

Stability of Cat, Ragweed, Dust Mite and Mold Allergens After Mixing with Cockroach Extracts

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Abstract

The physical and biochemical compatibilities of allergen extracts are essential to the development of stable immunotherapy vaccines and effective clinical outcomes. Although current practice parameters recommend that extracts with active endogenous proteases (molds, insects) should not be mixed with pollen, animal or environmental allergens, few studies have been performed to substantiate the nature and extent of degradative reactions for specific extract combinations at maintenance dose concentrations.

In this study, the immunochemical reactivities of standardized (cat, grass, dust mite, ragweed) and non-standardized (mold) allergens were examined after mixing with whole body American cockroach or German cockroach extracts and storing for up to 11 months at 2-8°C. Test extracts were analyzed for qualitative differences in IgE-binding structures (immunoblotting) and for quantitative changes in allergenic potency using validated assays sensitive to single (radial immunodiffusion) or multiple (ELISA inhibition) allergenic components. As expected, greater than 90% of grass extract reactivities were destroyed in the presence of high-protease cockroach extracts. By comparison, moderate to complete recoveries of cat, short ragweed, dust mite and fungal allergens were observed after mixing and storage with cockroach extracts under similar conditions. Glycerin provided concentration-dependent stabilization of critical cat Fel d 1 sequences or structures when combined with German cockroach extracts. Dust mite allergen potencies compromised by mixing with cockroach extracts were protected completely at 10-25% (or higher) glycerin concentrations. Cat and short ragweed extract potencies in these mixtures remained well within current lot release and stability limits for standardized products. Fungal antigens were also unaffected by cockroach proteases.

Based on these results, it is apparent that cat, ragweed, dust mite and fungal allergens generally considered to be incompatible with cockroach extracts may coexist effectively under most conditions. The compatibilities of these combinations may allow allergists to streamline the mixing, dilution and administration of immunotherapy mixtures, reduce the number of injections per office visit for many atopic patients, and actively address challenging reimbursement or managed care requirements.

Materials and Methods

Allergen mixtures and control samples were prepared using the following glycerinated Greer extract concentrates and diluents:

Meadow fescue grass	Lot GT14-25B-1UF3	100,000 BAU/mL
Dust mite D. farinae	Lot GB64-91B-1UR4	10,000 AU/mL
Cat dander	Lot RE63-138	1:10 w/w
Short ragweed	Lot C56-223B-1A5	1:20 w/w
Alternaria alternata	Lot GM1-205B-1A2	1:20 w/w
Aspergillus fumigatus	Lot GM3-68B-1A5	1:20 w/w
Penicillium notatum	Lot GM19-56B-1A5	1:20 w/w
American cockroach	Lot GB26-56B-2A2	1:20 w/w
German cockroach	Lot GB46-21B-1A2	1:20 w/w
Normal saline	Lots SD397 and SD515	
Glycero-normal saline	Lots GNS-2-2-2 and GNS-2-2-3	

Individual allergens were combined with American cockroach and/or German cockroach, with each extract component comprising 10% of the total volume (final concentrations = 1/10th of concentrate strengths). All extract mixtures and individual extract controls were formulated to contain 10%, 25% and 50% glycerin final concentrations in 1.0 mL or 5.0 mL total volumes via specific combinations of normal saline and (50%) glycero-normal saline diluents.

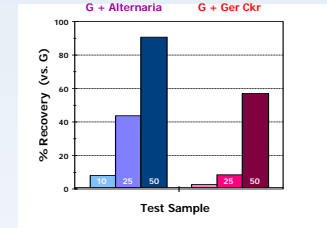
Mixtures and controls were stored at 2-8°C for up to 11 months, and analyzed periodically as follows:

Meadow fescue grass + Alternaria or German cockroach	147 days at 2-8°C	Human IgE immunoblots, 2 serum pools
	158 days at 2-8°C	Human IgE ELISA inhibition, 1 serum pool
Dust mite D. farinae + American cockroach or German cockroach	3 days at 2-8°C	Human IgE immunoblots, 2 serum pools
	31 days at 2-8°C	Human IgE ELISA inhibition, 1 serum pool
	191 days at 2-8°C	Human IgE ELISA inhibition, 2 serum pools
Cat dander + American cockroach or German cockroach	24 days at 2-8°C	Fel d 1 Radial immunodiffusion
	150 days at 2-8°C	Fel d 1 Radial immunodiffusion
	319 days at 2-8°C	Fel d 1 Radial immunodiffusion
Short ragweed + American cockroach or German cockroach	30 days at 2-8°C	Antigen E (Amb a 1) Radial immunodiffusion
	171 days at 2-8°C	Antigen E (Amb a 1) Radial immunodiffusion
	326 days at 2-8°C	Antigen E (Amb a 1) Radial immunodiffusion
Alternaria alternata + American cockroach or German cockroach		
Aspergillus fumigatus + American cockroach or German cockroach		
Penicillium notatum + American cockroach or German cockroach	10 days at 2-8°C	Human IgE immunoblots, 1 serum pool
		Rabbit IgE immunoblots, 3 antisera
	200 days at 2-8°C	Human IgE immunoblots, 1 serum pool
		Rabbit IgE immunoblots, 3 antisera
	317 days at 2-8°C	Human IgE immunoblots, 1 serum pool
		Rabbit IgE immunoblots, 3 antisera

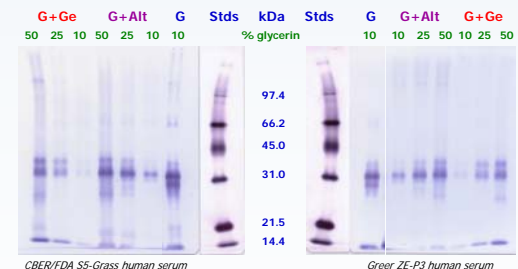
Analyses shown in red are current methods for allergen standardization, labeling and release by the U.S. Food and Drug Administration (FDA)

Grass-Cockroach Mixtures

Meadow fescue grass (G) allergen reactivities were reduced to extremely low levels after mixing with German cockroach extracts and storing for 5 months at 2-8°C. IgE-binding recoveries were improved in mixtures containing 25% and 50% glycerin compared to lower (10%) glycerin levels. Endogenous cockroach extract proteases were more detrimental to grass allergen structures compared to those derived from a common mold (Alternaria) known to contain high protease concentrations and activities.



IgE immunoblots with 2 human serum pools confirmed that prominent meadow fescue allergens, including group 1/5 (30-35 kDa), group 4/13 (50-60 kDa) and group 2/3/6/10/11 (11-18 kDa) molecules, were compromised by mixing with German cockroach (Ge) or Alternaria (Alt) extracts.



The compatibilities of American and German cockroach extracts with cat, short ragweed, dust mite and mold allergens were investigated to assess the suitability of current practice guidelines that recommend separation of high-protease (insects, fungi) and low-protease (pollens, mites, cat/dog) allergens in immunotherapy vaccines.

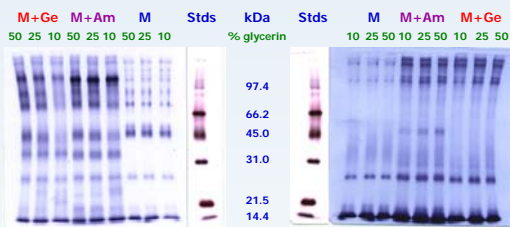
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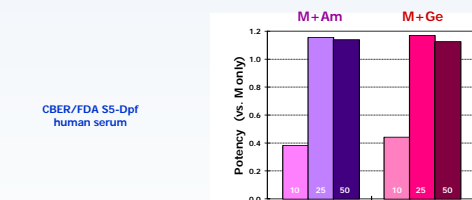
Dust Mite-Cockroach Mixtures

Dust mite *Dermatophagoides farinae* (M) allergens displayed improved compatibility with cockroach extracts compared to grass extracts. Some mite allergens were degraded rapidly at low (10%) glycerin concentrations based on human IgE immunoblot and ELISA analyses with mite-positive CBER/FDA serum pool S5-Dpf. This serum also contained cockroach-positive IgE antibodies, complicating the assessment of mite allergen recoveries from immunoblot band patterns. Serum pool ZE-P3 was more specific for dust mites and recognized mite allergens that exhibited near-complete compatibilities with American (Am) and German (Ge) cockroach extracts.

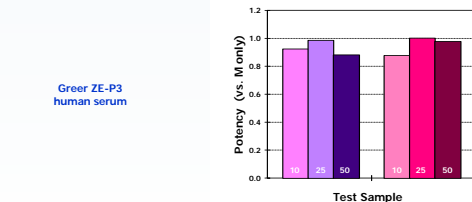


CBER/FDA S5-Dpf human serum

Greer ZE-P3 human serum



CBER/FDA S5-Dpf human serum

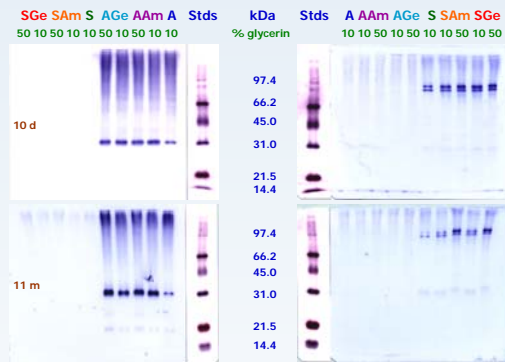


Greer ZE-P3 human serum

Mixtures and controls stored for 191 days at 2-8°C

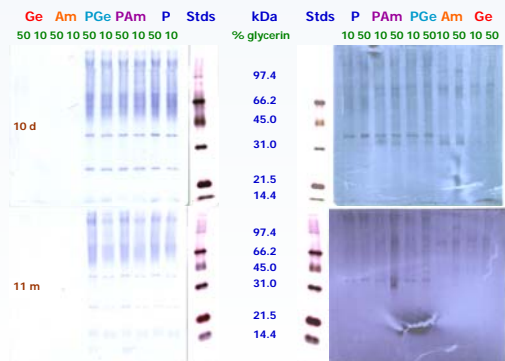
Mold-Cockroach Mixtures

Alternaria alternata (A), *Aspergillus fumigatus* (S) and *Penicillium notatum* (P) antigens recognized by rabbit IgG antibodies in hyperimmune sera remained essentially intact after mixing with American or German cockroach extracts and storing for 10 days (top blots) or 11 months (bottom blots) at 2-8°C. IgE-binding proteins in *Penicillium* extracts also displayed similar reactivities with human serum ZM-P1. Only minor (if any) differences were observed between paired samples containing 10% glycerin or 50% glycerin.



Rabbit anti-Alt a 1 serum

Rabbit anti-Aspergillus fumigatus serum

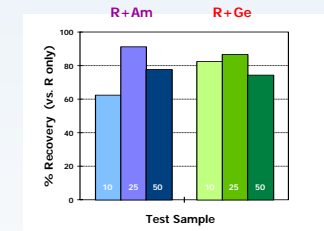
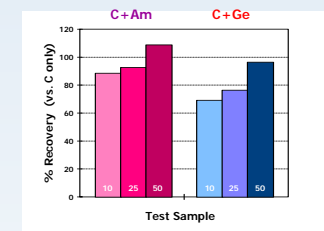


Rabbit anti-Penicillium notatum serum

Greer ZM-P1 human serum

Cat/Ragweed-Cockroach Mixtures

Cat (C) and ragweed (R) extract potencies were moderately stable after mixing with American or German cockroach extracts and storing for 11 months at 2-8°C. Cat Fel d 1 was more stable with American vs. German cockroach, while short ragweed Antigen E displayed the reverse trend. All cat and ragweed results were within current potency limits for standardized products (50-200% of reference or control).



Conclusions

Immunotherapy vaccines containing cockroach extracts and other high-protease (fungi) or low-protease (cat, ragweed, dust mite) allergens exhibited moderate to high levels of compatibility under most conditions examined in this study. Although glycerin improved overall recoveries, stabilities at low glycerin levels remained substantial for many of these mixtures. These results suggest that specific extract combinations generally regarded as unstable by current practice parameters may possess compatibilities sufficient to produce clinically-meaningful outcomes for allergists seeking more flexible, streamlined or simplified treatment regimens.