

Commercial laboratory preparation of immunotherapy vials

Jeanne A. Wohlfarth, MT (ASCP), Laura A. Peterson, MT, and W. T. Willoughby

The efficacy of allergen-specific immunotherapy injections for allergic patients continues to be documented. Recent reports on sublingual immunotherapy have acknowledged this route as a viable and safe alternative to subcutaneous immunotherapy for reducing respiratory symptoms. Allergens can be determined by direct skin testing (*in vivo*) or by serum analysis (*in vitro*). *In vivo* testing includes scratch, prick/puncture, intradermal, and skin endpoint titration. *In vitro* testing uses a form of a radioallergosorbent test to measure allergen-specific IgE. The use of a US Food and Drug Administration–licensed commercial laboratory for the preparation of immunotherapy offers numerous advantages for the physician: any method of testing can be accommodated according to the physician's prescription; immunotherapy injections are prepared in a certified cleanroom following stringent guidelines to ensure quality and sterility; and a physician's overhead costs for staff, space, and supplies, as well as staff time constraints, can be reduced. *Curr Opin Otolaryngol Head Neck Surg* 2001, 9:186–192 © 2001 Lippincott Williams & Wilkins, Inc.

Antigen Laboratories, Inc., Liberty, Missouri, USA.

Correspondence to W. T. Willoughby, Antigen Laboratories, Inc., 30–34 South Main Street, PO Box 123, Liberty, MO 64069, USA; e-mail: antigenlab@antigenlab.com

W. T. Willoughby is president and owner of Antigen Laboratories, Inc., a FDA-licensed commercial allergenic extract laboratory. Jeanne A. Wohlfarth and Laura A. Peterson are employees of Antigen Laboratories, Inc.

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Abbreviations

FDA	Food and Drug Administration
mRAST	modified RAST
RAST	radioallergosorbent test
SET	skin endpoint titration
SIT	allergen-specific immunotherapy
SLIT	sublingual immunotherapy
WHO	World Health Organization

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Immunotherapy for a specific allergen by route of subcutaneous injections was first described by Noon [1] of London in 1911. Since that time, immunotherapy injections have increased to an estimated 7 to 10 million administered each year [2•]. Allergic immunotherapy is the escalation of dosing to a level in which allergic symptoms are improved on subsequent exposure to the allergen [3•]. The effectiveness of allergen-specific immunotherapy (SIT) in the management of allergic rhinitis was confirmed in 15 of the 16 analyzed single- and double-blind placebo-controlled studies by Ross *et al.* [4]. The same authors concluded in a meta-analysis of 24 double-blind placebo-controlled studies that SIT was effective in 71% of the studies for the management of allergen-triggered asthma [5]. In a study by Mungan *et al.* [6•], subcutaneous SIT was effective for mite-sensitive patients with rhinitis or with asthma. A double-blind placebo-controlled study of patients whose SIT had ended was conducted by Durham *et al.* [7] to determine the long-term clinical efficacy of grass pollen immunotherapy. The study concluded that immunotherapy of 3- to 4-year duration induced a prolonged remission with a persistent alteration in immunologic reactivity. Local or systemic reactions have been observed in SIT at a rate of 0.3 to 14%, with most reactions occurring during the build-up phase [2•].

Other routes of immunotherapy are currently being investigated, including sublingual immunotherapy (SLIT). The World Health Organization (WHO) position paper acknowledges that SLIT, when administered in high doses for respiratory allergic diseases, may be a viable alternative to subcutaneous immunotherapy. WHO advocates more research into SLIT but cautions that SLIT is not the standard therapy in the United States, nor is it recognized by major American allergy or immunology organizations [8••]. In a placebo-controlled, parallel-group, single-blind study to compare the efficacy of subcutaneous injections and SLIT, Mungan *et al.* [6•] concluded that SLIT may be effective in patients with allergic rhinitis for reducing rhinitis symptoms and may be a potential therapy for patients with allergic asthma. André *et al.* [9] reviewed eight double-blind placebo-controlled SLIT trials and confirmed the safety of this method in children and adults with rhinitis or moderate asthma. Di Rienzo *et al.* [10] confirmed the safety of SLIT in a surveillance study of 268 pediatric patients. The clinical benefit and safety of SLIT seem evident, and the validity of SLIT is becoming more accepted by

the medical community [11••]. However, SLIT remains controversial because of a lack of consensus in such parameters as administration, duration of treatment, and dosing schedule; lack of systemic changes such as the ratio of T-helper cells 1 and 2; and the need for larger and more long-term studies [11••,12,13•].

Whether SIT or SLIT is prescribed following the diagnosis of an allergic patient, immunotherapy (prescription treatment sets) prepared by a commercial laboratory can be very advantageous to the physician. Quality immunotherapy products can be expected from a Food and Drug Administration (FDA)–licensed commercial laboratory.

Commercial immunologic laboratories

In a FDA-licensed commercial laboratory, allergenic immunotherapy extracts are prepared in accordance with current Good Manufacturing Practices as directed by the Code of Federal Regulations of the FDA. Quality and compliance are assured by regular and extensive FDA inspections.

Quality assurance requires that all facets of manufacturing have met code requirements and are monitored regularly. All closures, seals, vials, and labels must meet strict specifications. Any changes must be validated and approved by the Center for Biologics Evaluation and Research.

Manufacturing technicians are thoroughly trained in standard operating procedures, undergo annual reviews of their training, and attend regular meetings on current Good Manufacturing Practices. Technicians preparing immunotherapy must demonstrate their proficiency by passing stringent sterility standards on simulated treatment sets. Revalidation of the technician's proficiency is performed on a regular basis.

In accordance with the Federal Standard 209E (Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones), preparation of immunotherapy injections is performed in classed areas in which the air supply is filtered through high-efficiency particulate air filters. The critical, or aseptic, area is monitored continuously during the preparation to verify a 0.5- μ m airborne particulate count of no more than 100 per cubic foot (class 100) (Fig. 1). Air quality is monitored daily for viable microbes, temperature, humidity, and air pressure. Recertification of the classed areas is performed semiannually by an independent contractor to ensure continued compliance and quality.

Prescriptions are prepared one patient at a time and immediately labeled as each prescription is filled. A double-check system of two trained technicians ensures

Figure 1. Cleanroom/hood monitoring



Air sampler and agar strip for viable microbes, particle counter, and microbial touch plates for surfaces, garments, and gloves.

the accuracy of each prescription and allergen before and during production. A sterile syringe is used once per allergen per patient and immediately discarded in a sharps container, thus preventing carryover and contamination with other allergens. Technicians wear sterile coveralls, boots, hoods, and gloves, while masks and eyewear cover the face area (Fig. 2). Garments, gloves, and the environment are routinely monitored for microbial levels during production (Fig. 1).

For immunotherapy injection preparation, a sterility sample of each treatment set, allergenic extract, and diluent is tested for both anaerobic and aerobic organisms in certified media (Fig. 3). The FDA-required sterility hold, or quarantine period, is 14 days, during which time the media is regularly examined for microbial growth. If any microbial growth occurs, the protocol

Figure 2. Cleanroom apparel

Technicians wear sterilized garments and gloves while working in the cleanroom.

includes a thorough written investigation and preparation of a new treatment set. To ensure that the physician receives a sterile product, treatment sets must satisfactorily pass all sterility testing before release.

A commercial laboratory will prepare individual treatment sets in accordance with the physician's signed and dated prescription order, which lists the allergenic test results. Whether the physician evaluates allergenic responses via *in vivo* or *in vitro* testing, a licensed commercial laboratory will be able to accommodate these testing methods. *In vivo* testing includes scratch, prick/puncture, intradermal, and skin endpoint titration (SET). *In vitro* testing uses an assay, such as radioallergosorbent test (RAST), for measurement of specific IgE.

Scratch, prick/puncture, and intradermal skin testing

The scratch test is one of the oldest forms of testing and incorporates a technique whereby the skin is superficially lacerated followed by a drop of concentrated allergenic extract. The popularity of this testing has decreased due to unreliable reproducibility, frequent false-positive results from skin irritation, and patient discomfort. With

the development of more comprehensive testing, the scratch test is no longer recommended by the Allergy Panel of the American Medical Association Council on Scientific Affairs [14].

The prick/puncture test has been in existence for approximately 90 years and remains the standard method employed by many allergists. A modified method of the prick test involves inserting a hypodermic needle through a drop of allergenic extract into the epidermal skin surface. A prick/puncture testing device, such as the Lincoln Diagnostic Multi-Test II® (Lincoln Diagnostics, Decatur, IL), provides the ability to produce multiple results, rapidly and consistently. Advantages of the prick/puncture method of testing are simplicity, rapid screening, fewer false-positive results, low cost, and relative safety. Disadvantages include possible false-negative results, no quantification of reactions, and subjectivity of the grading of reactions [15].

Intradermal testing, in existence for approximately 85 years, has been used by physicians as a follow-up to positive patient history and negative prick test findings.

Figure 3. Sterility testing

Every prescription treatment set is sterility tested for aerobic and anaerobic microbes in culture media.

The technique involves injecting a small amount of allergenic extract intradermally. This testing is highly sensitive and reproducible; however, intradermal testing provides only nonstandardized qualitative results and is susceptible to subjectivity [14,15].

Certain medications may have an inhibitory effect on *in vivo* skin testing (Table 1) [15–17]. β -blockers and angiotensin-converting enzyme inhibitors may be contraindicated because of possible side effects (Course on SET and RAST by Edwyn L. Boyd, MD).

Based on any of these *in vivo* direct skin testing results, a commercial laboratory would prepare a four-vial tenfold dilution treatment set. All allergens in each vial have the same weight to volume, with the weakest vial at a concentration of 1 to 100,000 and the strongest vial at a concentration of 1 to 100 (Table 2). Immunotherapy build-up would proceed weekly until the maximum tolerated dose is achieved. The maximum tolerated dose is the highest dose that provides symptomatic relief without initiating a local or systemic reaction. The concentration of the maximum tolerated dose is the maintenance dose the patient continues taking until immunotherapy is completed.

Skin endpoint titration

During the 1930s, SET was developed to fill the need for more accurate, sensitive, and quantifiable direct skin testing. Rinkel [18] later expanded the SET method by introducing titration based on fivefold dilutions.

The concept of SET is to demonstrate the exact sensitivity level by intradermal skin reaction to progressive dilutions of an allergenic extract. The endpoint is the dilution at which positive whealing is first observed, indicating a safe starting dose for immunotherapy. Because SET provides very sensitive results, there are fewer false-negative results. SET also provides highly reproducible quantitative and qualitative results. Even as SET is recognized as the otolaryngologist's standard, this method has disadvantages: SET is time consuming; more training and experience in testing and

Table 2. Injection immunotherapy preparation

Testing	Vial set	Dilution	Concentration
Scratch	4-vial set	10-fold	1:100,000→1:100
Prick/puncture, intradermal	(optional 5-vial set)	10-fold	1:1,000,000→1:100
SET	1-vial set	5-fold	Per SET result
RAST	3-vial set	5-fold	R –1, R 0, R+1
	(optional 4-vial set)	5-fold	R –2, R –1, R 0, R+1

SET, skin endpoint titration; RAST, radioallergosorbent test.

reading results are needed; more supplies are required; some pediatric patients may find SET traumatic; and false-positive skin reactions may occur [14,15]. Certain medications may need to be discontinued before SET testing because of a possible inhibitory effect (Table 1) [15–17]. β -blockers and angiotensin-converting enzyme inhibitors may be contraindicated because of possible side effects (Course on SET and RAST by Edwyn L. Boyd, MD).

A commercial laboratory preparing immunotherapy based on SET results would prepare a one-vial, fivefold dilution treatment set (Table 2). Sublingual–swallow drops can also be prepared from SET results and would consist of a one-bottle, fivefold dilution set (Table 3) (Waickman, Personal communication, September 2000). All allergens using either treatment method are at the exact concentrations as determined by the endpoint testing. Build-up proceeds directly from the vial or bottle. The maintenance dose is the maximum tolerated dose obtained during the build-up phase. Any dosing past the SET results must be determined by the physician.

Radioallergosorbent testing

In vitro testing for serum-specific IgE protein present in immunologic responses has been measured by different methods of RAST for more than 35 years. Approximately 25 years ago, Fadal and Nalebuff [19] modified earlier RAST techniques to improve sensitivity and to adjust the grading scale with the basis on fivefold dilutions. The modified RAST (mRAST) correlates well with SET. Because SET testing tends to be slightly more sensitive, corresponding results from Fadal–Nalebuff mRAST typically are one dilution class lower than results from SET [20]. Immunotherapy based on the Fadal–Nalebuff mRAST paired with vial safety testing has a proven safety record. Through seminars, Fadal and Nalebuff have trained thousands of physicians (representing millions of injections), and the safety record continues to be successful (Fadal, Personal communication, November 2000).

Advantages of RAST include fewer false-positive results, less trauma for the patient, medications do not interfere, in-office time is saved for physician and staff,

Table 1. Effects of medications on skin testing

Medications affecting skin testing	Medications with no or little affect
Anti-H ₁ histamines	Anti-H ₂ histamines
Ketotifen	Decongestants
Tricyclic antidepressants	Corticosteroids
Tranquilizers	β_2 -agonists
Clonidine	Bronchodilators
Dopamine	Cromolyn
ACE inhibitors	Theophylline
β -blockers	Calcium channel blockers
Specific immunotherapy	

ACE, angiotensin-converting enzyme.

and it is safer than initial skin testing. RAST could be used for patients with skin conditions that make direct skin testing impractical [20]. Because Fadal–Nalebuff mRAST provides specificity with only a small loss of sensitivity, a physician will be treating only specific IgE-mediated allergies. Fadal–Nalebuff mRAST results can aid in determination of immunotherapy starting point, alert the physician to potential systemic reaction allergens, and demonstrate allergens that might be treated more aggressively [21]. mRAST results in combination with SET results and a good understanding of SET can aid in determining a treatment plan for the allergic patient [22,23]. With RAST, false-negative results are possible and results take time to obtain from a laboratory.

With Fadal–Nalebuff mRAST results, a commercial laboratory prepares a three-vial, fivefold dilution treatment set with concentrations starting at a dose five times weaker than the patient's RAST score (or RAST –1) and escalating through RAST 0 to RAST +1. Special adaptations may include preparation of a four-vial fivefold set (RAST –2 to RAST +1) (Table 2). All positive results may be combined into one set or separated into two different sets by high and low RAST scores [21].

Sublingual–swallow drops can be prepared from RAST scores (Waickman, Personal communication, September 2000). A two-bottle fivefold dilution set can be prepared with build-up starting at RAST –1 and ending with RAST 0 (Table 3). The maintenance dose would continue at the maximum tolerated dose achieved during build-up.

Sublingual administration

Sublingual–swallow immunotherapy is administered as drops under the tongue and swallowed after 2 minutes. Food and drink should be restricted for 10 minutes after swallowing. The advantages of the drops include patient compliance and convenience. With the ease of administration, sublingual–swallow drops are less traumatic than injections for pediatric patients. This form of immunotherapy has fewer and less severe side effects [6•,24]. The higher cost of SLIT may be offset by the need for fewer supplies and less staff time required, as well as the socioeconomic time saved for the patient [11••,24]. However, because of the lack of a Current Procedural Terminology code,

most insurance companies do not currently reimburse for sublingual drops.

Vial safety test for immunotherapy injections

Before the initiation of SIT injections, it is essential that the prescription treatment set be tested with a vial safety test (skin challenge test) to ensure *in vivo* tolerance. The treatment set is tested whether it is prescribed for a new or current patient.

Using caution, physicians can successfully switch the preparation of immunotherapy from their office to a commercial laboratory or from one commercial laboratory to another by using the vial safety test. By providing the allergen's scientific name and weight-to-volume concentration, a duplicate vial could be made for the patient. Variables in different laboratories' extracts would be compensated for by performing a vial safety test before initiating treatment and adjusting the dosage downward, if needed.

The vial safety test is performed by applying approximately 0.02 to 0.04 mL of the vial contents intradermally to create a 4-mm wheal. After a 10-minute wait, the wheal is measured. If the wheal is 15 mm or less, the vial contents are presumed safe and dosing may proceed at the next visit [21].

If the vial safety test produces a wheal larger than 15 mm, then dosing should be evaluated by the physician. The physician may elect to make a fivefold dilution (or tenfold dilution for a tenfold treatment set) of the vial contents and perform a vial safety test of the diluted extract at a subsequent visit. With a successful vial

Table 3. Sublingual–swallow drops preparation

Testing	Vial set	Dilution	Concentration
SET	1-bottle set	5-fold	Per SET result
RAST	2-bottle set	5-fold	R –1, R 0

SET, skin endpoint titration; RAST, radioallergosorbent test.

Table 4. Emergency tray*

Epinephrine
Antihistamine (diphenhydramine)
Steroids
Albuterol inhaler
Ipratropium inhaler
H ₂ inhibitors
Dopamine
Heparin
Aminophylline
Phentolamine
Glucagon
Nitroprusside
Ammonia
Intravenous set-up/fluids
Tourniquet
Syringes/needles
Tracheotomy kit
Suction apparatus
Blood pressure cuff
Stethoscope
Oxygen
Written protocol

*Guideline only. Consult [25,26] for details.

safety test, build-up would proceed using the diluted extract at the next visit.

A physician should be present during the vial safety test and build-up of SIT injections. After any injection, a minimal 20-minute waiting period (longer for certain situations) is recommended by the American Academy of Allergy, Asthma, and Immunology, and the WHO [3•]. An emergency tray, and a written and practiced emergency protocol, should be available in case of possible systemic reactions (Table 4) [25,26].

Advantages of commercially prepared immunotherapy

Commercially prepared immunotherapy offers numerous advantages, services, and substantial cost savings to the physician. First, a physician's overhead costs for staff, space, and supplies will be reduced. Costs for office staff's initial training and continuing education are greatly reduced. The cost savings for the physician will be substantial when immunotherapy is prepared by a commercial laboratory. Consistency of ingredients and concentrations is ensured, even with staff turnover and absences. Staff time constraints will be minimized by commercially prepared prescription treatment sets. Immunotherapy can be individualized to accommodate any type of allergenic testing and special adaptations or can be duplicated if initially prepared elsewhere. An extensive variety of allergenic extracts are available for each physician's requirements in every geographical location. Orders may be mailed, faxed, or phoned in. Order forms may be provided. All orders will be verified for a current physician's signature, and the physician will be alerted when an updated signature is needed. Orders are verified by qualified technicians for clarity of results and prescription requirements to ensure the orders will be filled to the physician's specifications. Accuracy of prescriptions is ensured by a double-check system of two trained technicians, per FDA Code of Federal Regulations, Title 21, 211.188 and 211.194. Prescription treatment sets are made from sterility-tested allergenic extracts, per Code of Federal Regulations, Title 21, 680.3c. Prescription injections are tested for anaerobic and aerobic organisms. Sterility is confirmed before shipment, per Code of Federal Regulations, Title 21, 610.12. Because of the 14-day, FDA-required sterility hold, physicians need to place reorders in advance of the anticipated need. Shipment of prescription treatment sets is by a reliable, insured courier with overnight or second day air delivery. Prescription records and orders are kept on file to provide a tracking system for each individual patient and physician. Most laboratories have a computerized system to further assist tracking. A good quality laboratory will provide customer service to answer any questions regarding immunotherapy and prescription orders.

Conclusions

It has been estimated that as much as 30% of the general population has allergies in one form or another [27]. Although pharmacotherapy can reduce allergic symptoms, SIT and avoidance are the only methods capable of modifying allergic disease [3•,28]. With SIT, the development of asthma in patients with allergic rhinitis may be prevented [2•,3•]. SLIT may be a safe alternative to SIT for reducing allergic respiratory symptoms [8••]. Physicians at any level of medicine should keep in mind the prevalence of allergies and how they can affect a physician's practice [27]. A licensed commercial laboratory can assist the physician by providing quality immunotherapy products for the treatment of allergic patients.

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