

Letter to the Editor

Monitoring bacteriostasis in allergen extract mixing: 10 years of culture data

To the Editor:

There has been recent controversy regarding the safety of current allergen extract mixing practices for immunotherapy prescriptions.¹ Allergen extract vials are often mixed in physicians' offices by transferring stock extract into multidose patient-specific vials with an aseptic technique. Similar methods have been used safely for nearly 100 years, going back to the time of Noon and Freeman.² There are no reports in the medical literature of infections caused by immunotherapy injections.³ A retrospective study of immunotherapy records of 272 patients who received 26,795 injections over 6 years showed no evidence of infections caused by allergen immunotherapy injections.⁴

The United States Pharmacopeia regulates compounded sterile preparations and in January 2004 issued regulations that would include stringent requirements for allergen immunotherapy mixing (use of a laminar flow biologic safety cabinet, positive-pressure room, and storage limited to 7-14 days after immunotherapy mixing).¹ These requirements would have rendered immunotherapy impractical for the majority of patients.

The Joint Council of Allergy, Asthma and Immunology responded to these concerns, and standards for safely mixing allergen immunotherapy preparations were published in "Allergen immunotherapy: a practice parameter second update"³ and recently finalized.⁵ These standards include proper training of mixing personnel, oversight by physicians, inclusion of proper concentrations of glycerin or phenol for bacteriostasis, use of an aseptic technique, and refrigerated storage and that allergen immunotherapy is only intended for subcutaneous injection. Importantly, vials can be discarded according to the manufacturer's expiration dates (not 7-14 days after mixing), and there is no specific requirement for a flow hood or positive-pressure environment. The United States Pharmacopeia considered these guidelines during a public comment period and excluded allergen extracts from the more stringent standards of other compounded sterile preparations as long as certain standards are met, including sterile needle transfer of commercial allergen preparations, inclusion of appropriate bacteriostatic agents, use of patient-specific multidose vials, use of an aseptic technique with handwashing, and use of gloves, hair covers, gowns, face masks, and disinfection with alcohol.⁶

To provide further reassurance supporting the safety of our profession's current immunotherapy preparation mixing practices, we report the results of 10 years of bacterial cultures performed on allergen immunotherapy vials. At our institution, allergen extracts are mixed by using an aseptic technique in a laminar flow hood in a room without positive-pressure ventilation. Gloves are worn during mixing, and vials are wiped with alcohol pads. As part of quality assurance monitoring, 20 vials are cultured each month. At the time of mixing, 0.5 mL is removed from a patient's 1:1 vol/vol and 1:1,000 vol/vol vials and injected into separate collection tubes (6-mL sterile BD Vacutainer red-top blood collection tubes; BD, Franklin Lakes, NJ) by using an aseptic technique. These are stored at 4°C for up to 30 days and submitted to the laboratory in a batch for culture. The clinical microbiology laboratory then transfers the contents of each tube

to both a chocolate agar plate and a thio broth culture medium; therefore the sample collected from 1 vial is cultured by using 2 different methods. Both culture media are obtained from BD. The chocolate agar plates are cultured in a 5% CO₂ environment, the thio broth is cultured in a non-CO₂-enriched environment for 3 days at 35°C, and then results are reported. The chocolate agar is intended to culture for only aerobic organisms, whereas the thio broth cultures for both aerobic and anaerobic organisms.

A total of 2,085 cultures were done between 1998 and the present, and 2,084 cultures were negative. Only 1 culture from 1999 grew *Enterococcus* species (representing <0.05% of all cultures). No information was available on whether the positive culture extract had been administered to the patient. There have been no known cases of infections caused by allergen immunotherapy injections at our institution.

The frequency of positive cultures at our center is even lower than that reported by Lay et al.⁷ In this study bacterial contamination was compared between vials prepared either in an office setting by using an aseptic technique without a ventilation hood or under an International Organization of Standardization classification 5 vacuum-ventilated hood in the hospital pharmacy. Three hundred twenty vials were prepared in the office, and 217 vials were prepared in the pharmacy (total = 537 vials). Two (0.6%) of 320 office-prepared vials and 1 (0.5%) of 217 pharmacy-prepared vials showed positive cultures for bacterial contamination. The 3 patients who received injections from the contaminated vials experienced no clinical infection. Our rate of contamination (1/2085 [0.048%]) compares favorably with their overall rate (3/537 [0.56%]).

Mixing personnel at our center use an aseptic technique with gloves and alcohol pads under a laminar flow hood after proper handwashing but thus far have not worn gowns, face masks, or hair covers. Because our flow hood is conveniently available, we will continue to use it. However, a flow hood, gowns, face masks, and hair covers are not required by the present guidelines.⁵ Cultures are also not necessarily recommended but were begun 10 years ago at our center as a reasonable means of quality assurance. The current guidelines recommend that mixing personnel qualify at least annually by performing a media-fill test.

In conclusion, our nearly completely negative culture results over 10 years support the safety of our office-based allergen immunotherapy mixing practices at a tertiary referral center. Although most practicing allergists presumably do not use a flow hood for preparing allergen extracts, our data, combined with those of Lay et al.⁷ (who demonstrated a very low rate of bacterial contamination without a flow hood), support the current allergen immunotherapy preparation guidelines in use by the allergy community.⁵ The allergen extract cultures are an imperfect (because of unknown *in vivo* correlation) but reasonable and practical marker for bacteriostatic safety. Periodic cultures could be considered by any office or center preparing allergen extracts for subcutaneous immunotherapy as a method of ensuring quality assurance but are not required by the present guidelines (which recommend a media-fill test). Further studies performing cultures without a flow hood would further confirm the safety of mixing without a flow hood. It is especially reassuring that no clinical infections caused by allergen immunotherapy injections have ever been reported in the literature.

Adrian G. Letz, MD
Michael S. Tankersley, MD
John P. Dice, MD
Ronald W. England, MD

From the Wilford Hall Medical Center, Lackland Air Force Base, San Antonio, Tex.

E-mail: adrian.letz@lackland.af.mil.

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