

Allergen stability of testing/treatment boards and immunotherapy vials with various diluents

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Background: Otolaryngologists commonly use glycerin and normal saline with phenol (NSP) in diluting solutions to help preserve allergenicity in immunotherapy vials. Studies have shown that diluting with human serum albumin (HSA) may provide better allergen stability. The purpose of this study was to assess the ability of various diluents to preserve allergen content in testing/treatment boards (TTBs) and immunotherapy treatment vials (ITVs) at multiple time points.

Methods: TTBs with 4 allergens were prepared with HSA, NSP, 10% glycerin, and 50% glycerin. The major allergen content of the TTBs was measured at creation (time 0), 3 days, 8 weeks, and 18 weeks. Multiallergen ITVs containing the 4 allergens were prepared from the NSP board (diluted to 10% glycerin) and the HSA board (diluted in HSA) at the time of making the freshly prepared TTBs and again 8 weeks later, simulating the creation of ITVs from a "new" and an "old" TTB. The major allergen content of these ITVs was determined at creation and at interval time points thereafter.

Results: TTBs and ITVs showed a substantial loss of allergen at day 3. The loss of allergen was more pronounced in the more dilute (#4, #5, and #6) vials. HSA and 50% glycerin showed superior allergen preservation compared to NSP and 10% glycerin in TTBs. HSA showed superior allergen preservation compared to 10% glycerin-NSP in ITVs.

Conclusion: The use of HSA as a diluent in TTBs and ITVs showed superior allergen preservation compared to NSP and 10% glycerin, particularly for more dilute vials. © 2015 ARS-AAOA, LLC.

Key Words:

allergen immunotherapy; human serum albumin; stability; preparation; glycerin

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Immunotherapy for allergic disease is defined as the gradual administration of increasing amounts of allergen to which the patient is sensitive, for the purpose of modulating the allergic immune response to that allergen and alleviating allergic symptoms.¹ Subcutaneous allergen immunotherapy (SCIT) is a specific disease-modifying practice that can

provide long-term benefits to patients whose allergic symptoms have not been effectively controlled with avoidance and pharmacotherapy.² Patients undergoing SCIT are injected with complex mixtures of allergen extracts derived from source materials that correspond to their clinical sensitivities determined by allergy testing.³

One important consideration for providing effective SCIT is the ability to deliver the intended allergen dose in a consistent manner. Multiple factors such as storage temperature, age, diluent, and enzymatic activity can affect the stability of the allergen content of allergy testing and immunotherapy solutions.⁴⁻⁷ Scientists from the U.S. Food and Drug Administration (FDA) have defined allergen stability as "the persistence of adequate quantities of relevant antigens in an allergen vaccine from the time of initial assay to the time of clinical use."⁸ The FDA, however, does not regulate extract expiration dating for allergen vaccines diluted for immunotherapy.

Glycerin, at concentrations at or approaching 50%, has long been known to be a very effective preservative. Allergen extracts prepared by commercial allergen supply

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companies are most commonly preserved in 50% glycerin solutions. Unfortunately, 50% glycerin commonly produces unacceptable local irritation when injected subcutaneously, so it is not a practical preservative for intradermal testing and injection immunotherapy products. Various other sterile diluents are used to prepare allergen solutions used for allergy testing and immunotherapy vial (ITV) production, and these solutions may be stored for varying times.⁹ There are several options for diluents, each with their advantages and disadvantages. A very commonly used diluent in the production of allergy testing/treatment boards (TTBs) and ITVs in the specialty of otolaryngology is normal saline with phenol (NSP). Human serum albumin (HSA) is a naturally occurring plasma protein that has shown good preservation of allergens, likely through decreased adsorption of allergen to the glass vial wall and/or acting as a protein carrier.^{5,10} Many allergen solutions contain natural enzymes that can degrade the allergen protein in the solution over time. HSA may provide an alternate protein source to compete with allergen proteins for interaction with these enzymes, and therefore reduce the degradation of the desired allergen proteins.

It is common practice among otolaryngologists to create TTBs to be used for intradermal skin testing and creation of ITVs. TTBs are commonly kept in use for 6 to 8 weeks before they are discarded and new boards are created. During this timeframe, skin testing and ITV creation may be performed early in the treatment board lifespan, with fresh allergens, or toward the end of the treatment board lifespan, when there may be significant degradation of the allergen content. This could potentially result in significant variation in the allergen content of the product used for testing, and for delivering immunotherapy. This variation could have implications for the accuracy of skin testing, and the effectiveness/safety of immunotherapy.

The present study was designed to assess the stability of allergen extracts diluted in HSA, NSP, 10% glycerin, and 50% glycerin. Specifically, we assessed the stability of allergens in TTBs using the various diluents and the allergen content of ITVs created from “new” and “old” TTBs.

Materials and Methods

Allergenic Extracts

The following extract concentrates in 50% glycerin were provided by ALK Abelló, Inc. (Round Rock, TX): Timothy grass (*Phleum pratense*), 100,000 bioequivalent allergy unit (BAU)/mL; white birch (*Betula verrucosa*), 1:20 wt/vol; mixed dust mite (*Dermatophagoides pteronyssinus* and *farinae*), 10,000 allergy unit (AU)/mL; and cat hair, 10,000 BAU/mL. White birch was the only nonstandardized extract. Extracts were stored at 1°C to 5°C, and all bulk concentrate extract lots were mixed and tested within their expiration dates.

Diluents

Extract dilutions were made (see sections below for details) with the following 4 diluents: (1) NSP = normal saline (0.9% sodium chloride) + 0.4% phenol; (2) HSA = 0.3% human serum albumin + normal saline (0.9% sodium chloride) + 0.4% phenol; (3) 10% Gly = 10% glycerin + 0.4% phenol; and (4) 50% Gly = 50% glycerin. NSP, HSA, and 50% glycerin were from ALK, and 10% glycerin was from Greer (Lenoir, NC).

TTB Creation

A TTB was created using each of the above diluents using the typical 1:5 serial dilutions as described by King et al.⁹ One mL of concentrate was added to a vial containing 4 mL of diluent and labeled vial #1. One mL of vial #1 was added to another 4 mL of the diluent and labeled vial #2. This was repeated 4 more times resulting in vials #3 to #6. This resulted in the creation of 6 vials per allergen, for a total of 24 vials per diluent.

ITV Creation

One set of ITVs was made using the 4 allergens mixed with fresh NSP or HSA TTBs, simulating the creation of ITVs from a “new” TTB. The creation of the NSP ITVs proceeded as follows: 0.2 mL from each #1 allergen vial on the NSP TTB (0.8 mL total) was placed in a sterile empty vial labeled vial #1, then 3.2 mL of NSP and 1 mL of 50% Gly were added. Next, 0.2 mL from each #2 allergen vial on the NSP TTB (0.8 mL total), 3.2 mL of NSP, and 1 mL of 50% were placed in a sterile empty vial labeled vial #2. This was repeated for each dilution on the NSP TTB, resulting in creation of 6 ITV mixes. 10% GLY-NSP = 10% glycerin in normal saline with phenol.

The creation of the HSA ITVs proceeded as follows: 0.2 mL from each #1 allergen vial on the HSA TTB (0.8 mL total) was placed in a sterile empty vial labeled vial #1 and 4.2 mL of HSA was added. Next, 0.2 mL from each #2 allergen on the HSA TTB (0.8 mL total) was placed in a sterile empty vial, followed by 4.2 mL of HSA. This was repeated for each dilution on the HSA TTB, resulting in creation of 6 ITV mixes.

A second set of ITVs was created from the NSP and HSA TTBs after 8 weeks of storage at 1°C to 5°C, simulating the creation of ITVs from an “old” TTB. The second set of ITVs was created in the same manner as the first.

Analytical Methods

Monoclonal direct binding enzyme-linked immunosorbent assays (ELISAs) were used for measuring allergen proteins. These ELISAs allow for the detection of nanogram amounts of specific protein.⁴ Major allergens Timothy Phl p 5 (Ramírez et al.¹¹), mite Der p 1 (Ventas et al.¹²), cat hair Fel d 1 (Ventas et al.¹²), and birch Bet v 1 (Ramírez et al.¹³) were measured in each vial. The assays are performed by immobilizing specific monoclonal antibodies

		Dilution 1 1:5v/v			Dilution 2 1:25v/v			Dilution 3 1:125v/v			Dilution 4 1:625v/v			Dilution 5 1:3,125v/v			Dilution 6 1:15,625v/v		
		3d	8 wk	18wk	3d	8 wk	18wk	3d	8 wk	18wk	3d	8 wk	18wk	3d	8 wk	18wk	3d	8 wk	18wk
Timothy Grass (Phl p 5)	HSA																		
	50% GLY	84	38		92	31		68	31		64	33		46	51	26	44	45	22
	NSP	133	62		130	59		84	29		75	26		48	26	4	32	0	0
	10% GLY	94	40		89	29		64	22		41	5		10	10	0	7	0	0
Dust Mite (Der p 1)	HSA	102	47		85	35		88	17		30	7		15	2	0	9	0	0
	50% GLY																		
	NSP																		
	10% GLY																		
Cat Hair (Fel d 1)	HSA	90	118		102	95		90	74	70	91	85	63				89		
	50% GLY	86	103		116	117		93	84	84	100	85	73				70		
	NSP	106	105		87	62		54	35	14	24	6	0				0		
	10% GLY	97	109		83	95		60	48	37	51	6	0				0		
White Birch (Bet v 1)	HSA																		
	50% GLY	100	100		89	89		100	94	76	117	79	85				98		
	NSP	91	112		92	102		90	81	83	85	48	56				24		
	10% GLY	95	104		81	81		49	58	57	24	30	22				0		
White Birch (Bet v 1)	HSA	91	81		89	63		65	115		69	85		58	76	61	58	78	66
	50% GLY	91	79		90	71		62	88		45	74		53	0	8	29	0	0
	NSP	85	73		65	43		27	31		12	0		0	0	0	0	0	0
	10% GLY	81	79		61	46		33	35		11	0		4	0	0	0	0	0

FIGURE 1. Potency of individual vials from Testing/Treatment Boards. The percent of remaining allergen was calculated by comparing the amount found in the vial to the expected amount determined from the concentration in the bulk concentrate and dilution factor. The sensitivity of the assays are about 1 ng/mL and for Birch and Timothy extracts allergen content in the number 6 vial can be measured. Content of major allergen in dilution 6 for cat and mite extracts fall below the level of detection therefore values are absent. Two standard deviation assay range was used as basis of the color coding. Green = $\geq 70\%$; White = 69–36%; Peach = 35–11%; Red = $\leq 10\%$.

onto microtiter plates. Extracts are added to the wells and following incubation to allow the major allergen to bind to the antibody, the plates are washed and a secondary specific antibody is added to detect the bound allergen protein. Assay quantification is done with calibrated purified major allergen proteins. The standard curves show dose response with a limit of detection of around 1 ng/mL. This allows measuring up to 6 vials with 5-fold dilutions (1:15,625 vol/vol) for most birch and Timothy pollen extracts. The level of Der p 1 in mite and Fel d 1 in cat extracts allows detection only in vial #5. These assays were validated and precision was shown to average 14% relative standard error (RSE).

The amount of major allergen in micrograms per milliliter ($\mu\text{g/mL}$) in each individual concentrate vial was determined. Because the precision of these biological immunoassays is 14% RSE, the average value was calculated from multiple determinations, which ranged from 3 (for Der p 1) to 11 (for Fel d 1). Each of these concentrate vial values was multiplied by the appropriate dilution factor to give the amount of allergen in a given vial at the time of creation. For example, an average of 25.6 $\mu\text{g/mL}$ of Fel d 1 was obtained from assaying the cat hair concentrate. To calculate the amount of Fel d 1 in the #4 vial (1:625) of a TTB at the time of creation, 25.6 $\mu\text{g/mL}$ was multiplied by 1/625, giving a value of 0.041 $\mu\text{g/mL}$ of Fel d 1 in the #4 TTB dilution. This method was used to calculate the amount of major allergen in all vials at the time of making the TTB dilutions.

Allergen content was then measured at day 3, week 8, and week 18.

Day 3

ELISAs were run on the most dilute TTB concentrations that could be measured. Major allergen ELISAs for the first set of ITVs were run.

Week 8

All TTB vials and set 1 ITVs were analyzed by the allergen ELISAs.

Week 18

The TTB vials (18 weeks old), set 1 ITVs (18 weeks old), and set 2 ITVs (10 weeks old) were analyzed by the ELISAs.

Statistical Analyses

Validation of the major allergen ELISAs has determined an RSE (standard deviation/mean $\times 100$) of 13% for Der p 1 and Fel d 1, to 14% for Phl p 5, and 15% for Bet v 1, resulting in an overall average of 14%. This assay precision is typical of biological immunoassays. Assay precision error bars reflecting the RSE are shown in the graphical analysis. A 2 times standard deviation (or a $\pm 30\%$ range) representing approximately 95% of expected values was used to show significant decline during the stability testing. Values outside of this range reflect changes in the microgram amount in vials. Values within the $\pm 30\%$ are colored dark gray in Figure 1.

Results

TTBs

TTB dilutions created with HSA and 50% Gly consistently show higher allergen preservation regardless of the specific allergen. Figure 1 shows the percentage of expected major allergen content derived from the analysis of the concentrate bulk vials. The individual allergen results at week eight are shown graphically in Figure 2A-D. In vials #1 to #3, all diluents across all allergens showed relatively good major allergen potency, with most values being greater than or approaching 40% preservation. However, at the #3 vial, HSA and 50% Gly began to show better stability, except for Timothy vials at 18 weeks. For example, in the #3 dilution of dust mite at 8 weeks (Fig. 2D), HSA and 50% Gly retained 74% and 84% of the allergen Der p 1, respectively,

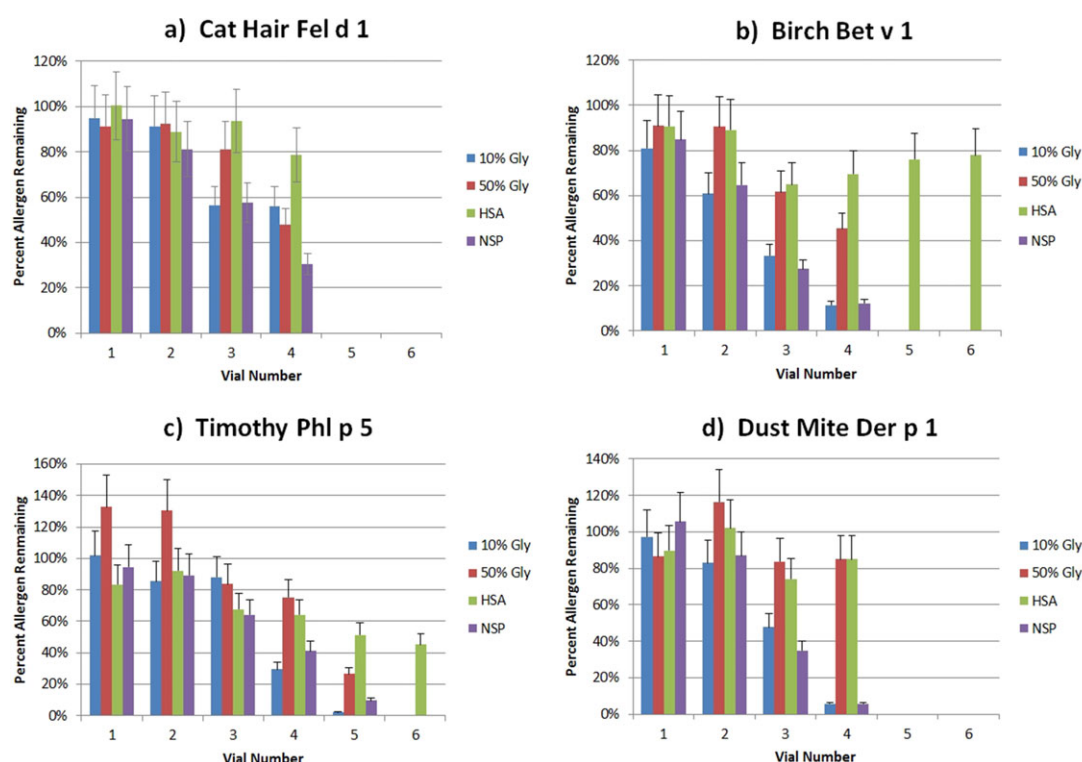


FIGURE 2. (A-D) Testing/treatment board major allergen content after storing for 8 weeks at 1°C to 5°C. Allergen content was determined using monoclonal-based ELISAs. Error bars are relative standard error. ELISA = enzyme-linked immunosorbent assay; Gly = glycerin; HSA = human serum albumin; NSP = normal saline with phenol.

whereas 10% Gly and NSP only retained 48% and 35%, respectively. In dilutions #4 to #6, very large differences in allergen content were noted between HSA and 50% Gly when compared to 10% Gly and NSP. For example, in the #4 dilution of white birch at 8 weeks, HSA and 50% Gly retained 69% and 45% of the allergen, respectively, whereas 10% Gly and NSP only retained 11% and 12%, respectively. The loss of allergen in these more dilute solutions was substantial. At 8 weeks in the #5 dilutions of white birch and Timothy grass, allergens diluted with 10% Gly and NSP were either undetectable or had <10% remaining.

Allergen deterioration in the non-HSA vials appears to happen very quickly. At day 3, only 24% of the cat hair major allergen was left in the NSP #4 vial. This pattern seemed to be the most substantial in the NSP vials. The percentages recovered at 3 days ranged from 0% to 54%, with more dilute solutions having much more allergen loss. The 10% Gly also shows substantial loss in diluted vials. Birch and Timothy retain only 4% and 15% major allergen in the #5 vial. Timothy seems to be less stable than the other allergens at week 18 with all dilutions and diluents showing losses greater than 50%, except the #1 and #2 vials of 50% glycerin.

ITVs

Overall, the ITVs showed a similar pattern to the TTBs in that there was a more significant loss of allergens in the more dilute vials regardless of the diluent. HSA consistently

proved to be a superior diluent in comparison to 10% Gly-NSP, especially in more dilute vials.

Set 1-Day 3

Table 1 shows the percentage of major allergen content still present after being stored for 3 days compared to the amount of major allergen content expected to be present based on the initial stock vial allergen content divided by each vial's dilution factor. The mean percent recovery was calculated from the combined results for each allergen Figure 3A. A substantial loss of allergen was noted in set 1 ITVs at day 3 in both HSA and 10% Gly-NSP vials, although the loss was greater in the 10% Gly-NSP vials. This illustrates a very rapid loss of allergen after creation of ITVs. Once again, HSA showed greater stability of allergens in more dilute solutions. For example, in the white birch ITVs only 19% of allergen remained in the 10% Gly-NSP #4 dilution while 69% of allergen remained in the HSA #4 vial dilution.

Set 1-Week 8

The amount of allergen remaining in the vials was relatively similar in comparison to amounts measured at day 3. HSA continued to stabilize the allergen proteins in more dilute solutions. For Timothy grass and white birch, no detectable allergen remained in the 10% Gly-NSP #5 vials (Fig. 3B).

TABLE 1. Major allergen content of individual treatment vials^a

	Allergen remaining after 3 days (%)											
	10% Glycerin in NSP						Human serum albumin in NSP					
Allergen	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6
Timothy Phl p 5	55	48	34	26	12	0	59	60	55	45	45	26
Birch Bet v 1	–	65	30	19	0			73	72	69	68	49
Mite Der p 1	130	103	74	42	–	–	120	107	93	96	96	–
Cat Fel d 1	122	87	73	55	–	–	121	110	100	104	–	–
Average	102	76	53	35	6	0	100	87	80	78	70	38

^aVials were mixed from the corresponding dilutions of each allergen from the treatment boards. After storing for 3 days the major allergen content was measured and the percent recovery calculated from the concentrate content divided by the dilution factor. Missing data in vials 1, 5, and 6 are because of values below the level of detection in mites and cat and invalid data in birch.

NSP = normal saline with phenol.

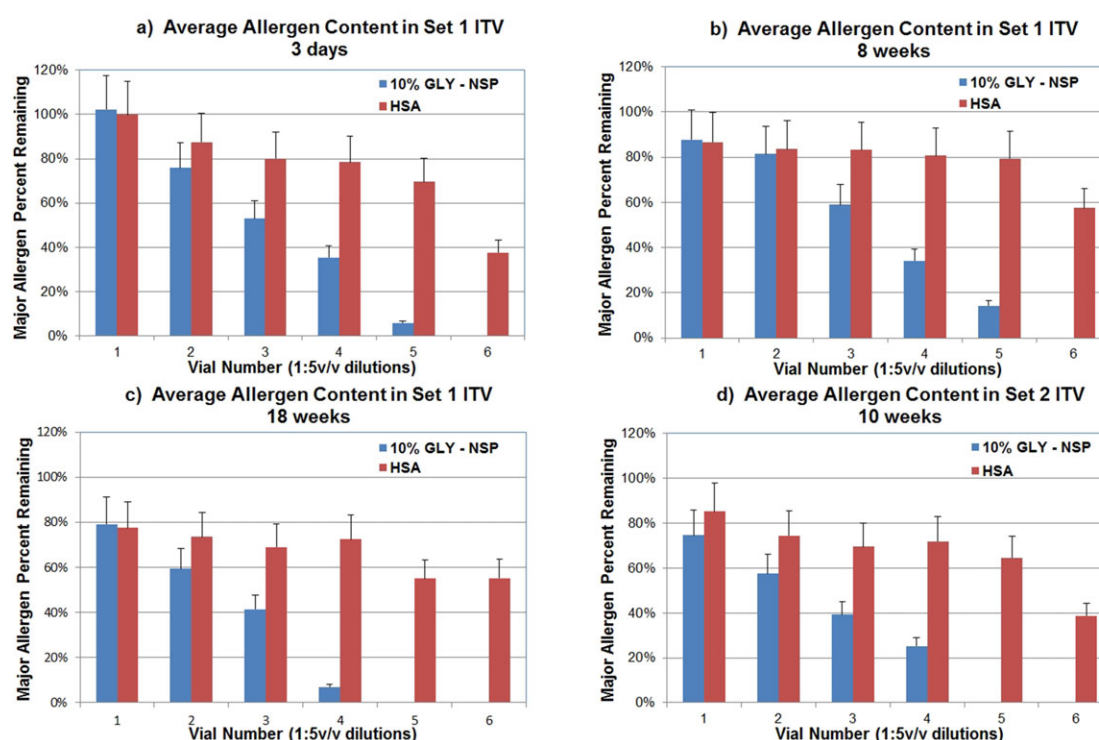


FIGURE 3. Individual patient vials allergen content. Vials were mixed from the corresponding dilutions of each of the 4 allergens. Major allergens for each extract were measured at (A) 3 days, (B) 8 weeks, (C) 18 weeks, and (D) 10 weeks after mixing a new patient vial from an 8-week-old treatment board. The average percent remaining of the expected major allergen content was calculated from the stock concentration divided by the dilution factor. The graphs show the average percent remaining of all 4 allergens. 10% GLY-NSP = 10% glycerin in normal saline with phenol; HSA = human serum albumin; ITV = immunotherapy treatment vial.

Set 1-Week 18

The losses in comparison to week 8 data were surprisingly modest. HSA showed excellent allergen stability at Week 18 (Fig. 3C).

Set 2-Week 10

The second set of ITVs made from the “old” (week 8) TTB was analyzed after 10 weeks of cold storage (Fig. 3D). The vials diluted with HSA contained approximately 70% to

80% of the expected allergen content for dilutions 1 to 4. For the same dilutions, NSP-diluted vials contained approximately 25% to 75% of the expected allergen, with a near linear decrease from dilution #1 to dilution #4. In general, the 10% Gly-NSP ITVs showed a substantial difference in the amount of allergen in the set 1-week 8 and set 2-week 10 vials, whereas HSA ITVs showed a less substantial difference. The general pattern of HSA superiority in allergen stability, especially in more dilute vials, was consistent in set 1 and set 2 vials.

Discussion

Various sterile diluents are available to prepare extract dilutions and these dilutions may be stored for varying amounts of time. A common diluent used in TTBs among otolaryngologists is NSP.⁹ 0.4% Phenol acts as an antibacterial, but does not stabilize proteins. It has been the typical practice of otolaryngologists to assume a shelf life of 6 to 8 weeks for these TTB solutions.⁹ When mixing ITVs, it is common practice among otolaryngologists to use NSP as the main diluent, with the addition of sufficient amounts of glycerin to obtain a final solution with approximately 10% glycerin concentration.⁹ In practice, these vials are assumed to maintain potency for about 3 months.

Skin testing is the most commonly used method of identifying type I, immunoglobulin E (IgE)-mediated allergy. Skin testing can give both qualitative and quantitative information about allergen sensitization. Intradermal delusional testing (IDT) and modified quantitative testing (MQT) are the most commonly used skin testing techniques to produce both qualitative and quantitative results. Many otolaryngologists base the starting dose of immunotherapy on the degree of sensitization as determined by IDT or MQT testing. Accurate results from MQT and IDT testing logically require that the various dilutions in the testing vials have relatively consistent concentrations of the allergen being tested. Single intradermal allergy tests are also commonly used to identify sensitivities to suspicious allergens when initial skin-prick testing is negative. Single intradermal tests also require administering a predictable range of allergen concentration for reliable interpretation of results. Conventional injection immunotherapy regimens typically include an initial escalation phase, when the allergen dose is increased until a targeted maintenance dose or a maximum tolerated maintenance dose is reached. Maintaining allergen potency in TTB solutions used for IDT, MQT, and during the escalation phase of immunotherapy is important for obtaining accurate results, and for safety.

The potential for biologically active allergen degradation during the 6-week to 8-week lifespan of a TTB allows for potential inaccuracy and safety concerns during allergy testing and immunotherapy escalation. Obtaining accurate quantitative results from IDT and MQT testing is based on utilizing a defined allergen concentration for testing. If there is significant degradation of allergen during the lifespan of a TTB, tests performed with solutions from a freshly created TTB could produce different endpoint results compared to tests performed from solutions drawn from the same TTB after 8 weeks of storage. Because the practitioner is basing the starting dose of immunotherapy on the testing endpoint, this could present the possibility of safety concerns. If there has been significant degradation of allergen content, the testing could potentially produce a falsely lower endpoint (endpoint from a less dilute vial), suggesting a lesser degree of sensitivity.

The most significant risk factor for a systemic reaction during immunotherapy is injections during the escalation

phase.¹⁴ During escalation immunotherapy, safety is based on slowly increasing the amount of allergen delivered with each injection. As the immunotherapy injections transition from a lower-concentration to a higher-concentration vial, it is important that there is a relatively predictable step-up in allergen content between the 2 vials. There are 2 particular potential sources of inaccuracy in the assumed steady allergen increase. First, immunotherapy escalation commonly involves starting with a 0.05-mL initial dose from a vial, followed by 0.05-mL incremental increases in dosing until a 0.5-mL dose is achieved. This typically would allow 10 doses (over 10 weeks) from a particular ITV, before advancing to the next higher concentration vial. If there is significant degradation of allergen in a treatment vial during that 10-week period of time, the final injection from that vial, before moving onto the next concentration, may not have the anticipated amount of allergen. In other words, as the allergen degrades, the actual escalation of allergen may not actually occur, because even though a higher volume of solution is given, the amount of biologically active allergen in the injected solution may actually be stable or decreasing. When the patient is transitioned to the next vial, there could potentially be a large jump in actual allergen amount being delivered in the next injection. This could be particularly true if the first vial is created at the end of a TTB lifespan (therefore potentially created from a solution that has already lost significant allergen content before the treatment vial is even produced), and then the next more concentrated vial is produced created from a freshly created TTB (which would not yet have had significant allergen degradation). This could potentially be a cause of the sudden large local reactions sometimes encountered in patients who have previously had no significant reactions during the escalation process. To further compound this potential, this study shows that higher-concentration solutions do not degrade as much as lower-concentration solutions. Therefore, as the escalation transitions from a more dilute solution to a higher-concentration solution, there could be even more potential for unanticipated large increases in actual allergen content in the injection.

Several articles over the last 3 decades have contributed to scientific knowledge concerning allergen stability with various diluents. Nelson and colleagues published the first studies on extract stability.⁵⁻⁷ Nelson's early studies showed the stabilizing effect of 50% glycerin. In addition, it was shown that glycerin concentrations down to 10% and HSA-containing diluents may have benefit over simple saline solutions in preserving activity. Further studies verified the stabilizing effect of HSA.^{1,10,15,16} Multiple studies have shown that more dilute extract solutions have a greater loss of potency compared to more concentrated solutions, and that 50% glycerin and HSA more effectively stabilize dilute solutions when compared to lower concentrations of glycerin and saline.^{1,5,7} It is suspected that the high concentrations of sugars, such as glycerin or sucrose, confer allergen stability by preventing denaturation and inhibiting enzymatic activity that modifies the

active IgE binding regions.¹⁷ The ability of HSA to stabilize dilute extracts is presumably due to HSA functioning as a protein carrier (either preventing adsorption of the allergen to the vial glass or inhibiting protein aggregation) or providing nonspecific protein to compete for enzyme activity.⁴

The current study was performed to investigate the ability of various diluents to maintain allergen content when utilized in the setting of common otolaryngology allergy practices. A consistent pattern of greater loss of allergen potency was noted in the more dilute vials. Also, HSA and 50% Gly consistently showed superior preservative activity in TTBs when compared with 10% Gly and NSP, especially in the more dilute vials. In our ITVs, we observed a similar pattern of loss in dilute vials, and the superior allergen-preserving activity of HSA in comparison to 10% Gly-NSP. The NSP TTB and 10% Gly-NSP ITVs showed poor preservation of allergen in storage. Loss of allergen content occurred more significantly in the dilute vials, beginning typically in the #3 dilution, and becoming more severe in the #4 and subsequent higher dilutions. Of particular interest, and only previously shown by Naerdal and Vilsvik,¹⁵ is how rapid the loss of allergen is observed in TTBs and ITVs. Naerdal and Vilsvik¹⁵ showed a “marked difference” between NSP and HSA potencies within hours and speculated that such a rapid discrepancy was likely due to adsorption rather than enzymatic activity within the NSP group. The magnitude of discrepancy between the NSP and HSA groups within their study was similar to our findings, with HSA frequently showing approximately double the potency. In our study, day 3 data showed that the most significant loss of allergen potency commonly occurred within the first 3 days after vial creation, especially in NSP TTB vials and 10% Gly-NSP ITVs (see Fig. 3A).

Our data shows that both HSA and 50% Gly do a better job of allergen preservation, even at day 3, in comparison to the other diluents. Greater degradation of allergen potency in NSP and 10% Gly-NSP solutions lead to considerable changes in the actual allergen content from the creation of an ITV until its end. The most commonly used method of TTB creation with NSP and ITV creation with 10% Gly-NSP by otolaryngologists may not be the optimal method for maintaining allergen potency in allergy testing and immunotherapy. HSA’s ability to better maintain potency in dilute solutions makes it an attractive diluent option for use in TTB and ITV creation (particularly during the escalation phase of immunotherapy).

HSA is a derivative of human blood. As such there is concern about the transmission of disease agents that may be associated with the HSA fraction of processed blood. HSA is a remarkably stable protein in a liquid solution at room

temperature over the shelf life of the product. The stability allows the protein to be heated at 60°C for 10 hours to facilitate virus inactivation during manufacturing. This process has shown the elimination of both lipid-enveloped and certain non-enveloped viruses in validation experiments.¹⁸ Given its established safety profile and unique properties, HSA is frequently used as a stabilizer for proteins. In ITVs, HSA’s amphiphilic nature inhibits adsorption of the allergens to the glass vial wall.¹⁹ Another possible mechanism for allergen preservation could involve HSA’s ability to act as a protein carrier inhibiting protease activity. HSA may also compete with allergen for the enzymatic activity of the proteases, thereby reducing the amount of allergen degraded. In addition to concerns about disease transmission, another consideration for choice of diluents is cost.⁹ Information from unpublished price lists from 4 manufacturers showed that HSA 4.0 mL vials are 10% to 15% higher in cost than NSP vials. 10% Glycerin vials are 11% to 44% higher in cost than HSA and 28% to 59% higher than NSP.

Limitations of this study include the measurement of only 1 of the several allergenic proteins to measure potency. For each allergen, though, the most important allergen protein was measured. Another limitation is the lack of human clinical trials to support the assumptions that the variability in allergen content produced by allergen degradation results in clinically significant differences in allergy testing results, and safety/efficacy concerns during immunotherapy. The data presented here may serve as a starting point for future studies with in vivo testing methods, such as skin testing the same patient utilizing solution from the same TTB at various points during its lifespan to examine for variations in test results. Other areas of interest include the testing of the stabilizing effect of alternate protein sources such as soy protein.

Conclusion

In summary, ELISA testing of allergen preservation of various diluents showed the superior stabilizing effect of HSA over other diluents, primarily NSP. The clinical significance is yet to be determined, but these findings support consideration of the use of HSA as a protein stabilizing diluent for the production of TTBs and ITVs. 🌐

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