

PREPARATION AND MIXING OF AEROALLERGEN VACCINES

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The term *immunotherapy* was introduced by Norman and Lichtenstein⁶ to designate the treatment of allergic respiratory disease by injections of extracts of vaccines of the antigens responsible for illness. The usage of the term has been extended to similar treatment in the therapy of symptoms owing to certain drug reactions and allergic reactions to insect stings. In practice, immunotherapy consists of the injection of progressively larger amounts of antigen (allergen) until a predetermined dose is reached, symptoms are alleviated, or the patient's tolerance to the injected amount is increased. The intent of this article is to discuss the practical aspects of the preparation of allergen vaccines. No attempt has been made to discuss the mechanisms of action or the efficacy of this form of therapy.

This article focuses on vaccines for airborne allergens. The authors discuss the potency of individual vaccines and the factors that may alter potency. In addition, the authors describe standardized allergen vaccines. Practical suggestions are offered for vaccine preparation and storage.

POTENCY OF ALLERGEN EXTRACTS

The potency of allergen vaccines is dependent upon multiple factors (Box 1). The nature of the allergen source and the physicochemical properties of the raw material affect the potency of the allergen vaccine. Most vaccines of airborne allergens are prepared with a buffered aque-

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ous solution extraction of source material. Selected allergens are more water soluble, resulting in higher concentrations of the allergens in solution. For example, grass or weed vaccines are more potent than tree, mold, or dust mite vaccines.

Box 1. Factors Affecting Potency

- Nature of allergen source
- Storage conditions
- Dilution
- Mixing of extracts

Potency also may be dependent on physical storage conditions such as temperature. Allergen vaccines degrade much quicker at elevated temperatures.

The addition of excipients, such as glycerine, phenol, or albumin, to allergen vaccines may enhance stability. In addition, mixing of vaccines may positively and negatively affect potency. The mixing of protease containing allergens (e.g., molds) and other allergens (e.g., grasses) may lead to more rapid deterioration of the latter.

NATURE OF ALLERGEN SOURCE

The nature of the allergen may affect the potency and quality of vaccines. The pollens of weeds and grasses are more potent than trees, molds, and dust mites owing to water solubility. Pollens must be collected under optimal conditions to decrease microbial contamination or other allergen introduction. For optimal preparation of allergen vaccine, harvests from 3 different years are blended to minimize effects of the growing season and climate.⁷

Fungal allergens are complex, and fungal vaccines are more variable than pollen vaccines. The reasons for this variability include heterogeneity among the same species, difficulty in accurate identification of a species, variability in allergen content caused by effects of growth conditions, and differences between spore and mycelial material in water solubility.^{1,7} Identification of the major allergens from *Alternaria*, *Cladosporium*, and *Aspergillus* should facilitate producing consistent fungal vaccines.

The stability of mite allergens also varies. *Dermatophagoides farinae* allergens of the major two groups, designated Der f 1 and Der f 2, are more stable than the equivalent major allergens of *D. pteronyssinus*, Der p 1 and Der p 2.³ Der f 1 is the most stable, maintaining potency for up to 3 years when stored at 4°C. Der p 1 and Der p 2 are not stable, even with refrigeration. There are no data available documenting a relationship between this instability and an effect on clinical efficacy, but the amount of allergen administered in a vaccine has been shown to affect efficacy.

PHYSICAL STORAGE

Physical storage may alter the potency of allergen vaccines. Vaccines vary with respect to stability over time and over temperature ranges. Degradation of allergen occurs at extremes of temperature. At room temperature loss of allergen potency occurs within weeks whereas at increased temperatures, more than or equal to 35°C, this process occurs within days. Refrigeration of vaccines is essential. Having vaccines at room temperature during routine daily practice probably does not significantly affect allergen potency as long as the vaccines are refrigerated when not being used.

Most allergens are glycoproteins with differing heat lability. Thus, temperature will have variable effects on allergen potency. Cat vaccine is relatively heat stable and is resistant to the effect of higher temperature, whereas pollens of trees, grasses, weeds, and dust mites lose potency at extremes of heat. Temperature susceptibility has been attributed to heat-labile proteins and the activation of proteases in these vaccines. The latter results in autodegradation by way of proteolysis.

The effect of increased temperature on Group 1 and Group 2 allergens in mite extracts has been assessed by measuring epitope stability in an ELISA with monoclonal antibodies.³ Samples were stored at 4°C, 26°C, 37°C, and 50°C. The potency of the allergen vaccine decreased within 3 months of storage at the higher temperature (Table 1). In contrast, storage at -20°C preserved allergen potency more effectively than 4°C storage (Table 2). Freezing of other allergen vaccines has not been studied.

DILUTION

In addition to time and temperature, the dilution of allergens may affect potency. Potency is generally proportional to the concentration of the allergen vaccine. Thus, the more dilute vaccines are less potent. The decrease in potency has been attributed to the adherence of allergen to the glass vial. Human serum albumin (HSA) may be added to the vaccines to limit this effect.

Table 1. RELATIVE POTENCIES OF MITE EXTRACTS STORED AT DIFFERENT TEMPERATURES

Extract	Storage (mo)	Relative Potency			
		4°C	26°C	37°C	50°C
E5-Df	6	1*	0.9	0.7	0.1
E5-Dp	6	1	0.9	0.6	0.1
	24	1	1.0	0.3	
	30	1	0.9		

*Sample stored at 4°C was used as reference and assigned as 1.

From Liu T, Lin Y: The epitope stability of Group 1 and Group 2 allergens in mite extracts. *Ann Allergy Asthma Immunol* 80:117-183, 1998; with permission.

Table 2. DER P 1 AND DER P 2 CONTENTS IN E5-DP AFTER 30 MONTHS OF STORAGE AT 4°C AND -20°C

Reference Extract	Mite Allergen	Allergen Concentration ($\mu\text{g/mL}$)*		
		At Zero Time	After 30 Months	
			4°C	-20°C
E5-Dp	Der p 1	20.7	6.3	11.6
	Der p 2	85.4	15.0	30.0

*Average of at least three determinations.

From Liu T, Lin Y: The epitope stability of Group 1 and Group 2 allergens in mite extracts. *Ann Allergy Asthma Immunol* 80:117-183, 1998; with permission.

Nelson et al⁵ studied the effect of dilution on the potency of allergen vaccines. Grass and cat vaccines lost potency with dilution faster than ragweed vaccine.⁵ These investigators recommended reconstituting preparations of stock vaccines used for intradermal testing and maintenance injections at 3-month intervals.

STABILIZERS

Although lyophilization is the best technique for maintaining vaccine potency, preservatives also can be effective as stabilizing agents. Glycerol is the most commonly used stabilizer. The ideal concentration of glycerol in extracts is between 10% and 25%. Concentrations of 50% glycerol are effective but may be irritating, resulting in a burning sensation, and are associated with false-positive results on intradermal testing.² Normal saline and sodium bicarbonate solutions have not been effective in stabilizing allergen vaccines. HSA also stabilizes allergen vaccines.

The effect of mixing allergens was studied by Nelson et al.⁵ Most vaccines were susceptible to deterioration, with the exception of *D. farinae*, which was stored in a 25% glycerin concentration. The glycerin protected the mite allergen from degradation by proteases of other allergens present in the mixture.⁵ Allergen vaccines that contain proteases are listed in Box 2.

Box 2. Allergen Vaccines that Contain Proteases

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| <ul style="list-style-type: none"> • Insects/Mites <i>Dermatophagoides pteronyssinus</i> <i>Dermatophagoides farinae</i> <i>Periplaneta americana</i> | <ul style="list-style-type: none"> • Fungi <i>Alternaria alternata</i> <i>Penicillium notatum</i> <i>Cladosporium herbarium</i> <i>Fusarium moniliforme</i> <i>Aspergillus fumigata</i> |
|---|---|

Because mold and mite extracts contain proteases it would seem best to segregate these from pollens of trees, grasses, and weeds. There is limited data available in this area. In a study by Nelson et al,⁵ however, mixing of these vaccines did result in a loss of potency. *Alternaria* significantly decreased the potency of timothy and Bermuda grasses, with no effect on the potency of short ragweed. *Penicillium*, *D. farinae*, and *D. pteronyssinus* did not affect the potency of any of the extracts. Allergen vaccines with protease activity, such as cockroach, *Alternaria*, *Cladosporium*, and *Penicillium*, did not lose potency as a result of mixing. These investigators concluded that pollen vaccines, with the exception of ragweed, should not be mixed with *Alternaria*, *Cladosporium*, or cockroach unless glycerin was added.

STANDARDIZED VACCINES

Allergy vaccines vary in potency. This variability is limited with the use of standardized vaccines. These vaccines offer reproducibility and the ability to compare skin test responses of different patients and the same patient over time.^{4*} The standardization of allergen vaccines is regulated in the United States by the Center for Biologics Evaluation and Research of the Food and Drug Administration. The vaccines may be standardized by the content of major allergens, for example, cat and short ragweed. Allergen vaccines also may be standardized by intradermal testing in selected patients using vaccine dilutions. Using this method, vaccines are assigned a bioequivalent allergy unit based on the erythema diameter of 50 mm. Grasses and dust mites have been standardized using this method.

Standardized vaccines facilitate consistent, safe dosing of immunotherapy. The use of standardized vaccines will limit problems resulting from the transfer of immunotherapy from one physician to another and from the preparation of replacement vaccines during the course of treatment. Studies of the efficacy of immunotherapy are facilitated with standardized vaccines, permitting more accurate determination of a minimally effective dose. Finally, monitoring and comparison of the degree of allergic sensitivity may be feasible because test results from different time points would be comparable.

PRACTICAL CONSIDERATIONS

Safe administration of a potent allergen vaccine is essential for effective immunotherapy. Key factors to consider include:

- Standardized extracts should be used when available.

- Safe but effective doses must be employed.

- Vaccines should be refrigerated to maintain potency.

- Allergen vaccine exposure to room temperature should be limited.

Potency may be enhanced by not mixing allergens such as trees, grasses, and weeds (with the exception of ragweed) with fungi, cockroaches, and mites, which contain proteases.

10% to 25% glycerol can enhance allergen stability.

Stock vaccine dilutions used for intradermal testing and maintenance injections should be prepared every 3 months.

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