

Mixing Compatibility of Phenolated, Glycerinated Allergenic Extracts Stored at Refrigeration or Ambient Temperatures

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Overview and Methodology

Scope

Glycerinated allergenic extracts are utilized by allergy clinics with increasing frequency because of their enhanced physical and biochemical stabilities relative to aqueous products. Phenolated extract mixtures containing 50% glycerin are often employed as stock concentrates for testing and treatment, or as patient-specific formulations. For many common extract mixtures, the stabilities of allergens present in these solutions during typical storage or daily-use conditions have not been investigated.

This handout summarizes the compatibilities of various phenolated (0.2%), glycerinated (50%) allergenic extracts determined after mixing with high-protease (fungal, insect) and/or low-protease (pollen, animal, dust mite) glycerinated products and storage for up to 12 months at refrigeration (2-8 C) or ambient (20-25 C) temperatures.

Materials and Methods

Allergen mixtures and control samples were prepared using licensed glycerinated (50% v:v) Greer extract concentrates and AP dog hair-dander extract obtained from Hollister Stier. Each product was sourced at its highest available glycerinated strength.

Two-component extract mixtures and single-component extract controls were formulated at one-tenth of concentrate strengths in 2-5 mL total volumes using 50% glycerol-normal saline diluent. All samples were analyzed after storage for 1-12 months at 2-8 C or 20-25 C.

Test sample potencies were determined by quantitative human IgE ELISA inhibition and radial immunodiffusion assays, the methods established by FDA for extract standardization in the United States.

IgE ELISA inhibition assays were performed to determine the allergenic potencies of a variety of allergenic extracts. Microtiter plates coated with saturating levels of target allergens were co-incubated with serial dilutions of reference (controls) or test allergens (mixtures) and IgE-positive human serum pools reactive with the target allergens. Parallel dose-response curves bracketing 50% inhibition levels were observed for controls and mixtures. Relative IgE-binding potencies were calculated using a parallel line bioassay spreadsheet similar to those employed for lot release and stability testing of standardized grass pollen and dust mite extracts.

Radial immunodiffusion assays for short ragweed allergen Amb a 1 and cat hair allergen Fel d 1 employed specific allergen standards, anti-Amb a 1 and anti-Fel d 1 sheep antisera, and incubation conditions identical to those used for standardization of short ragweed and cat hair extracts.

Two-site ELISA analyses for major allergenic proteins in several products were also performed on some mixtures. These assays utilized allergen-specific mouse or rabbit capture and biotinylated probe antibodies, with results obtained by log-linear regression of calibrated allergen standards and multiple test sample dilutions.

Percent recoveries for extract mixtures were expressed relative to the activities of single-extract controls containing identical concentrations of test allergens. Recoveries were typically within 10% of mean values for replicate samples.

Results were assessed for significance by T test ($p \leq 0.05$).

MPN 102411H445

Allergen Compatibilities for Mixtures Stored at 2-8 C

Extract activities in mixtures were determined after storage for 1-12 months at 2-8 C. Mean recoveries of 70% or higher (stable, green shading), 50-69% (risky, yellow shading) and below 50% (unstable, red shading) are illustrated below.

Target	Mixed w/	Mean % Recovery after mixing and storage for ...										
		Months at 2-8 C				Months at 2-8 C						
		+	1	3	6	12	+	1	3	6	12	
Meadow fescue grass	Alternaria		94	79	70	36	American cockroach		150	33	54	
	Aspergillus		68	77	63	33	German cockroach		115	50	45	
	Penicillium		62	71	50	32	Fire ant invicta		156	63	75	
	Cladosporium		76	136	49		D. farinae			101	89	
	Epicoccum		69		96		D. pteronyssinus			87	103	
	Aureobasidium		72		177		Short ragweed		59	51	61	
	Fusarium		152		73		Giant ragweed		102	63	75	
	Mucor		70		78		False ragweed		68	59	73	
	Bipolaris		94	100	82	49	Western ragweed		69	47	72	
							Desert ragweed		99	95	79	
Timothy grass	Alternaria			48	72	58	Short ragweed		100	99	90	86
	Aspergillus			78	62	45	Giant ragweed		100	104	111	111
	Penicillium			39	32	21	False ragweed		116	110	88	
	Cladosporium			68	102	104	Western ragweed		73	114	90	
	Bipolaris			66	69	66	Desert ragweed		93	104	111	
	American cockroach			51	53	54	Fire ant invicta		114	70	57	
	German cockroach			48	59	44						
Bermuda grass	Short ragweed		95	96	96	95	Short ragweed		95	96	96	95
Johnson grass	Short ragweed		106	56	97	86	Giant ragweed		91	53	105	90
Cat	Alternaria			102	100		American cockroach		97		102	
	Aspergillus			98	102		German cockroach		96		94	
	Penicillium			100	102		Short ragweed			105	108	108
							Giant ragweed			114	110	110

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Allergen Compatibilities for Mixtures Stored at 2-8 C

Target	Mixed w/	Mean % Recovery after mixing and storage for ...									
		Months at 2-8 C					Months at 2-8 C				
		+	1	3	6	12	+	1	3	6	12
Short ragweed	Alternaria			112	93		American cockroach	126		89	
	Aspergillus			112	95		German cockroach	100		90	
	Penicillium			93	86		D. farinae		100	113	88
	Timothy	87	108	95	96		Cat		104	117	97
	Bermuda			93	103	79	Dog epithelia		99	94	99
	Johnson			90	112	85	Dog dander		98	97	103
	+	1	3	6	12	+	1	3	6	12	
	+	1	3	6	12	+	1	3	6	12	
Eastern cotton-wood	Alternaria				102		American cockroach				134
	Aspergillus				87		German cockroach				126
	Penicillium				97		Fire ant invicta				105
White oak	Alternaria				77		American cockroach				116
	Aspergillus				57		German cockroach				101
	Penicillium				72		Fire ant invicta				104
D. farinae	Alternaria				101	91	American cockroach	101		105	
	Aspergillus				150	96	German cockroach	121		110	
	Penicillium				125	107	Short ragweed	107	140	123	175
	+	1	3	6	12	+	1	3	6	12	
Dog epithelia (IgE)	Alternaria				104	107	American cockroach			114	80
	Aspergillus				114	113	German cockroach			98	79
	Penicillium				103	92	Fire ant invicta			91	100
	Short ragweed	97	104	92	134		Giant ragweed	87	95	77	101
Dog epithelia (Can f 3)	Short ragweed					183	Giant ragweed			142	226

Target	Mixed w/	Mean % Recovery after mixing and storage for ...											
		Months at 2-8 C					Months at 2-8 C						
		+	1	3	6	12	+	1	3	6	12		
Dog dander (IgE)	Alternaria				96	102	American cockroach				112	97	
	Aspergillus				94	97	German cockroach				110	94	
	Penicillium				87	118	Fire ant invicta				133	112	
	Short ragweed	96	123	91	105		Giant ragweed	95	133	87	108		
Dog dander (Can f 1)	Short ragweed					95	Giant ragweed				122	100	
	+	1	3	6	12	+	1	3	6	12			
Alternaria (IgE)	Aspergillus				94	102	101	American cockroach	92	96	97		
	Penicillium				112	139	124	German cockroach	93	111	106		
	Cladosporium				98	93	107	Fire ant invicta	86	105	90		
	Bipolaris				172	184	190						
Alternaria (Alt a 1)	Aspergillus				116	100	121	94	Fusarium	74			74
	Penicillium				80	121	92	98	Mucor	78			75
	Cladosporium				97	106	90	87	American cockroach	40	39	37	
	Bipolaris				90	99	103	86	German cockroach	79	84	89	
	Epicoecium				77			74	Fire ant invicta	101	126	109	
	Aureobasidium				68			71					
German cockroach (IgE)	Alternaria				79	105		Bipolaris		93	84		
	Aspergillus				99	108		American cockroach		188	215		
	Penicillium				105	87		Fire ant invicta		129	165		
	Cladosporium				96	113							
German cockroach (Bla g 1)	Alternaria				83	124		Bipolaris		111	94		
	Aspergillus				94	103		American cockroach		91	95		
	Penicillium				75	94		Fire ant invicta		110	96		
	Cladosporium				108	93							

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Allergen Compatibilities for Mixtures Stored at 20-25 C

Target	Mixed w/	Mean % Recovery after mixing and storage for ...									
		Months at 20-25 C					Months at 20-25 C				
		+	1	3	6	12	+	1	3	6	12
Meadow fescue grass	Alternaria	35	36	13		Short ragweed	70	60	38		
	Aspergillus	16	18	5		Giant ragweed	57	57	46		
	Penicillium	21	11	7		False ragweed	60	48	34		
	Cladosporium	54	56	36		Western ragweed	82	50	42		
	Bipolaris	54	23	6		Desert ragweed	98	69	61		
	American cockroach	32	17	7		Fire ant invicta	43	39	12		
	German cockroach	28	33	8							
Timothy grass	Alternaria	47	32	16	6	Short ragweed	92	85	70	84	
	Aspergillus	38	20	9		Giant ragweed	91	101	103	175	
	Penicillium	14	8	2		False ragweed	97	101	71		
	Cladosporium	86	77	70		Western ragweed	83	50	83		
	Bipolaris	71	44	22		Desert ragweed	135	100	77		
	American cockroach	47	19	9		Fire ant invicta	51	45	9		
	German cockroach	33	18	10							
Bermuda grass	Short ragweed	101	180	334		Giant ragweed	120	172	347		
Johnson grass	Short ragweed	103	76	126		Giant ragweed	97	81	151		
Cat	Alternaria	100	100	96		American cockroach	104	100	105		
	Aspergillus	91	92	91		German cockroach	96	100	100		
	Penicillium	96	92	91		Fire ant invicta	100	104	100		
	Cladosporium	104	104	105		Short/giant ragweed		105	110	117	
Short ragweed	Alternaria			94		D. farinae	103	96	89		
	Timothy	100	96	98	103	Cat	101	117	96		
	Bermuda		107	99	87	Dog epithelia	95	99	99		
	Johnson		106	93	109	Dog dander	104	104	99		

Target	Mixed w/	Mean % Recovery after mixing and storage for ...									
		Months at 20-25 C					Months at 20-25 C				
		+	1	3	6	12	+	1	3	6	12
D. farinae (IgE)	Alternaria	93	65	78		American cockroach	105	69	80		
	Aspergillus	102	93	86		German cockroach	96	86	98		
	Penicillium	105	86	91		Fire ant invicta	102	118	130		
	Cladosporium	108	76	90		Short ragweed	94	158	138		
D. farinae (Der f 1)	Alternaria	110	111	107		American cockroach	119	91	113		
	Aspergillus	102	79	108		German cockroach	108	102	118		
	Penicillium	108	74	112		Fire ant invicta	103	104	129		
	Cladosporium	120	94	110							
Dog epithelia (IgE)	Alternaria	106	83	95		American cockroach	129	127	97		
	Aspergillus	109	76	94		German cockroach	106	94	82		
	Penicillium	112	114	125		Fire ant invicta	107	102	101		
	Cladosporium	136	120	120		Short/giant ragweed	105	127	101	185	
Dog epithelia (Can f 3)	Alternaria	98	85	57		American cockroach	89	103	112		
	Aspergillus	99	82	69		German cockroach	92	106	105		
	Penicillium	81	107	72		Fire ant invicta	95	126	103		
	Cladosporium	107	112	100		Short/giant ragweed			190	245	
Dog dander (IgE)	Alternaria	92	92	67		American cockroach	109	93	108		
	Aspergillus	84	60	34		German cockroach	79	83	82		
	Penicillium	94	74	62		Fire ant invicta	109	110	88		
	Cladosporium	116	92	113		Short/giant ragweed	101	189	134	232	
Dog dander (Can f 1)	Alternaria	103	97	86		American cockroach	91	68	83		
	Aspergillus	98	98	79		German cockroach	81	82	88		
	Penicillium	103	105	107		Fire ant invicta	82	99	114		
	Cladosporium	99	86	102		Short/giant ragweed			178	123	

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Allergen Compatibilities for Mixtures Stored at 20-25 C

Target	Mixed w/	Mean % Recovery after mixing and storage for ...									
		Months at 20-25 C					Months at 20-25 C				
		+	1	3	6	12	+	1	3	6	12
Alternaria (IgE)	Aspergillus		96	84	90		American cockroach	96	80	88	
	Penicillium		117	114	109		German cockroach	98	111	101	
	Cladosporium		104	86	106		Fire ant invicta	88	103	87	
	Bipolaris		158	169	220		Short ragweed			98	
	Timothy				81						
Alternaria (Alt a 1)	Aspergillus		77	113	77		American cockroach	51	46	46	
	Penicillium		91	123	110		German cockroach	67	87	55	
	Cladosporium		109	100	88		Fire ant invicta	104	119	79	
	Bipolaris		117	97	98						
German cockroach (IgE)	Alternaria			75	43		Bipolaris		71	38	
	Aspergillus			28	14		American cockroach		172	118	
	Penicillium			6	7		Fire ant invicta		156	127	
	Cladosporium			108	73						
German cockroach (Bla g 1)	Alternaria			111	127		Bipolaris		111	114	
	Aspergillus			103	117		American cockroach		107	102	
	Penicillium			68	127		Fire ant invicta		97	111	
	Cladosporium			104	117						
German cockroach (Bla g 2)	Alternaria			105	90		Bipolaris		102	97	
	Aspergillus			85	77		American cockroach		98	98	
	Penicillium			91	90		Fire ant invicta		104	97	
	Cladosporium			107	112						

Conclusions

The allergenic extract compatibility tables provided in this summary are derived from quantitative immunochemical laboratory testing of defined extract mixtures and corresponding single-extract control samples.

Validation of these assays has confirmed that they recognize specific allergenic (IgE-binding assays) or antigenic (specific allergen assays) components of the target extracts within each product mixture.

Extract mixtures comprised of two products from different allergen categories were selected for these studies.

Although most allergen immunotherapy mixtures typically contain a more complex and diverse mixtures of extraction products, the two-component mixtures were formulated to combine particular target and companion extracts known or suspected to be unstable or incompatible under storage conditions relevant to clinical practices.

The presence of fewer extracts and a more limited group of soluble extract constituents also facilitates expression of more specific and consistent antibody-allergen interactions, which promotes accurate and precise quantitation of these activities in the laboratory.

Target extract recoveries for each mixture were analyzed by T tests assuming equal variances, with statistical significance achieved at p values at or below 0.05. Most combinations shaded in yellow and all mixtures highlighted in red were found to exhibit significant levels of allergen degradation.

Glycerinated grass pollen allergens were degraded significantly after mixing with several glycerinated fungal or insect extracts and storage for 1-12 months at 2-8 C, or for shorter time periods at 20-25 C. Meadow fescue and Timothy grass extracts exhibited slightly different recoveries under certain conditions, but clearly possessed more significant susceptibilities to mixing with protease-rich fungal or insect extracts compared to other pollen, dust mite, cat, dog, Alternaria or German cockroach allergens.

These data support the current clinical utilization of glycerinated allergen mixtures for diverse testing and injection treatment regimens, as well as investigations of non-injection modes of immunotherapy, including multi-allergen sublingual-oral administration.

Based on the results from these studies, the expected mixing compatibilities of glycerinated extracts from selected product categories are presented on the next page, with specific allergen combinations noted as compatible (green), risky (yellow) or compromised (red).

Mixing Compatibility of Phenolated, Glycerinated Allergenic Extracts Stored at Refrigeration or Ambient Temperatures

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Compatibility Charts: 2-8 C

Stability of ...
↓

After mixing and storage for 1 month with ...
High-protease Low-protease

	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Green	Yellow	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat	Green	Green	Green	Green	Green
Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ...
↓

After mixing and storage for 3 months with ...

	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Yellow	Yellow	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat	Green	Green	Green	Green	Green
Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ...
↓

After mixing and storage for 6 months with ...

	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Yellow	Yellow	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat	Green	Green	Green	Green	Green
Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ...
↓

After mixing and storage for 12 months with ...

	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat	Green	Green	Green	Green	Green
Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Compatibility Charts: 20-25 C

Stability of ...
↓

After mixing and storage for 1 month with ...
High-protease Low-protease

	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat	Green	Green	Green	Green	Green
Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ...
↓

After mixing and storage for 3 months with ...

	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat	Green	Green	Green	Green	Green
Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ...
↓

After mixing and storage for 6 months with ...

	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat	Green	Green	Green	Green	Green
Dog	Yellow	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ...
↓

After mixing and storage for 12 months with ...

	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat	Green	Green	Green	Green	Green
Dog	Yellow	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green