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# Stability of standardized grass, dust mite, cat, and short ragweed allergens after mixing with mold or cockroach extracts

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**Background:** Limited data are available on the immunochemical compatibilities of standardized and nonstandardized allergen extracts in immunotherapy vaccines. Extract combinations recommended in immunotherapy practice parameters are based primarily on theoretical considerations rather than on actual product compatibilities.

**Objectives:** To determine the stabilities of standardized grass, short ragweed, dust mite, and cat extracts after mixing with fungal and cockroach extracts at final product concentrations similar to those recommended for maintenance immunotherapy injections.

**Methods:** Mixtures were prepared using individual products from multiple sources at variable glycerin concentrations and were analyzed after storage for up to 1 year at 2°C to 8°C. Quantitative analyses included radial immunodiffusion assays for cat Fel d 1 and short ragweed Amb a 1 and human IgE enzyme-linked immunosorbent assay inhibitions for meadow fescue grass and dust mite allergens. Immunoblot analyses provided qualitative patterns of IgE binding.

**Results:** Meadow fescue grass allergens were unstable after mixing with fungal or cockroach extracts but were highly compatible with dust mite extracts from numerous commercial sources. Fescue and dust mite allergen recoveries varied considerably when mixed with different mold extracts. The presence of cockroach extracts reduced dust mite allergen potencies but retained moderate levels of cat and short ragweed allergen activities. In all cases examined, glycerin provided concentration-dependent improvements in allergen recoveries.

**Conclusions:** Several allergen extract combinations generally regarded as unstable by current practice parameters seem to possess considerable biochemical compatibilities. Use of these mixtures in immunotherapy vaccines is supported for practitioners seeking to optimize formulations, doses, and treatment regimens for their patients.

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## INTRODUCTION

The practice of allergen immunotherapy is essential to the management of IgE-mediated hypersensitivities in many adults and children with allergic rhinitis or asthma. In addition to reducing symptom severity and the risks of life-threatening exposures to specific allergens, immunotherapy has been shown to block the natural progression of allergic diseases and the onset of new sensitizations with increasing age.<sup>1</sup> The effectiveness of immunotherapy regimens for most patients is affected by several factors, including the accuracy of skin or serum IgE testing, close correlations between test results and clinical histories, extract compositions containing the specific allergens responsible for symptomatic events, and immunotherapy vaccine formulations producing meaningful immunologic changes.

Extraction of allergenic materials typically yields complex, heterogeneous solutions composed primarily of water-soluble

proteins and carbohydrates.<sup>2</sup> Some extracts contain substantial levels of biologically active molecules that may affect the structures or activities of other substances through enzymatic or nonenzymatic interactions. The preparation of extract mixtures for immunotherapy vaccines creates the potential for interactive effects to change the inherent allergenic properties of individual components. In particular, the presence of active proteolytic enzymes in whole-body insect or fungal extracts can compromise the integrity of specific allergenic regions or 3-dimensional structures required for positive clinical outcomes.<sup>3–5</sup>

Several studies<sup>3,5–9</sup> have examined the stability of allergens in defined mixtures representing common or potential immunotherapy vaccines. These investigations used commercial or noncommercial extracts in combinations pairing low-protease (pollen and animal) and high-protease (dust mite, mold, and insect) allergens. Although useful in documenting the risks of combining particular extracts in immunotherapy vaccines, these reports have not addressed several issues fundamental to the clinical significance of the study findings.<sup>3</sup> The extracts used in these studies, particularly the high-protease products, were obtained from only single sources. Comparisons of analogous products from multiple manufacturers or sources have not been reported, raising the possibility that the results of these studies may depend on the allergen source. The

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published reports included various laboratory analyses, but very few assessments using the methods currently recognized by the US Food and Drug Administration (FDA) for lot release and stability testing of standardized extracts (human IgE enzyme-linked immunosorbent assay [ELISA] inhibition analyses for grass pollen and dust mite extracts and rabbit IgG radial immunodiffusion tests for the prominent allergenic proteins cat Fel d 1 and short ragweed Amb a 1). The ability of glycerin to inhibit protease digestions in immunotherapy mixtures has been observed in several studies but not at multiple concentrations with corresponding single-extract controls. The objectives of the present study were to evaluate the effects of dust mite, fungal, or cockroach extracts and glycerin levels on the structural integrity of current standardized products, to correlate observed potency reductions with qualitative molecular changes determined by human IgE immunoblotting, and to compare these results with those of previous studies and practice parameter recommendations.

## METHODS

### *Allergen Extracts*

Most of the allergenic extracts used in this study were licensed aqueous and glycerinated product concentrates obtained from multiple US manufacturers, including Greer Laboratories Inc, Hollister-Stier (Spokane, Washington), ALK Abello (Round Rock, Texas), Allermid (San Diego, California), Allergy Laboratories (Oklahoma City, Oklahoma), and Antigen Laboratories (Liberty, Missouri). Commercial extracts included meadow fescue grass (100,000 BAU/mL), dust mite *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (10,000 and 30,000 AU/mL, respectively), short ragweed (1:20 wt/vol), American cockroach and German cockroach (1:20 wt/vol), and the following fungi (1:10 to 1:40 wt/vol): *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aureobasidium pullulans*, *Bipolaris sorokiniana*, *Cladosporium herbarum*, *Cladosporium sphaerospermum*, *Epicoccum nigrum*, *Fusarium moniliforme*, *Fusarium solani*, *Mucor plumbeus*, *Penicillium chrysogenum*, and *Penicillium notatum* (1:20 wt/vol). A nonlicensed freeze-dried cat dander extract (Greer Laboratories) containing elevated Fel d 1 concentrations was selected instead of a commercial 10,000 BAU/mL product to permit quantitation of cat allergen levels after dilution or mixing with other products. All product lots were mixed and tested within their expiration dates. Extract concentrates were stored at 2°C to 8°C.

### *Extract Mixtures and Controls*

Extract concentrates and diluents were combined to produce 2-component mixtures or single-component controls, with each extract present at one tenth of concentrate levels. Final glycerin concentrations were controlled using nonglycerinated and 50% glycerinated isotonic sodium chloride solution with phenol diluents (Greer Laboratories). For 5.0-mL mixtures and controls, 0.5 mL of glycerinated extract mixed with 0.5 mL of glycerinated extract or diluent and 4.0 mL of

nonglycerinated diluent yielded 10-fold extract dilutions and 10% glycerin final concentrations. Inclusion of a total of 2.5 or 5.0 mL of glycerinated solution produced final glycerin levels of 25% or 50%, respectively. All test sample mixtures and controls were stored in 10-mL glass vials at 2°C to 8°C and were analyzed periodically during the next 12 months.

### *Human and Rabbit Sera*

Freeze-dried human serum pools containing specific IgE to grass pollen or dust mite allergens were obtained from the FDA (grass-positive serum lot S5-Grass and mite-positive serum lot S5-Dpf) or prepared at Greer Laboratories (mite-positive serum lot ZE-P3). Rabbit antisera directed against cat Fel d 1 (lot S-2b) and short ragweed Amb a 1 (lot S-8) were also procured from the FDA.

### *Analytical Methods*

Test mixtures and controls were evaluated side by side at identical dilutions in all evaluations. The ELISA inhibition analyses were performed using a procedure comparable with the compendial FDA method.<sup>10-12</sup> Briefly, microtiter plates (Costar; Corning Inc, Corning, New York) were coated with saturating levels of dust mite or meadow fescue extract controls in carbonate buffer (pH 9.6) for 15 to 20 hours at 2°C to 8°C. After washing with phosphate-buffered saline, pH 7.4, containing 0.05% Tween-20, plates were incubated with human serum pools (1:30 to 1:50 vol/vol dilutions) containing serial dilutions of controls (reference) and test mixtures for 4 to 6 hours at 20°C to 25°C. Bound IgE was then detected by means of successive incubations with biotinylated anti-human IgE (KPL, Gaithersburg, Maryland), avidin-alkaline phosphatase (Zymed, South San Francisco, California) and p-nitrophenyl phosphate (Amresco, Solon, Ohio). Absorbance values were determined at 405 nm in a microplate reader (ELx808; BioTek Instruments Inc, Winooski, Vermont). Using dose-response curves established by linear regression, the IgE-binding potencies of test mixtures relative to the corresponding single-extract references were determined using parallel-line bioassay. Statistical tests (paired *t* tests) were performed to confirm parallelism and validity of test and reference curves. Radial immunodiffusion assays for Fel d 1 and Amb a 1 were performed using 1% agar gels containing specific rabbit antisera cast onto support films (GelBond; Cambrex BioScience, Rockland, Maine) and allergen standards established by the FDA (C10-Cat: 4.6–13.5 Fel d 1 U/mL; C14-RAS: 5–30 µg Amb a 1 U/mL).<sup>11,13,14</sup> Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed with 12% acrylamide gels using a Mini-Protean II slab cell system (Bio-Rad, Hercules, California) under nonreducing conditions.<sup>12,15</sup> Test mixtures and controls were diluted in Laemmli sample buffer (Bio-Rad), boiled for 3 minutes at 100°C, and loaded onto the gels (10 µL per lane). Samples and low-kilodalton range molecular weight standards (Bio-Rad) were electrophoresed at constant voltage (200 V) for 35 to 45 minutes and were transferred by bidirectional, passive (nonelectrophoretic) diffusion to polyvi-

nylidene difluoride membranes (Immobilon-P<sup>50</sup>; Millipore, Medford, Massachusetts) at 20°C to 25°C.<sup>16-18</sup> After blocking, standard lanes were removed and stained with colloidal gold (Bio-Rad). Blot membranes containing test samples were incubated with human serum for 15 to 24 hours at 20°C to 25°C and, after 3 washes with Tris-buffered saline-0.05% Tween, probed with goat anti-human IgE-alkaline phosphatase conjugate (Sigma-Aldrich Corp, St Louis, Missouri) for 6 hours at 20°C to 25°C. The IgE-binding proteins were visualized using bromochloroindoxyl phosphate/nitroblue tetrazolium substrate (Amresco Inc, Solon, Ohio).

## RESULTS

### Dust Mite Extract Mixtures

The compatibility of meadow fescue grass allergens with *D. farinae* and *D. pteronyssinus* extracts was examined using

standardized dust mite products from multiple US manufacturers at 2 concentrations (1,000 and 3,000 AU/mL) within current immunotherapy practice parameter recommendations.<sup>3</sup> Test mixtures and controls were analyzed by means of ELISA inhibition and immunoblotting after storage for up to 12 months at 2°C to 8°C. High levels of meadow fescue allergen activity were retained after mixing with dust mite extracts from all sources at both concentrations (Fig 1). All test samples contained 20% glycerin, the lowest level possible after mixing meadow fescue and 10,000 AU/mL of dust mite extracts to a final mite concentration of 3,000 AU/mL. The ELISA potencies (80% to 140% of meadow fescue controls) are within current assay limits for comparability. Minor differences in immunoblot band patterns seemed to relate more to mite extract differences than to changes in meadow fescue allergen reactivities.

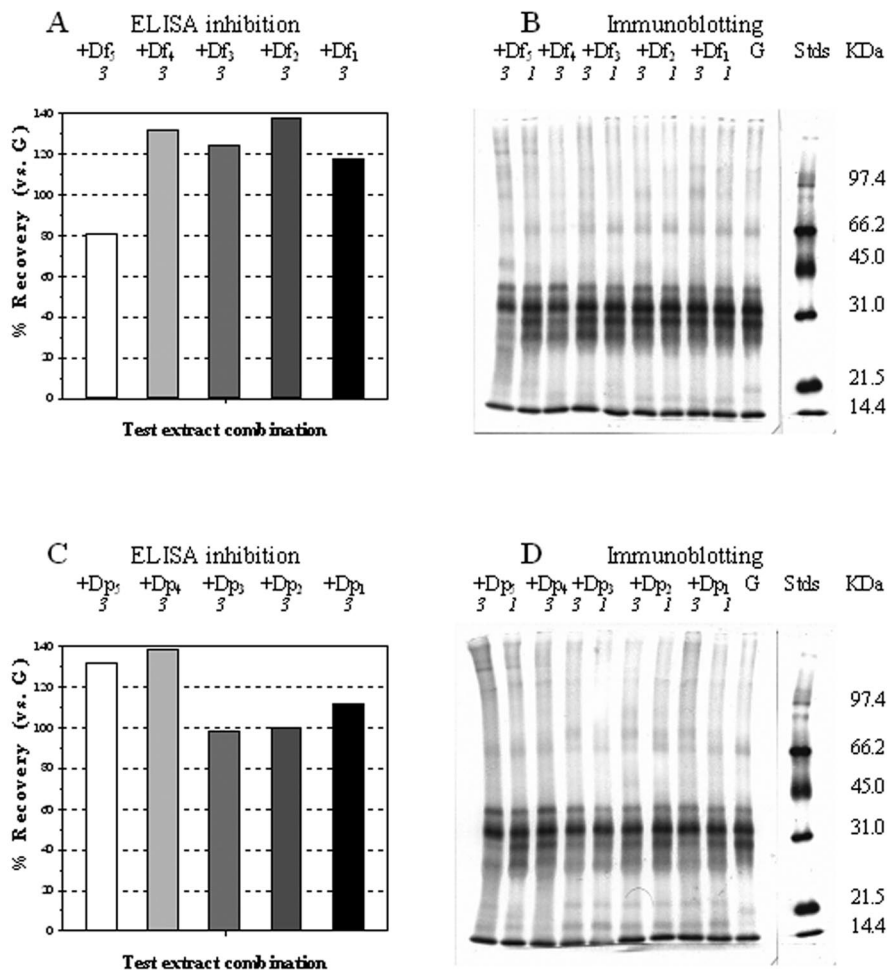


Figure 1. Results of enzyme-linked immunosorbent assay (ELISA) inhibition (A and C) and immunoblot analysis (B and D) of meadow fescue grass (G) allergens after mixing with *Dermatophagoides farinae* (Df) or *Dermatophagoides pteronyssinus* (Dp) extracts and storage for 12 months at 2°C to 8°C. The ELISA inhibition recoveries are expressed relative to control (G-only) samples. Molecular weight standards (Stds) and corresponding kilodalton values for immunoblots are illustrated. All mixtures and controls contained 20% glycerin. Mite extracts are designated Df<sub>1</sub> to Df<sub>5</sub> and Dp<sub>1</sub> to Dp<sub>5</sub>. Final concentrations (in allergy units per milliliter ×1,000) in all samples are shown in italics.

### Fungal Extract Mixtures

To evaluate the relative compatibilities of allergenically important fungi, meadow fescue allergens were mixed with a variety of fungal extracts and were analyzed after storage for up to 12 months at 2°C to 8°C. High levels of meadow fescue allergen degradation were observed with *Penicillium*, *Aspergillus*, *Alternaria*, and *Cladosporium* extracts (Fig 2). Lot-to-lot variations were less than 15% of mean values for these mixtures. Longer storage periods (6 months) resulted in progressive reductions to less than 10% of control values for mixtures containing *Penicillium*, *A fumigatus*, and *Alternaria*. Several fungal extracts (*Aureobasidium*, *Fusarium*, and *Mucor*) induced very little change in meadow fescue allergen reactivities with 10% glycerin, even after 6 months.

*Alternaria* extracts from 6 manufacturers showed some quantitative differences in meadow fescue allergen inactivation in mixtures containing 10% glycerin levels but much smaller differences at 50% glycerin (Fig 3). Most prominent meadow fescue allergens were reduced in activity by the presence of *Alternaria* extract. No major differences in IgE-binding activity were observed between control (grass-only) solutions at 10% glycerin and 50% glycerin. *Aspergillus* extracts from 3 manufacturers produced greater than 95% reductions in meadow fescue extract potency at 10% glycerin and at least 50% reductions at 50% glycerin, with changes detected to all major IgE-binding proteins (Fig 4). One extract (*A fumigatus* 3) produced virtually no changes in ELISA potency or immunoblot reactivity compared with control samples at either glycerin level. *Penicillium* extracts from the same 4 manufacturers displayed relative reactivities similar to

*Aspergillus*, with product *P notatum* 3 producing results closely related to those observed for *A fumigatus* 3 (Fig 4). Extracts *A fumigatus* 3 and *P notatum* 3 contained considerably lower total protein concentrations compared with analogous products from other manufacturers (data not shown).

The stability of dust mite allergens after mixing with fungal extracts was investigated using 2 mite-positive human serum pools: FDA lot S5-Dpf and Greer Laboratories lot ZE-P3. Mite allergen recoveries at 10% glycerin varied noticeably between the 2 sera (Fig 5). Mixtures at 25% glycerin displayed relatively minor differences, and the presence of 50% glycerin resulted in complete recoveries of mite allergen activities. The absence of a potency reduction with the Greer Laboratories serum was consistent with apparent differences in the mite IgE specificities of the 2 pools. The addition of *Penicillium* extract at 10% glycerin resulted in rapid degradation of the high-molecular-weight (>45-kDa) mite proteins recognized by the FDA serum with little or no change to the low-molecular-weight protein detected by the FDA and Greer Laboratories sera.

After 5 months at 2°C to 8°C, only *Penicillium* extract reduced cat Fel d 1 concentrations below 80% of control values at 10% glycerin (Fig 6). Short ragweed Amb a 1 was susceptible to proteases present in *Penicillium* and *Alternaria* extracts at relatively low (10%) glycerin levels. Higher glycerin levels produced improved recoveries for these extract combinations. Under identical conditions, no significant changes in Amb a 1 potency were observed after mixing with *Aspergillus* at all 3 glycerin levels.

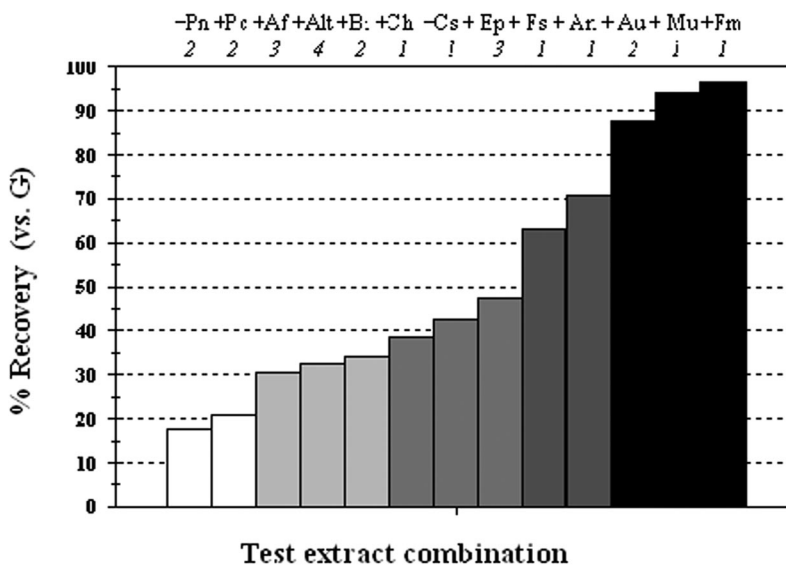


Figure 2. Results of enzyme-linked immunosorbent assay (ELISA) inhibition analysis of meadow fescue grass (G) allergens after mixing with fungal extracts and storage for 1 month at 2°C to 8°C. Mean ELISA inhibition recoveries are expressed relative to control (G-only) samples. All mixtures and controls contained 10% glycerin. Fungal extracts included *Penicillium notatum* (Pn), *Penicillium chrysogenum* (Pc), *Aspergillus fumigatus* (Af), *Alternaria alternata* (Alt), *Bipolaris sorokiniana* (Bi), *Cladosporium herbarum* (Ch), *Cladosporium sphaerospermum* (Cs), *Epicoccum nigrum* (Ep), *Fusarium solani* (Fs), *Aspergillus niger* (An), *Aureobasidium pullulans* (Au), *Mucor plumbeus* (Mu), and *Fusarium moniliforme* (Fm). Numbers of product lots included in this analysis are shown in italics.

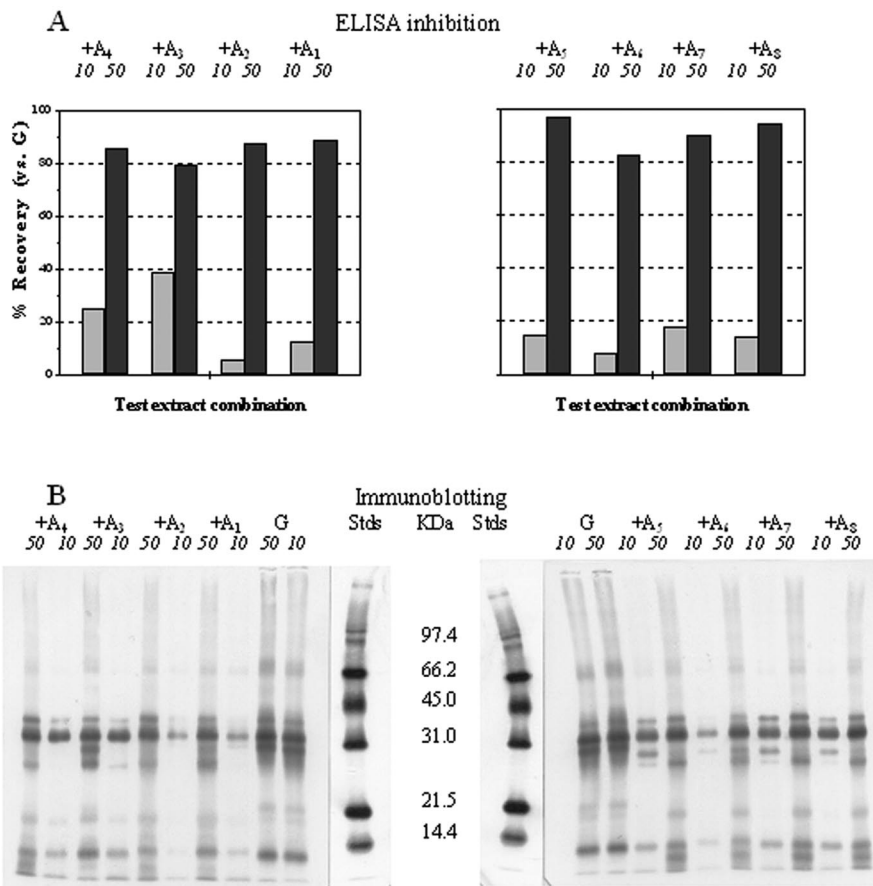


Figure 3. Results of enzyme-linked immunosorbent assay (ELISA) inhibition (A) and immunoblot analysis (B) of meadow fescue grass (G) allergens after mixing with *Alternaria* (A) extracts and storage for 4 months at 2°C to 8°C. The ELISA inhibition recoveries are expressed relative to control (G-only) samples at identical glycerin concentrations. Molecular weight standards (Stds) and corresponding kilodalton values for immunoblots are illustrated. *Alternaria* extracts are designated A<sub>1</sub> to A<sub>8</sub>. Final glycerin concentrations (percentage) in all samples are shown in italics.

### Cockroach Extract Mixtures

Mixtures of *D. farinosa* and cockroach extracts exhibited selective reductions in mite allergen activity with the FDA serum at 10% glycerin (Fig 7), similar to sensitivities with fungal extracts (Fig 5). Cat extract potencies were reduced to a small degree (<30%) after addition of German cockroach extract at 10% glycerin (Fig 8). Recoveries were improved at higher glycerin concentrations. American cockroach had no significant impact on Fel d 1 reactivity. Short ragweed Amb a 1 recoveries were high (>80% of controls) with cockroach extracts from either species at all glycerin levels tested.

### Summary

Based on the results of this study and previous investigations,<sup>4-9</sup> the compatibilities of allergen extracts are summarized in Figure 9. Extract combinations demonstrating high recoveries are designated in green. Differences in compatibility within a given allergen category (such as fungi) are shown in yellow to reflect uncertain or inconsistent recoveries with these products. High-risk combinations or those with

low recoveries for the only extracts tested to date (trees and weeds) are illustrated in red.

### DISCUSSION

This study was conducted to determine the stabilities of common and potentially unstable extract combinations using laboratory methods that correlate standardized extract potencies with qualitative changes in these mixtures. Immunoblots were performed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis to identify changes relevant only to allergenic (IgE-binding) components. Mixtures and controls were tested without freezing or freeze-drying to eliminate physical or biochemical modifications caused by phase changes. Multiple glycerin levels were included to determine the concentration dependence of this stabilizer in specific extract interactions. Product lots from several manufacturers were evaluated to examine the range of effects that may occur with similar mixtures prepared at different clinics. Direct measurements of protease activities were not performed be-

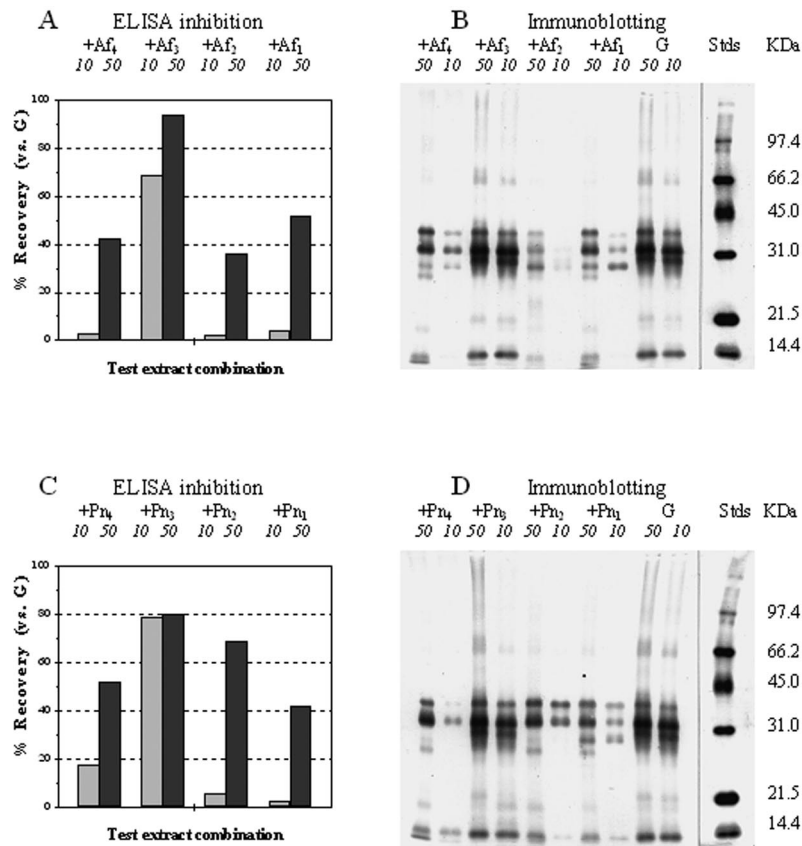


Figure 4. Results of enzyme-linked immunosorbent assay (ELISA) inhibition (A and C) and immunoblot analysis (B and D) of meadow fescue grass (G) allergens after mixing with *Aspergillus fumigatus* (Af) or *Penicillium notatum* (Pn) extracts and storage for 4 months at 2°C to 8°C. The ELISA inhibition recoveries are expressed relative to control (G-only) samples at identical glycerin concentrations. Molecular weight standards (Stds) and corresponding kilodalton values for immunoblots are illustrated. Fungal extracts are designated Af<sub>1</sub> to Af<sub>4</sub> and Pn<sub>1</sub> to Pn<sub>4</sub>. Final glycerin concentrations (percentage) in all samples are shown in italics.

cause of the inconsistent relationships between enzyme activities and allergen recoveries reported previously and the lack of availability of analytical reagents sensitive to relevant protease specificities.<sup>5,7,9</sup>

The presence of 20% glycerin may have affected the high recoveries of meadow fescue grass extract reactivity with all dust mite products. Earlier studies<sup>5,8,9</sup> reported reductions in grass extract potency after mixing with *D. farinae* or no major changes with *D. farinae* at 25% glycerin. Data from this study extend these results to *D. pteronyssinus* extracts and products from multiple manufacturers at 20% glycerin. Similar mixtures at 10% glycerin also provided near-complete recoveries of all major allergens (data not shown). Immunoblotting confirmed the stability of prominent meadow fescue allergens in these samples.

Extracts from 13 common allergenic fungi produced major differences in meadow fescue grass compatibilities. Recoveries ranged from low (*Penicillium*, *Aspergillus*, and *Alternaria*) to near complete (*Mucor*, *Fusarium*, and *Aureobasidium*) after 1 month. Longer storage periods reduced

recoveries for unstable combinations but did not produce adverse effects on the more stable mixtures. Inclusion of multiple fungal extract lots verified the repeatability of these results. The practice parameter recommendation of separating molds from pollen and other low-protease extracts thus seems appropriate for most major fungal species. Products from different *Fusarium* species produced relatively high recoveries in the present study but much lower recoveries in an earlier study.<sup>7</sup> Variations in mold cultures, cultivation, and extraction conditions may contribute to these differences.

Reductions in meadow fescue extract potency were similar for all *Alternaria* products tested and for *Aspergillus* and *Penicillium* products from all but 1 manufacturer, with very low overall recoveries supporting the separation of these products from low-protease extracts in immunotherapy vaccines. Products from 1 manufacturer (*A. fumigatus* 3 and *P. notatum* 3) produced much higher recoveries at 10% glycerin and the highest yields at 50% glycerin. The low protein concentrations of these extracts may affect the minimal changes observed. However, the possibility remains that

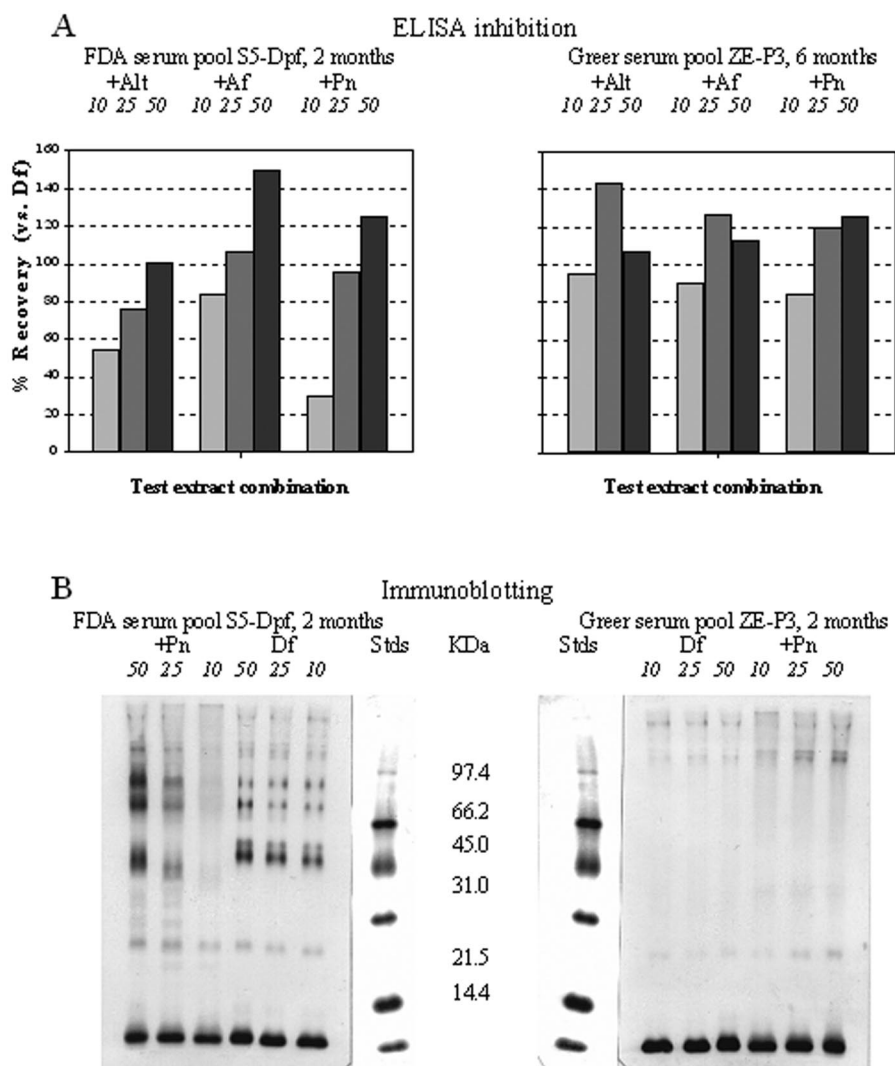


Figure 5. Results of enzyme-linked immunosorbent assay (ELISA) inhibition (A) and immunoblot analysis (B) of *Dermatophagoides farinae* (Df) allergens after mixing with *Alternaria* (Alt), *Aspergillus fumigatus* (Af), or *Penicillium notatum* (Pn) extracts. Samples were tested after storage for 2 or 6 months at 2°C to 8°C using Food and Drug Administration (FDA) serum pool S5-Dpf and Greer Laboratories serum pool ZE-P3. The ELISA inhibition recoveries are expressed relative to control (Df-only) samples at identical glycerin concentrations. Molecular weight standards (Stds) and corresponding kilodalton values for immunoblots are illustrated. Final glycerin concentrations (percentage) in all samples are shown in italics.

these products may contain lower protease-allergen ratios compared with analogous extracts from other manufacturers. A 1996 study<sup>8</sup> reported a high level of compatibility between Timothy grass and *Penicillium* extracts. The manufacturer of the *Penicillium* extract is the same as that of one product in the present study that produced low recoveries with meadow fescue extract. Compositional differences between the 2 extract lots prepared during a 10-year period may account for these differences in allergen recovery but cannot be confirmed because of the lack of availability (and age) of the 1996 study product.

Analysis of dust mite allergens mixed with fungal or cockroach extracts revealed differences in mite extract recovery

and specific allergen degradation with 2 human serum pools. The clear differences in the IgE specificities of these pools demonstrate the critical importance of including multiple patient samples or pools from distinct patient populations in compatibility or stability studies. In this instance, use of only the Greer Laboratories serum would have produced a much different result and immunotherapy recommendation.

The stabilities of cat hair and short ragweed allergens with cockroach extracts were different from those described in a previous study or the 2003 immunotherapy practice parameters.<sup>3,8</sup> In the present study, cat Fel d 1 was highly compatible with mold and cockroach extracts, with greater than 75% recoveries observed after 5 months even at 10% glycerin. The

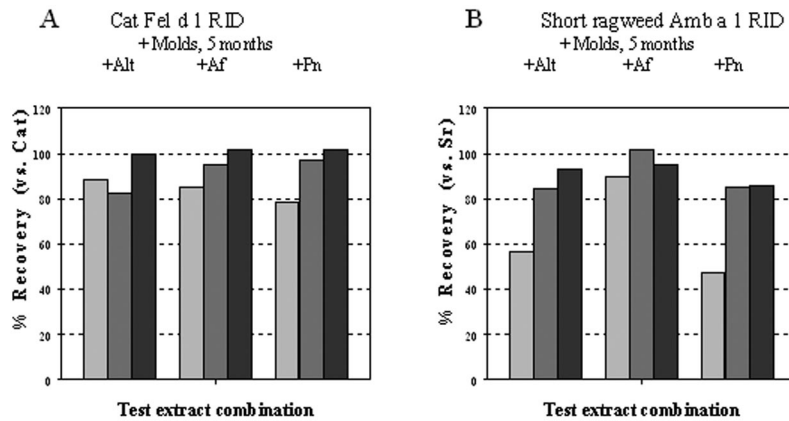


Figure 6. Results of Fel d 1 radial immunodiffusion (RID) analysis of cat hair (Cat) extracts (A) or Amb a 1 RID analysis of short ragweed (Sr) extracts (B) after mixing with *Alternaria* (Alt), *Aspergillus fumigatus* (Af), or *Penicillium notatum* (Pn) extracts. Samples were tested after storage for 5 months at 2°C to 8°C. The RID recoveries are expressed relative to control (Cat-only or Sr-only) samples at identical glycerin concentrations. Final glycerin concentrations (percentage) in all samples are shown in italics.

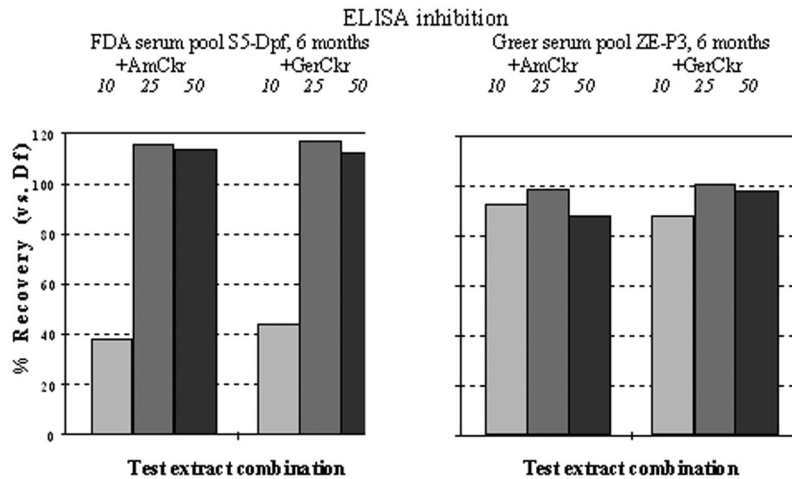


Figure 7. Results of enzyme-linked immunosorbent assay (ELISA) inhibition analysis of *Dermatophagoides farinae* (Df) allergens after mixing with American cockroach (AmCkr) or German cockroach (GerCkr) extracts. Samples were tested after storage for 6 months at 2°C to 8°C using Food and Drug Administration (FDA) serum pool S5-Dpf and Greer Laboratories serum pool ZE-P3. The ELISA inhibition recoveries are expressed relative to control (Df-only) samples at identical glycerin concentrations. Final glycerin concentrations (percentage) in all samples are shown in italics.

earlier study reported significant potency reductions in cat mixtures analyzed by means of ELISA inhibition using a cat-positive human serum pool. Although cat allergen specificity differences between this human serum and rabbit anti-Fel d 1 antiserum used for radial immunodiffusion cannot be ruled out, the practice parameter recommendation of separating cat from high-protease extracts may be too conservative. For short ragweed, the compatibility of Amb a 1 with high-protease extracts was high with cockroach but not with all fungal extracts. *Penicillium* and *Alternaria* extracts at 10% glycerin produced noticeable reductions in Amb a 1 concentrations. These differences may be related to the different *Penicillium* extracts used in the present and previous studies, as noted earlier, or to differences in the radial immunodiffu-

sion and human serum ELISA inhibition test methods, as suggested for cat. It is clear that some combinations of fungal and short ragweed extracts may be less than optimal.

There are several limitations to the experiments and data in this study. All the experiments involved laboratory measurements of allergen concentration or activity. The clinical significance of these results requires correlation with an in vivo analysis, such as quantitative intradermal skin testing, on patients sensitive to 1 extract in an immunotherapy mixture.<sup>19</sup> The interpretations of this study are also limited to the extracts and serum samples used for the analyses. Expansion of these studies should include additional extracts from multiple suppliers, individual patient samples, and alternative analytical methods specific for a variety of allergens in low- and



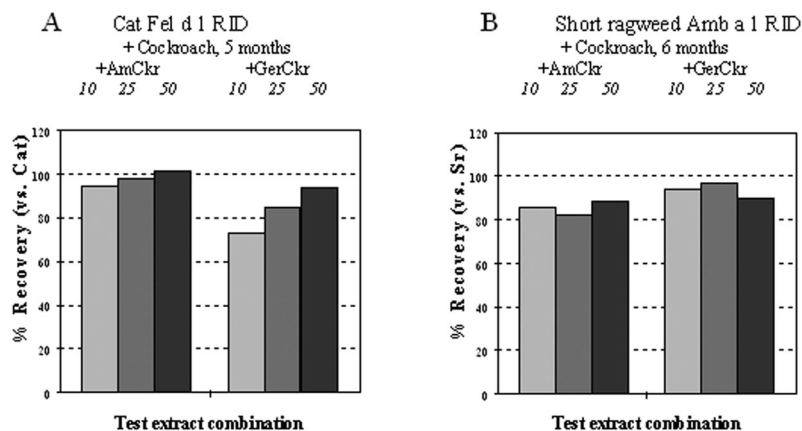


Figure 8. Results of Fel d 1 radial immunodiffusion (RID) analysis of cat hair (Cat) extracts or Amb a 1 RID analysis of short ragweed (Sr) extracts after mixing with American cockroach (AmCkr) or German cockroach (GerCkr) extracts. Samples were tested after storage for 5 to 6 months at 2°C to 8°C. The RID recoveries are expressed relative to control (Cat-only, Sr-only) samples at identical glycerin concentrations. Final glycerin concentrations (percentage) in all samples are shown in italics.

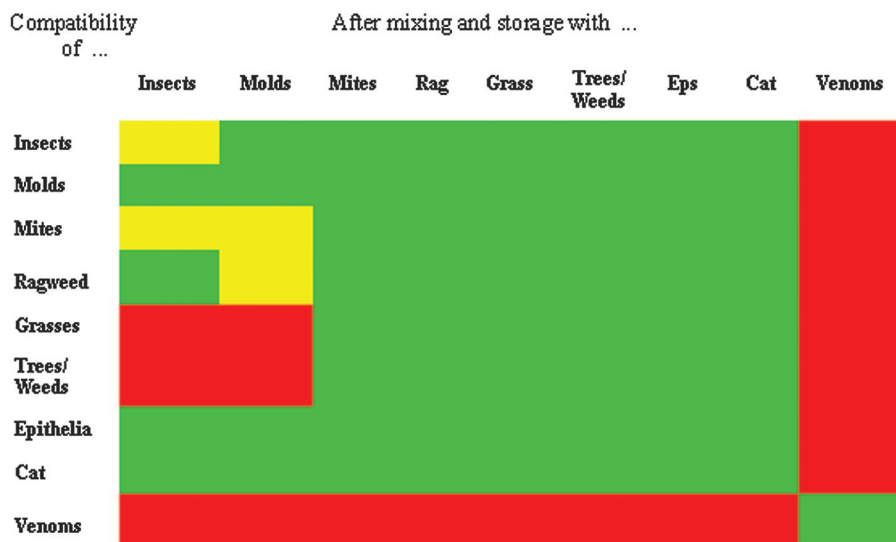


Figure 9. Extract compatibility chart. Combinations producing low (red), moderate or risky (yellow), and favorable (green) compatibilities of both extract components are illustrated.

high-protease extracts. Reports of different analytical results attributed to method or reagent performance variations rather than extract differences are not uncommon.<sup>20,21</sup> Extracts remain complex and, for the most part, poorly characterized in terms of molecular compositions, allergen levels and activities, and susceptibilities to untoward environmental changes or the consequences of mixing and dilution. Concerted efforts to analyze allergens, extracts, and mixtures in a systematic, comparable, and open manner would improve our knowledge and understanding of allergen profiles, identify clinically relevant test procedures, and facilitate improvements in extract compatibilities and treatment outcomes for allergic patients.

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