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BACTERIOSTATIC AGENTS AND STERILITY REQUIREMENTS FOR ALLERGEN IMMUNOTHERAPY

Phenol and glycerin are common bacteriostatic preservatives added to allergen extracts. United States Pharmacopeia guidelines, Immunotherapy 2007 Practice Parameters, and the American Academy of Allergy Asthma & Immunology/American College of Allergy,

Asthma and Immunology/Joint Council of Allergy, Asthma and Immunology (JCAAI) guidelines do not specify recommended concentrations of phenol or glycerin.^{1–3} The Joint Council of Allergy, Asthma and Immunology and the American Academy of Otolaryngic Allergy (AAOA) have recommended that “allergen extract dilutions must be bacteriostatic, meaning 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 To our knowledge, no published studies support the use of phenol concentrations of at least 0.25%, or if less than that, glycerin concentrations of at least 20%. Limited studies have been published on the sterility of immunotherapy.⁵ Immunotherapy vials are multidose vials kept for up to 1 year, with the potential for multiple manipulations over that time. To our knowledge, no reports have been made of patients developing infections from immunotherapy injections. We investigated the potential for microbial growth in expired allergenic extracts previously used in patient care and studied the effects of using recommended and lower than recommended concentrations of phenol and glycerin on microbial growth in allergenic extracts.

Fifty expired immunotherapy vials from one institution were collected from patients undergoing immunotherapy. The phenol and glycerin concentrations in each expired vial were calculated. Albumin saline with 0.4% phenol (Allergy Laboratories, Oklahoma City, OK) was used for diluent. Phenol concentrations ranged from 0.12% to 0.4%, and glycerin concentrations ranged from 0% to 35% (Fig. 1). Forty-four vials contained greater than 0.25% phenol. Six vials contained less than 0.25% phenol but at least 20% glycerin. An additional 50 expired immunotherapy vials, from a different institution, were collected for culture purposes only. All 100 expired vials were sent for bacterial culture in thioglycolate broth, as well as in blood agar and anaerobic plates.

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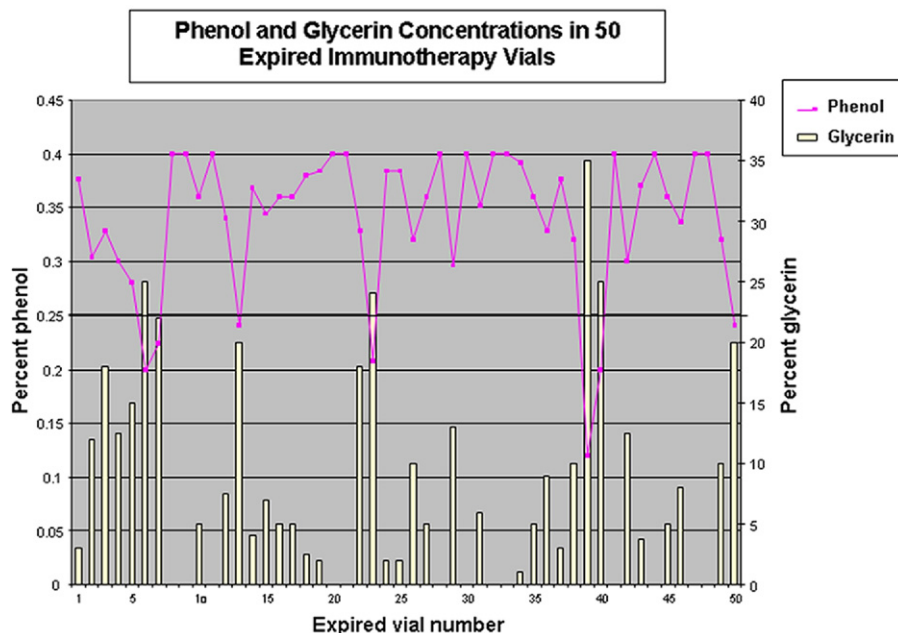


Figure 1. Phenol and glycerin concentrations in 50 of the expired patient immunotherapy vials. All expired immunotherapy vials met the JCAAI and AAOA recommendations of the use of at least 0.25% phenol, or if the phenol concentration was less than 0.25%, the extracts contained glycerin concentrations of at least 20%.

To study the effects on microbial growth of using lower than recommended concentrations of phenol and glycerin, two sets of experiments were set up in parallel with and without a laminar flow hood (Nuair Biologicals, Class II type AZ, Plymouth, MN). The first experiment was conducted with the use of a laminar flow hood with appropriate attire, including gown, mask, sterile gloves, and sterile alcohol. The second experiment was prepared on the bench top and included alcohol wipes of the vials and reagent bottles, with dilutions performed on the bench top. For each experimental setup, a sterile, 5-mL vial was prepared with 5 mL of preservative-free (Allergen Extract Diluent Injection preservative free, sterile, nonpyrogenic, isotonic, Mayo Pharmacy Services, Rochester, MN) diluent with the addition of varying concentrations of phenol from 0%, 0.05%, 0.1%, and 0.25% (albumin saline with phenol: NaCl 0.9%, normal serum albumin 0.03%, phenol 0.4%, from Allergy Laboratories Inc., Oklahoma City, OK) or glycerin from 0%, 1%, 5%, 10%, and 20% (sterile diluent for allergenic extract with glycerin 50% (wt/vol), sodium bicarbonate 0.091%, NaCl 0.166%, from ALK-Abelló, Inc., Round Rock, TX) with or without 1 mL of cat hair (1,000 bioequivalent allergy units) allergen extract (Standardized Cat Hair 5,000 bioequivalent allergy units/mL, ALK-Abelló, Inc., Round Rock, TX), for a total of 36 vials (18 using the hood, 18 on the bench top). The vials were stored at 4°C for a 1-month period. During this 1-month period, a total of 10 manipulations were performed to each vial on the bench top. An alcohol pad was swiped across the top of each vial, and a 27-gauge needle plus 1-mL syringe was used to puncture the tops of the vials and was allowed to contact the solution, simulating an allergy shot withdrawal. These punctures were performed every 3 to 5 days over 1 month.

The results showed that none of the 100 total expired immunotherapy vials showed microbial growth, and none of the 4 different concentrations of phenol or the 5 different concentrations of glycerin tested showed microbial growth. No difference was found between

hood or bench top preparation. Only the spiked positive control vials developed bacterial growth.

Based on our study, we conclude that one institution's 50 expired immunotherapy vials met the JCAAI and AAOA recommendations of at least 0.25% phenol or glycerin concentrations of at least 20%, and that current immunotherapy practice is highly effective at preventing microbial growth. Second, a sterile hood may not be necessary to ensure the sterility of allergenic extracts, and the sterility of immunotherapy extracts is preserved even with low concentrations of phenol or glycerin over a period of 1 month.

KARI ROSSOW, PA-C
MARY ANN BUTLER, BS
DAVID LOWE, MD
JAMES T. LI, MD, PHD

Division of Allergic Diseases
Department of Internal Medicine
Mayo Clinic
200 1st Street SW
Rochester, MN 55905
pearson.kari@mayo.edu

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