatment and prevention of anaphylaxis are critical quality management issues for all clinics involved in the delivery of allergen immunotherapy. Until recent recommendations from specialty societies are uniformly adopted, many facilities that provide immunotherapy are forced to deal with a variety of allergen immunotherapy extracts in a variety of packaging and labeling formats that further increase the risk for incorrect dosing during administration. Thus training in what to look for and how to assure that these varied prescriptions are administered safely and effectively is even more crucial.

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

- On the job training from a qualified co-worker or supervisor
- AAAAI and ACAAI workshops and seminars
- Manuals from allergen extract manufacturers
- Journal articles (i.e., Allergen Immunotherapy: A Practice Parameter Second Update)
- Online course at [https:// www.instanted.com](https://www.instanted.com) , Click on Project Immune Readiness 2007-2008, password = paper. Two modules are currently available: anaphylaxis and allergen immunotherapy overview (allergen extract preparation manual and exam currently in development)

The most current and widely adopted recommendations in the United States for all aspects of allergen immunotherapy are embodied in "Allergen Immunotherapy: A Practice Parameter Second Update" (<http://jcaai.org> or J Allergy Clin Immuno Sept 2007; 120:S25-S85).⁷ This joint effort by experts from the American Academy of Allergy, Asthma and Immunology (AAAAI), the American College of Allergy, Asthma and Immunology (ACAAI), and the Joint Council of Allergy, Asthma and Immunology (JCAAI) focuses on evidence-based recommendations that will optimize immunotherapy efficacy and safety. All healthcare providers involved in immunotherapy preparation and administration should be oriented to the contents of this practice parameter, which contains practical clinical information and sample forms. The sample forms can be downloaded from www.aaaai.org (members only section).

Some suggested qualifications of extract preparation personnel from these Practice Parameters include:

- training in and demonstrated understanding of appropriate antiseptic hand cleaning, surface disinfection and aseptic technique
- pass a written test on aseptic technique and extract preparation
- annually pass a media-fill or equivalent test verifying use of aseptic technique
- reinstruct and re-evaluate if fail written test or media-fill test equivalent

Competency assessment and documentation: Training of personnel involved in the administration of allergen immunotherapy is widely recognized as a critical requirement for safety and efficacy. Content should include core cognitive knowledge as well as demonstration of procedure performance competency. Appendix 1 contains a sample document for assessing and documenting competency of personnel in the preparation of allergen immunotherapy treatment sets. It is adapted from competency elements for allergy technicians/nursing personnel at the United States Army Centralized Allergen Extract Laboratory at Walter Reed Army Medical Center. These competency elements are based on recommendations of the Joint Commission on Accreditation of Hospital Organizations requirements. As with all sample forms, this form is merely an example. Since different practice settings will have site specific standard operating procedures, competency forms should be developed to meet the needs of each practice and practitioner. For example, a practice may have an extra focus on sterility by adding a sterile glove test that involves hand preparation, donning of sterile gloves, cutting off the tip and culturing for sterility.

ALLERGEN EXTRACTS

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

Allergen extract used for treatment and testing are liquid solutions containing dissolved allergenic proteins from pollens, dust mites, animal dander, molds, and insects. The manufacturing process usually includes crushing raw materials and "extracting" allergenic proteins by adding solvents that release them from the solid raw material into the liquid solvent. This is followed by a variety of purification steps resulting in a liquid solution that is stable under normal storage conditions (4° C) without precipitation that can change the concentration of allergens in the mixture.

Each allergen extract can contain a number of allergenic proteins that can induce allergic symptoms with exposure. However, it is important to realize that the end product is a complex mixture of the diluents or solvents, additives, preservatives, allergenic proteins, and other components of the raw material that survive the manufacturing process.

Stock allergen extracts are licensed by the Center for Biologics Evaluation and Research (CBER) within the Food and Drug Administration (FDA) in the United States.

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Commercially available stock extracts are supplied by a handful of manufacturers throughout the country. These concentrated extracts are used to mix individual patient treatment sets and are available in only a few forms:

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

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

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

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

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

Diluents are solutions used to keep the allergens in suspension and form the liquid backbone of allergen extracts. Diluents are used to re-suspend lyophilized extracts, dilute extracts for diagnostic use, dilute vials in treatment sets, and to fill maintenance vials to final volume after addition of stock allergen quantities. There are a few different diluents that are commonly used today:

- Glycerin (e.g., 50% glycerin ± phenol)
- Phenol saline (e.g., 0.4% phenol, saline)
- Human serum albumin (e.g., 0.03% human serum albumin, 0.4% phenol, saline)

Each diluent has advantages and disadvantages related to preservation of extract potency and sterility. For example, glycerin is both a preservative and stabilizer. Meanwhile, human serum albumin is a stabilizer, and phenol is a preservative. These additives are discussed in further detail in the discussion of extract stability.

Standardized allergen extracts: Several commonly used extracts have been standardized such that allergen content is consistent between manufacturers and between lots made from the same manufacturer. Extracts are standardized based on intradermal skin test responses in allergic individuals. Specifically, reference standards from the Center for Biologics Evaluation and Research of the U.S. Food and Drug Administration (FDA) are obtained for standardized allergen extracts by identifying concentrations that reproducibly produces erythema with a sum of perpendicular long axes of 50mm (ID₅₀EAL).¹¹ These reference standards are then used by manufacturers to assure that the allergen content of each new lot falls within specified ranges for potency labeling. Laboratory 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 & ragweed Amb a 1), total protein/hyaluronidase/phospholipase content (Hymenoptera venom), and other assays (pooled sera immunoassay inhibition activity). Units of potency applied to standardized extracts vary, and include BAU/ml (Bioequivalent Allergy Unit/ml), AU/ml (Allergy Unit/ml), mcg/ml (microgram protein/ml) or in the case of some standardized short ragweed stock extracts in w/v (weight per volume). 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 range, it is possible that actual allergenic protein content can vary several- fold for the same potency label. Only a few allergen extracts have been standardized to date (See Appendix 3 for

probable effective dose range from the Allergen Immunotherapy Practice Parameter Second Update):

- Cat hair & 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 BAU/ml or w/v)
- Grass (Bermuda, Kentucky bluegrass, perennial rye, orchard, timothy, meadow fescue, red top, sweet vernal; potency in BAU/ml)
- Hymenoptera venoms (yellow jacket, honeybee, wasp, yellow hornet, white-faced hornet, and mixed vespids; potency in mcg/ml)

ALLERGEN EXTRACT MIXING CONDITIONS

In addition to standardization of allergen stock extract manufacturing, there are new requirements for conditions under which allergen extracts should be prepared. Mixing condition recommendations are designed to decrease the risk of bacterial contamination during the preparation of allergen extract treatment and diagnostic sets.

Recommended measures include good personal hygiene, hand washing and the use of antiseptics to clean working surfaces and vial tops prior to transfers. Two sets of guidelines can be referenced in the preparation of clinic specific standard operating procedures.

The first is the "Allergen Immunotherapy Extract Preparation Guidelines" prepared by the Joint Task Force on Practice Parameters, representing the American Academy of Allergy, Asthma and Immunology; the American College of Allergy, Asthma and Immunology; and the Joint Council of Allergy, Asthma and Immunology (Table 1):

Table 1: Allergen Immunotherapy Extract Preparation Guidelines

1. Qualifications of extract preparation personnel:

- Compounding personnel must pass a written test on aseptic technique and extract preparation.
- Compounding personnel must be trained in preparation of allergenic products.
- Compounding personnel must annually pass a media-fill test, as described in Addendum A.*

- Compounding personnel who fail written or media-fill test would be re-instructed and re-evaluated.
- Compounding personnel must be able to demonstrate understanding of antiseptic hand cleaning and disinfection of mixing surfaces.
- Compounding personnel must be able to correctly identify, measure, and mix ingredients.
- Compounding personnel should be appropriately trained health professionals including, but not limited to, registered nurses, licensed practical nurses, medical technicians, medical assistants, physician assistants, advanced practice nurses and physicians.

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

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

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

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

6. Mixing of extracts with high and low proteolytic enzymes: Separation of aqueous extracts with high proteolytic enzyme activities from other extracts is recommended.

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

8. Subcutaneous injection: According to FDA-approved package insert allergen extracts are to be administered by prick-puncture or intradermal routes or administered subcutaneously for immunotherapy injections.

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

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

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

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

12. Policy and procedure manual: Practices preparing allergy extracts must maintain a policy and procedure manual for the procedures to be followed in

mixing, diluting, or reconstituting of sterile products and for the training of personnel in the standards described above.

***Addendum A:** Example of a media-fill test procedure. This or an equivalent test is performed at least annually by each person authorized to compound allergen immunotherapy extracts under conditions that closely simulate the most challenging or stressful conditions encountered during compounding of allergen immunotherapy extracts. Once begun, this test is completed without interruption. A double-concentrated media such as from Valiteq is transferred in ten 0.5-mL increments with a sterile syringe to a sterile 10-cc vial. Five mL of sterile water (preservative free) is added. This is the "concentrate." The vial is incubated within a range of 20-35C C or 68-95° F for 14 days. Failure is indicated by visible turbidity in the medium on or before 14 days

Adapted from Allergen Immunotherapy: A Practice Parameter Second Update; Table VIII erratum in J Allergy Clin Immunol.2008;122(4):842)

The second set of guidelines is outlined in a 2008 revised bulletin from the United States Pharmacopeia with an effective date of June 2008 (USP <797>). It should be noted that these standards are less rigorous than standards required for typical sterile drug compounding in pharmacies. In so doing, the bulletin recommends that mixers be aware of the greater potential risk of contamination and adhere to recommendations listed.

USP created scaled back recommendations for allergen extracts under the assumptions that mixing of allergen extracts involves simple transfer of sterile substances in the presence of preservatives. Therefore, "allergen extracts as compounded sterile preparations are not subject to the personnel, environmental and storage requirements for all CSP Microbial Contamination Risk Levels in this chapter when all of the following criteria are met:" (USP <797>)

- Clean nails, hands and arms to elbow for 30 seconds using soap & water before mixing
- Wear head & facial hair covers, gowns, and face masks
- Use alcohol based surgical hand scrub before gloving
- Use powder-free sterile gloves compatible with 70% isopropyl alcohol (IPA)
- Disinfect gloves intermittently with IPA, especially when mixing for long periods

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- Wipe vials &/or ampules with 70% IPA ensuring wetting for a minimum of least 10 sec
- Use aseptic technique to minimize contact with secretions, skin, glove fingertips, etc.
- Label each vial: name, beyond use date, storage temp (based on manufacturer)
- Do not store single dose allergen extracts for additional future use

USP emphasizes that unless appropriate measures are taken, full compliance with the much more stringent “ low risk compounding requirements” are indicated, This includes laminar flow hood use & testing with buffer area, media fill testing, ISO class V air quality, training, garb, etc., for clinics/facilities requiring compliance with USP 797.

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

Sites where allergen extract patient treatment sets are prepared should be compliant with the recommendations contained in the “Allergen Immunotherapy Extract Preparation Guidelines”. However, these conditions and practices may not be required for all clinics or offices. Additionally, some hospital based clinics may be required to be fully compliant with USP recommendations. Clinic and facility supervisors can help determine the applicability of these two sets of guidelines. Regardless, your clinic or office should have in place appropriate measures that focus on proper mixing technique, minimizing the risk of contamination, and appropriate vial labeling in accordance with the Allergen Immunotherapy Practice Parameter recommendations.

ALLERGEN IMMUNOTHERAPY PRESCRIPTIONS

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

Each prescription should contain:

- Patient identifying and contact information (and picture if possible)
- Name of preparer
- Date of preparation
- Name, concentration and volume for each allergen
- Type and volume of diluents
- Stock allergen manufacturer and lot number
- Expiration date

All prescriptions should be reviewed for accuracy prior to preparation. This includes the review of all essential elements listed above including patient identifiers and contact information, vial contents and volumes, schedule for administration (and suggested adjustments in the schedule for adverse reactions or interruptions). Even though some of these elements may be routine for a clinic, it is important to review them for each and every patient.

Optimal mixing of allergens to create an individual patient treatment set is based on proper identification of relevant allergens, appropriate dosing of allergens, avoidance of combinations that could affect overall potency, and selection of allergens using knowledge of those that are cross-reactive. For example, molds and cockroach extracts contain degrading enzymes and should generally not be mixed with pollens or animal dander according to the Practice Parameters and reported studies.¹² For similar reasons, stinging insect venom extracts should not be mixed with each other or other aeroallergen extracts (e.g., pollens, pet dander, dust mite). Aeroallergens with high cross-reactivity allow prescribers to treat with one allergen at an effective dose and have some confidence that they are also treating for related allergens. For example, northern pasture grass allergen extracts contain cross-reactive allergenic proteins.

COLOR CODING, LABELS AND EXPIRATION DATES

As recommended by the "Allergen Immunotherapy: A Practice Parameter" guidelines and in accordance with Joint Commission national patient safety goals, a consistent uniform labeling system should be used for immunotherapy treatment vials.

Standardizing the label contents and vial coding will improve communication between care providers and patients, and likely prevent errors in extract administration.

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

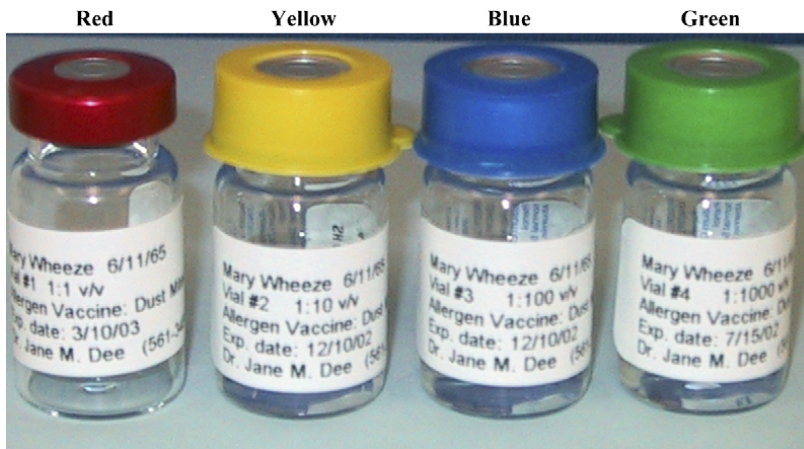
- 2 patient identifiers (e.g., name and date of birth)

- Concentration in vol/vol
- Color code or alphanumeric code (1 for highest concentration if number)
- Expiration or "beyond use" date

Immunotherapy treatment vial concentrations are now labeled in vol/vol with 1:1 vol/vol representing the maintenance concentrate. Alternatively the vial concentration can be labeled in actual units (e.g., 1000 BAU, 100 BAU) but this system may be complicated if allergens with different potency units are used (e.g., w/v, BAU, AU or PNU) and make it difficult to interpret the vial label.

All the vials in the treatment set are numbered and/or color coded in the following manner:

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



If a numbering system is used, the **highest concentration should be numbered #1** and the next 10 fold dilution (i.e., yellow vial) would be labeled #2, and so forth. Variation from patient to patient occurs when labeling more concentrated vials with

larger numbers. This practice resulted in patients often having a different number on their maintenance vial that was based on the total number of dilutions prepared.

Expiration dates should follow the manufacturer's recommendations. The rule of thumb is that the expiration date for a treatment or skin testing vial is the earliest expiration date recommended for any extract in the mix. Less concentrated extracts are more sensitive to temperature and might not maintain potency until the listed expiration date. 1:10 to 1:100 dilutions of stock extracts are generally stable for at least 12 months. This usually includes at least the patient maintenance treatment vial and the 1:10 vol/vol or yellow vial. Expiration dates for venom extracts are sometimes shorter. Perhaps this is due to the use of diluents with low levels of glycerin. The venom extract package inserts provides guidelines for expiration dates for the different dilutions.

Expiration dating periods for allergen extract products are regulated by the United States Food and Drug Administration (FDA). Even under ideal refrigerated conditions some loss of potency occurs over time. The potency and stability of these products are not assured beyond their labeled expiration date. Non-standard extract products are assigned expiration dating in accordance with FDA regulations (21 CFR, Section 610.53) with regards to whether products are glycerinated or non-glycerinated. A total of six years from the time of extraction is allotted to 50% glycerin bulk extracts. This six-year period is divided into a maximum of three years for manufacturer storage and three years for final container dating. Non-glycerinated products are allowed only a total of three years or half the dating of the manufacturer cold storage and 18 months maximum for final container dating. Manufacturers assign an expiration date to each container within FDA guidelines.

Sample expiration dates for diagnostic and treatment sets prepared by the U.S. Army Centralized Allergen Extract Laboratory are based on stock concentrate manufacturer recommendations for its suppliers. It is important that expiration dating practices for your clinic are in accordance with your manufacturer's recommendations and the earliest expiration date principle for mixes discussed above.

Diagnostic Products	Expiration Date*
Prick Test Materials	1 Year
ID Test Materials	6 Months
Immunotherapy Treatment Sets	
1:10 W/V-1:5000 W/V	1 Year

1:50,000 W/V and weaker	3-6 Months**
1000 PNU/ml - 20,000 PNU/ml	1 Year
<1000 PNU/ml	3-6 Months**
500 AU/ml and Stronger	1 Year
< 500 AU/ml	3-6 months**
1000 BAU/ml and Stronger	1 Year
< 1000 BAU/ml	3-6 months**

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

****The stability of lower extract concentrations (e.g., 1:1000 and 1:10,000 vol/vol) has not been extensively studied. Loss of potency in these lower concentrations may be due to absorption of the allergenic proteins to the glass wall. 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**

MIXING INDIVIDUAL PATIENT ALLERGEN EXTRACT TREATMENT SETS

Every clinic should develop a specific standard operating procedure document or manual to ensure standardization and safe practices of allergen extract mixing. Responsible providers developing the procedures should consult stock extract manufacturer recommendations and the most recent Allergen Immunotherapy Practice Parameter Update to incorporate the most up to date recommendations. USP <797> requirements should also be reviewed if relevant for your clinic.

These procedures should emphasize the importance of individual treatment vials and vial sets, especially when mixing of allergens is required. The mixing of antigens in a syringe is not recommended due to the potential for cross-contamination of extracts.

Here are a few guiding principles for mixing allergen extracts

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

Initial preparation:

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

Pre-mixing preparation:

- 1) Verify that a supervising physician is present in the same building as the mixing location(s).
- 2) Prepare specific mixing location(s).
- 3) Cleanse and maintain an aseptic work environment using an approved disinfectant solution (i.e., 70% isopropanol) without additives like dyes and glycerin.
- 4) Prepare vial labels in accordance with prescription and verify accuracy.
 - a. Name and second identifier

- b. Concentration
 - c. Antigens on label match those that are to be added
 - d. Expiration date is consistent with clinic procedures and source antigens
- 5) Apply label to treatment set vials
- a. If using color coded vials, verify color and concentration match
 - b. Alternatively, labels may be applied after mixing. For example, the label for the empty maintenance concentrate (red) vial (or all vials) can be left off until all contents are injected into the vial to improve visibility during checks for impurities, final volume and color comparison of dilution series.

Example of allergen extract mixing step by step procedures. This sample set of procedures does not constitute "recommended" procedures, but can be used as a starting point develop procedures that best fit a specific clinic/facility needs:

Mixing the maintenance (red) vial

- 1) Pull new empty sterile vials (usually 5, 8 or 10ml) for each vial in the patient's treatment set and put in order from strongest (maintenance/red) to most dilute
- 2) Pull the stock extract vial for each antigen on the prescription and stock diluents from refrigerator
 - a. Check stock antigens for turbidity/particulate matter. If present, consult package insert or manufacturer guidelines including possible recommendations for re-suspension or filtering.
 - b. For prolonged 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. A separate syringe is used for each antigen & diluent
 - b. Label each syringe (i.e. abbreviation for antigen or diluents)
 - c. For immediate use only, stock extracts should not be pre-drawn for extended periods due to risk of potency loss and misidentification

- 4) Document lot number & manufacturer for each antigen (preferably one per antigen)
- 5) Note expiration dates of stock extracts and that label expiration date does not exceed earliest stock vial extract
- 6) Wear appropriate personal protective equipment
 - a. Wash hands/nails to elbows for at least 30 seconds with soap & water
 - b. Don hair & facial hair covers, gowns, face masks (if following USP <797) guidelines)
 - c. Use alcohol based surgical hand scrub prior to gloving
 - d. Don powder-free sterile gloves compatible with 70% isopropyl alcohol (IPA)
- 7) Disinfect gloves before mixing with IPA (& intermittently for lengthy mixing)
- 8) Wipe vials &/or ampules with 70% IPA with wetting for at least 10 sec
- 9) Maintain aseptic technique by minimizing contact with secretions, skin, glove fingertips, etc. during mixing
- 10) Draw up the correct amount of each antigen and the diluent in syringe and place each syringe by the respective stock antigen vial
- 11) Verify drawn up doses are correct volume and antigen (Quality checkpoint opportunity: have a co-worker verify, if available)
- 12) Inject contents of all drawn up antigens one by one into the maintenance concentrate (red) vial.
 - a. The empty syringes should be discarded immediately into an appropriate sharps disposal container.
 - b. If the sterile maintenance vial is not a vacuum (air filled), an equal volume of air may need to be withdrawn prior to injecting stock extract volumes
- 13) If there is precipitate present in the stock antigen vials
 - a. Particulates and precipitates suspended in an extract solution are not uncommon.

- b. These particulates and precipitates often do not cause any significant loss in potency. Consult manufacturer recommendations in package insert or bulletins for additional information.
 - c. Attempted re-suspension by agitation (shaking or rolling) may be indicated in accordance with the package insert and your clinic operating procedures.
- 14) After mixing is complete, conduct final quality assurance check (preferably by mixer and trained co-worker)
- a. Solution color check
 - b. Label check
 - c. Vial color-code check
 - d. Liquid turbidity, precipitate & consistency check
 - e. Vial physical integrity (leaks, cracks, etc.) check
- 15) If applicable, package treatment set for transport or shipping
- 16) Document preparation details according to clinic specific procedures on prescription or preparation form and in mixing log (see Practice Parameter appendices for sample forms).
- a. Name of preparer & date prepared
 - b. Stock allergen extract manufacturer, lot number & beyond use or expiration date
 - c. Mixing log should be maintained in the unlikely event of a stock antigen recall or for extract or adverse event troubleshooting

Special procedure notes concerning alum precipitated extracts

Diluent: Alum- precipitated extracts generally require phenol saline diluent for all 10-fold dilution vials. 10% glycerol-saline or human serum albumin diluent cannot be used for alum- precipitated prescriptions as it interferes with the aluminum hydroxide-antigen absorbed complex.

- 1) 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 out to the bottom of the vial.

- 2) Unlike aqueous and glycerinated extracts that generally do not lose potency with filtering, large antigen-alum complexes may be lost during the filtering process and thus result in loss of potency. Therefore, **do not filter alum precipitated extracts.**

Preparing serial 10-fold dilutions of the maintenance (red) vial

Serial 10-fold dilutions are prepared to complete a patient's initial allergen immunotherapy treatment vial set. **Dilutions are made by serial dilution** (taking from a parent vial and placing into a new vial prefilled with diluent to create a 10-fold dilution ($1/10^{\text{th}}$ the amount of allergen contained in the parent vial). This newly diluted vial becomes the parent vial and another dilution is made, and so on until the desired number of 10-fold dilutions is achieved. **Diluted allergen immunotherapy vials should not be made by pulling directly from a manufacturer's concentrated stock vial extract.** The biggest 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 this is virtually impossible to do to the precision needed for the most dilute vials. Thus a dilution vial prepared by this method may contain less or more than expected and potentially increase the risk of adverse events during vial transitions within the build-up phase.

The volume used to make serial dilutions from parent vials depends on both the desired dilution (10-fold in this case) and the final volume. Typical treatment set vials are: 2, 5, 8 or 10 ml. Treatment set vials are now available with original color caps or snap-on caps to create sets according to the recommended color scheme. Vials also come empty or prefilled with diluents suitable for intradermal or subcutaneous administration. Pre-filled volumes correspond to the amount of diluent needed to make a 10-fold dilution. For example, a prefilled 5 ml yellow vial will contain 4.5ml of diluent and have a yellow cap. To make the yellow 10-fold dilution vial, 0.5ml would be taken from the parent red maintenance vial (1:1 vol/vol) and added to yellow vial for a total final volume of 5 ml ($0.5 = 1/10^{\text{th}}$ of 5ml, a 10 fold dilution or 1:10 vol/vol). To make the same 10-fold diluted yellow vial using one that was not prefilled, 0.5 ml is added from the red maintenance vial and 4.5 ml is added from a stock diluent vial.

To determine how much is 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, $10 \text{ ml} / 10 = 1 \text{ ml}$). Then calculate the amount 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.

To determine how much is taken from the parent vial for a Y fold dilution for final volume X, divide X by Y. For example, a 5 fold (Y=5) dilution of a 10ml (X=10) vial, will require 2ml to be transferred from the parent vial ($X/Y = 10\text{ml} / 5 = 2 \text{ ml}$). To calculate the amount of diluent needed, subtract the X/Y volume from the final volume X. In this example 8ml of diluent is required (10 ml – 2 ml = 8 ml). The final concentration of the diluted vial is 1/Y that of the parent vial (1/5th in this example). The following formula can be used to create dilutions

Figure 1: Allergen immunotherapy dose-calculation table⁷

TABLE II. Allergen immunotherapy dose-calculation table

- Express weight per volume as a fraction: $\frac{1}{N}$ or 1:N.
- Calculate concentration of individual allergens in mixtures. If a mixture is made of equal amounts of N individual allergens to make a total concentration (C), the final concentration of an individual allergen (Ca) can be calculated by using the following equation:
 $C_a = C \times \frac{1}{N} = \frac{C}{N}$ or $C:N$.
 For example, if a mixture of equal amounts of 5 (N) allergens has a total concentration (C) of 100,000 BAU/mL, then the final concentration of each individual allergen (Ca) is: $C_a = \frac{C}{N} = \frac{100,000}{5} = 20,000 \text{ BAU/mL}$.
 Likewise, if C = 1:10 (wt/vol), then: $C_a = \frac{C}{N} = \frac{1/10}{5} = \frac{0.1}{5} = 0.02$ or $\frac{1}{50}$ or 1:50.
- Dilution of individual allergen: If an initial volume, Vi (in milliliters), of an individual antigen with concentration, Ci, is added to an allergen extract to make a final volume of Vf (in milliliters), the final allergen concentration (Ca) in the allergen extract mixture will be: $C_a = C_i \times \frac{V_i}{V_f}$.
- Final concentration of an allergen in a mixture of mixtures is determined by multiplying the initial concentration by the dilution factors from each mixing step.
 For example, consider a mixture containing equal amounts of 5 (N) allergens with a total concentration (C) of 100,000 BAU/mL (first dilution). If an initial volume (Vi) of 0.5 mL of this mixture is further mixed with other components and diluent to make a final allergen extract mixture volume (Vf) of 5.0 mL (second dilution), the final concentration of an individual allergen (Ca) will be:

$$C_a = C \times \underbrace{\frac{1}{N}}_{\text{Mixture dilution}} \times \underbrace{\frac{V_i}{V_f}}_{\text{Allergen extract dilution}} = 100,000 \times \frac{1}{5} \times \frac{0.5}{5.0} = \frac{100,000}{50} = 2000 \text{ BAU/mL}$$

Likewise, if C = 1:10 (wt/vol), then: $C_a = \frac{1}{10} \times \frac{1}{5} \times \frac{0.5}{5.0} = \frac{1}{300}$ or 1:300.

Preparation of 5 ml dilution vials for patient treatment sets (serial 10-fold dilutions):

- 1) Verify the labeling and order (color coded, label concentration) for vials is correct
- 2) Ensure the maintenance vial is mixed by inverting or rolling
- 3) Using a fresh syringe and aseptic technique, remove 0.5 ml from the mixed 5 ml maintenance concentrate red or 1:1 vol/vol vial.

- 4) Using aseptic technique, inject this 0.5 ml from the maintenance vial into the 4.5 ml pre-filled (10% glycerol-saline or HSA) yellow or 1:10 vol/vol vial. This vial will be a 10- fold dilution of the maintenance concentration vial.
- 5) Ensure this newly made 10 fold diluted (yellow) vial is mixed by inverting or rolling
- 6) Subsequent 10-fold dilutions are done in the same manner for the rest of the vials in the treatment set (0.5 ml into 4.5 ml of the 10 fold weaker labeled 10% glycerol-saline prefilled vial).
 - a. 0.5ml from yellow 1:10 vol/vol into 4.5ml diluent filled blue 1:100 vol/vol vial
 - b. 0.5ml from blue 1:100 vol/vol into 4.5ml diluent filled green 1:1000 vol/vol vial
 - c. 0.5ml from green 1:1000vol/vol into 4.5ml diluent filled silver 1:10,000 vol/vol vial
 - d. And so on for additional more dilute (silver) vials
- 7) Whereas using a fresh syringe for each dilution transfer is often preferred, use of the same syringe for serial dilution transfers is an alternative if a "mix/rinse" step is included. A mix/rinse step consists of pulling up a full syringe volume (1 ml for a 1 ml syringe) and re-injecting back into the vial without removing the syringe. This is often repeated (i.e., for a total of 3 times) prior to pulling up the final volume for the transfer to the next dilute vial. (REMINDER: do not reuse syringes when mixing antigens for the initial maintenance vial.)

Below is an example of completed allergen immunotherapy prescription form, which includes a formula that can be used to calculate the volume needed to deliver the target dose. For example, if the desired maintenance dose for cat was 2000 BAU: for a 0.5 ml dose the desired concentration would be 4000 BAU/ml. This amount is divided by the manufacturer's extract 10,000 BAU and then multiplied total volume 5 ml to determine the amount needed to add e.g., $4000/10,000 \times 5\text{ml} = 2\text{ml}$. So adding 2 ml into 3 ml to produces a final volume of 5 ml and concentration of 4000/BAU for the cat extract.

In the below example of a completed immunotherapy prescription form, a **0.5ml maintenance dose** would deliver:

Cat: 2000 BAU

Ragweed: ~ 9 mcg Amb 1

Timothy: 2000 BAU

D. farinae: 1000 AU

D. Pteronyssinus: 1000 AU

Figure 2: Completed Allergy Immunotherapy Prescription Form

Example of a Completed Allergen Immunotherapy Prescription

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

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

Vaccine Name: C, R, G, T, W

**Maintenance Concentrate
 Prescription Form**

Prepared by: Mary Lancet Date Prepared: 6/10/08

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

* Components of mixes listed on a separate sheet

Antigen Number	Extract Name Allergen or Diluent (Common name or Genus/species)*	Concentration and Type Manufacturer's Extract (AU, BAU, W/V, PNU/ (50% G, Aq, Ly, AF)	Volume of Manufacturer's Extract to Add*	Extract Manufacturer	Lot Number	Expiration Date
1	Short ragweed	1:10 w/v G (350 Amb al)	0.25ml	Greer	12345	1/01/10
2	<i>D. farinae</i>	10,000 AU/ml G	0.5 ml	Allermed	5765	2/06/10
3	<i>D. Pteronyssinus</i>	10,000 AU/ml G	0.5ml	Antigen labs	4222	3/24/08
4	Cat	10,000 BAU/ml G	2.00ml	Hollister-Stier	9898	2/27/10
5	Timothy grass	100,000 BAU/ml G	0.2 ml	ALK	56789	7/09/09
6						
7						
8						
9						
10						
Diluent	HSA		0.6ml	ALK	68597	12/12/11
Total Volume			5.00 ml			6/09/09

** Assumes 0.5 ml injection as target maintenance dose

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

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

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

Adapted from completed allergen immunotherapy prescription for which can be downloaded from www.aaaai.org members only section

Allergen extract treatment set preparation hints

- 1) Do not mix prescriptions for more than one patient at the same time.
- 2) Train multiple qualified personnel in allergen extract preparation in case of absences and for participation in quality checks.
- 3) Avoid putting hand lotion on before the compounding of allergen extract vaccines and skin test antigens. Lotion tends to harbor bacteria.
- 4) Regularly review operating procedures for opportunities to make the process safer and more efficient.
- 5) Establish a regular inventory check.
 - a. identify stock allergen extracts, diluents and mixing supplies in need of reordering
 - b. check for expiring stock allergen extracts and diluents and mixing supplies
- 6) Return antigen stock trays to the refrigerator when away from the compounding area for an extended period of time.
- 7) Minimize diversions during extract preparation.
- 8) Stock refrigerators are NOT to be used for food or drink storage.

Additional quality assurance checks

Before use or shipping, additional quality assurance checks should be conducted, ideally by a co-worker. In accordance with Practice Parameter label recommendations, a final **label check** should be performed. This should consist of verifying that the label contains the right name, right contents (allergens), right concentration, right alphanumeric number in the right order with lowest = 1 (if numbers used), right expiration date (dilute vials may have earlier expiration dates than more concentrated vials). Additionally, a "**color check**" of the solution in each vial should be conducted. The solution in the maintenance concentrate vial should be the darkest in color and vials should be lighter in color with each 10 fold dilution. The weakest strength vial should contain the lightest colored solution. When using color-coded vials, a **vial color code check** should be performed. Vials in the treatment set should be arranged in order (Red/maintenance, Yellow, Blue, Green and Silver). For each color coded vial the label concentration in vol/vol or number should match what is recommended in the Practice Parameters for that color code (see Table X1 and Table XII from Practice

Parameters). Additionally, the color of the solution should be a shade consistent for that dilution (lighter if not the red maintenance vial).

Figure 3: Suggested nomenclature for allergen extract dilutions⁷

TABLE XII. Suggested nomenclature for labeling dilutions from the maintenance concentrate

Dilution from maintenance concentrate	Vol/vol label	No.	Color
Maintenance concentrate	1:1	1	Red
10-fold	1:10	2	Yellow
100-fold	1:100	3	Blue
1000-fold	1:1000	4	Green
10,000-fold	1:10,000	5	Silver

From: Allergen Immunotherapy: A Practice Parameter Second Update (2007)

All vials should also undergo a **precipitate check**. 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 re-mixing that patient's vial set.

STINGING INSECT ALLERGEN EXTRACT PREPARATION

Lyophilized or freeze-dried stinging insect venom extracts are available commercially for diagnostic testing and patient treatment. 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 vespidae" extract at a reconstituted concentration of 300 mcg/ml. 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.

Insect venom (and fire ant) extracts should not to be mixed with other venom or aeroallergen extracts for either testing or treatment due to the lack of sufficient stability, safety and efficacy studies to support mixing.

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 micrograms per ml (mcg/ml). Reconstitution and dilution of all insect venom extracts is most commonly performed with HSA (human serum albumin/phenol) diluent.

Extracts are also available for **Imported Fire Ant** Hymenoptera species. Two fire ant species, *Solenopsis richteri* and *S. invicta*, are commercially available as individual extracts for testing or treatment or as a fire ant mix containing both species. Fire ant extracts are made from whole fire ant bodies. Fire ant venom extracts are being investigated for clinical use, but require a significant amount of time and resources for mass production. Fire ant stock concentrate extracts typically are available as glycerinated extracts in w/v concentrations (i.e., 1:20 w/v). Practice Parameters for Insect Allergy has individual expert recommendations for the maintenance dose range from 0.5 ml of 1:100 w/v to 1:10 w/v of a maintenance concentrate.

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.¹³ Several measures are taken by stock extract manufacturers and healthcare personnel to minimize the risk of loss of potency of extracts during normal storage and use.

Dilution of extracts alone can affect the long term potency of extracts. For example, diluted extracts have lower concentrations of important preservatives and stabilizers. Furthermore, lower concentrations of proteins decrease 3-dimensional protein structure stabilization achieved through protein-protein interactions that are facilitated at higher protein concentrations. Finally dilutions may also magnify the effect of allergenic protein loss due to binding to sites on glass vials that is essentially insignificant at higher protein concentrations.

Manufacturer processing steps include **additives** that stabilize the allergenic proteins and preservatives that prevent contamination of the stock extract and individual patient

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

Phenol is a common bacteriostatic preservative added to allergen extracts and is used at a final concentration of approximately 0.4%. One possible ill-effect of using phenol is that it may denature (unfold or breakdown) allergenic proteins even if in 50 % glycerin.^{13, 14} Human serum albumin may protect against phenol's adverse effects on allergenic proteins.¹³ Other recognized preservatives such as thimersol and methylparaben are not generally used in allergen extract preparation. **70% isopropanol** is a disinfectant but not a preservative. **Disinfectants** are antimicrobial agents applied to non-living objects (e.g., countertops). Thus they are not "preserving" viability, potency or purity. Disinfectants should also be distinguished from antibiotics that kill microorganisms within the body. Sanitizers are high level disinfectants that kill over 99.9% of a target microorganism. Sterilization refers to the complete elimination of all microorganisms.

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

Stabilizers are added to diluents to maintain the structure of allergens in solution and prevent sticking or adherence to the glass vials they are added to. Common stabilizers include glycerin and human serum albumin. 50% glycerin is often considered the best stabilizer alternative and is also considered a preservative while human serum albumin is not a preservative. Glycerin potently stabilizes proteins in solution, inhibits proteases found in some allergen extracts, and is bacteriostatic at concentrations greater than or equal to 20%. It should be noted that these preservative and stabilizing properties are diminished as the concentration of glycerin is decreased. One drawback of glycerin is that it is irritating to the skin in higher concentrations. Although most extracts used for prick or percutaneous skin testing have 50% glycerin, extracts used for intradermal testing contain considerably less, often 100 to 1000-fold dilutions of those used for percutaneous skin testing.

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

Allergen extracts are stored in refrigerators **at a temperature of 4°C** or in accordance with manufacturer recommendations. A temperature range of 2-8°C is considered acceptable by most experts. Given the expense and temperature sensitivity of stock allergen extract concentrates and mixed patient treatment sets, it is also reasonable to conduct some form of temperature monitoring to ensure that extracts are not exposed to temperature extremes. For example a log of daily temperatures can be maintained or an automated continuous temperature monitoring device can be installed. Facilities might also consider installing temperature alarms.

Many allergen extracts are heat sensitive. The loss in potency when allergen extracts are exposed to high temperatures (i.e. >78°F or 26°C) may be due to the heat labile

Comment [T1]: Assume that Mike wanted a straight F to C conversion??? Either 26 deg C or 95 deg F.

(sensitive) proteins that unfold or degrade at these temperatures. Loss of potency can also occur at lower temperatures, including room temperature (i.e., 68-72°F and 22°C). This is possibly due to proteases in the extract that are activated at these temperatures and degrade relevant allergen proteins in the extract. Allergen extracts exposed to room temperature over time may thus lose potency, such as extracts frequently left out of the refrigerator for long periods during testing or treatment. For example, skin testing trays with extracts that are taken out of the refrigerator in the morning everyday 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. 50% glycerin may help protect against the effects of prolonged exposure to room temperature, possibly due to its effect on proteolytic enzymes. Less is known about the effects of freezing (< 0°C) on allergen extract potency but at least one study found a moderate loss of potency when an extract was stored frozen and thawed for use.¹⁵ An increase in the number of multiple freeze-thaw cycles increases the observed loss in potency of extracts. Thus extracts that are accidentally frozen should be replaced with new extract prior to use.

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

Investigations have shown that extracts stored in vials only partially filled with solution are less stable. In other words, one milliliter 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 re-ordering and preparing treatment and diagnostic materials as the extract volume in current vials becomes low.

SUMMARY

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

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

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

All personnel involved in allergen extract preparation should be familiar with the contents of the most recent Practice Parameters. A companion examination has been developed based on this training document to assist in satisfying competency assessment and documentation requirements. It will be available along with this document on the Joint Council of Allergy, Asthma and Immunology web site (<http://www.jcaai.org>) as a joint collaboration of the American Academy of Allergy, Asthma and Immunology; the American College of Allergy, Asthma and Immunology and the Joint Council.

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Appendix 1: Initial and Ongoing Competency Assessment: Allergen Extract Mixing

Name _____ Job Title: _____ Clinic: _____

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

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

Employee signature _____ Date _____

*** Self Assessment:**

1 = Experienced

2 = Needs Practice/Assistance

3 = Never Done

NA = Not Applicable

+ Evaluation/Validation Methodologies:

T = Tests

D = Demonstration

V = Verbal

I = Interactive Class

February 20, 2009

Appendix 2: Probable Effective Dose Range For Allergen Extracts US Standardized Units. From Allergen immunotherapy: A practice parameter second update.⁷

Antigen	Labeled potency or concentration ^{a,b}	Probable Effective Dose Range
Dust mites: <i>D. farinae</i> and <i>D. pteronyssinus</i> ^c	3,000, 5,000, 10,000 and 30,000 AU/ml	500-2,000 AU
Cat ^d	5,000 to 10,000 BAU/ml	1,000-4,000 BAU
Grass, standardized ^e	10,000-100,000 BAU/ml	1,000-4,000 BAU
Short ragweed ^f	1:10 –1:20 w/v 100,000 AU/ml	6-12 mcg of Amb a 1 1000-4000 AU Concentration of Amb a 1 is on the label of w/v extracts in FDA units ¹⁶
Non-standardized extract -Dog ^g	1:10- 1:100 w/v	15 mcg of Can f 1
Non-standardized extracts	1:10 –1:40 w/v or 10,000-40,000 PNU/ml	Highest tolerated dose
Wasp, yellow jacket, hornet and honeybee venoms ^h	100 mcg/ml	50-200 mcg
Imported Fire ant whole body extracts ⁱ	1:10 w/v	0.5 ml of 1:100 w/v to 0.5 ml of 1:10 w/v

- a.** Multiple studies have demonstrated that the efficacious dose for allergen immunotherapy is between 5 and 20 mcg of the major allergen per injection. Only 2 extracts licensed in the United States are standardized based on major allergen content (measured by means of radial immunodiffusion): short ragweed (Amb a 1) and cat (Fel d 1).
- b.** The labeled concentrations for the nonstandardized extracts have no established standards for biologic potency. Nonstandardized extracts are labeled on the basis of PNU values or the weight of the source material extracted with a given volume of extracting fluid (wt/vol).
- c.** There have been no dose-response studies with United States–licensed dust mite extracts, and dosing recommendations in AU value are extrapolated from published European studies that used aqueous¹⁷ and alum-precipitated^{18, 19} extracts. One study designed to investigate the effect of 3 doses of an alum precipitated *D pteronyssinus* extract (0.7, 7, and 21 mcg of Der p 1) found a dose-response effect on efficacy and side effects.¹⁹ The authors suggested the optimal maintenance dose was 7 mcg of Der p 1. Corresponding doses were based on specific allergen measurements of US commercially available standardized extracts provided by manufacturers. Extrapolating effective and safe doses in this manner might not be scientifically valid. *D farinae* and *D pteronyssinus* are similar in group 1 allergen content according to the FDA’s current reference standards. Appropriate dose reductions would need to be made when combining antigens that have a strong degree of cross-reactivity, such as *D pteronyssinus* and *D farinae*.

- d.** The major cat allergen Fel d 1 is reported in FDA units, with 1 Fel d 1 unit equaling approximately 2 to 4 mcg of Fel d 1.²⁰⁻²² The amount of Fel d 1 in 10,000 BAU/mL ranges from 10 to 19.9 U/mL. One study demonstrated clinical efficacy of a maintenance dose of 4.56 FDA units of Fel d 1 dose in terms of decreased cat extract PD20, titrated skin test results, and allergen-specific IgE and IgG levels.^{23, 24} In a recent study that investigated the efficacy in terms of immunologic changes of 3 doses of a United States–licensed cat extract (0.6, 3, and 15 mcg) demonstrated that a significant effect on titrated skin prick test results, allergen specific-IgG4 levels, and CD41/IL-4 levels was only seen in the group treated with 15 mcg of Fel d 1, although the 3-mcg dose group did demonstrate a significant change in titrated skin test response and increase in cat-specific IgG4 levels.²⁵
- e.** There have been no dose-response studies with United States–licensed standardized grass extracts. Recommended doses are extrapolated from published European studies that have used aqueous²⁶, alum-precipitated,^{27, 28} and calcium phosphate–precipitated grass pollen extracts.²⁹ One of these studies compared a dose of 2 mcg with 20 mcg of major timothy allergen (Phl p 5) and found clinical efficacy at both doses.²⁸ The efficacy was greater in the 20 mcg of Phl p 5 dose, but the systemic reaction rate was also higher in the high-dose group. The package inserts for United States–licensed grass pollen extracts contain a table to convert the nonstandardized units (wt/vol and PNU), for which there have been studies that have demonstrated efficacy, into BAU. Extrapolating effective and safe doses in this manner might not be scientifically valid. Appropriate dose reductions would need to be made when combining antigens that have a strong degree of cross-reactivity, such as the northern pasture grasses (subfamily Pooideae; eg, perennial rye, meadow fescue, or timothy).
- f.** Ragweed is reported in FDA units, with 1 U of Amb a 1 equaling 1 mcg of Amb a 1. The potency units for short ragweed extracts were originally assigned based on their Amb a 1 content. Subsequent data suggested that 1 unit of Amb a 1 is equivalent to 1 mcg of Amb a 1, and 350 Amb a 1 units/mL is equivalent to 100,000 BAU/mL.³⁰ The package insert of the short ragweed 100,000 AU/mL extract states the optimal immunotherapy dose is 2000 AU, with a range of 1000-4000 AU. One open study of patients with ragweed-induced allergic rhinitis demonstrated a significant improvement in ragweed nasal challenge in patients treated with a mean dose of 6 mcg of Amb a 1 for 3 to 5 years compared with an untreated matched control group.³¹ A ragweed dose-response study (0.6, 12.4, and 24.8 mcg of Amb a 1) demonstrated efficacy, as measured by nasal challenge, at 12 and 24 mcg of Amb a 1.³² The efficacy of the 24-mcg dose was not significantly better than the 12-mcg dose, and the authors concluded that the optimal dose for ragweed extract was greater than 0.6 mcg but not more than 12.4 mcg of Amb a 1.
- g.** Dog extracts are not standardized. However, one dose-response study with a United States–licensed acetone-precipitated dog extract investigated the efficacy of 3 doses (AP dog; Hollister-Stier, Spokane, Wash; 0.6, 3, and 15 mcg) in terms of immunologic changes and found the dose of 15 mcg of Can f 1 to be most efficacious.³³ The 3-mcg dose also demonstrated significant

efficacy, although not as great as the 15-mcg dose. The extract used in the dosing study was assayed at 160 mcg/mL. Subsequent lots have assayed between 128 and 208 mcg/mL (average Can f 1, 162 mcg/mL [SD \pm 26 mcg/mL]; information provided by the extract manufacturer, Hollister-Stier

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Preparation of Allergen Extract

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