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GEMTEK® Products Composition Studies

Dustroy

Anti-Allergen Spray

Prepared for:

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2 May, 2000

GEMTEK® Products Composition Studies

Allergens Studied:

Fel d 1 =	Cat allergen
Can f 1 =	Dog allergen
Der p 1 =	Dust Mite allergen
Der f 1 =	Dust Mite allergen
Bla g 1 =	Cockroach allergen

Products Studied and Compared:

Dustroy AA =	Manufactured by ***GEMTEK®*** Products Formulated as a stand alone ready-to-use anti-allergen spray
Dustroy EX =	Manufactured by ***GEMTEK®*** Products Formulated as a stand alone anti-allergen spray or used as an additive for other cleaning formulations
Dustroy EX2 =	Manufactured by ***GEMTEK®*** Products Used as a blinded control
Dustroy aa =	Manufactured by ***GEMTEK®*** Products Same formulation as Dustroy AA with a different concentration level
AllerCare™ Dust Mite Allergen Spray =	Manufactured by S. C. Johnson (product recalled during test trials) Formulated with benzyl benzoate as a ready-to-use spray
Allersearch® ADS™ = Allergen Spray =	Manufactured by Alkaline Corporation Formulated with 3% tannic acid as a ready-to-use spray

Terms:

Polyclonal =	Of, or derived from several different clones of cells.
Monoclonal =	Of, forming, or derived from a single clone.
BSA =	Bovine Serum Albumin

GEMTEK® Products Composition Studies

Purpose:

These studies were designed to investigate a variety of products that make claims to denature or destroy a number of common household allergens. Since these products are designed to affect proteins that can cause allergy, they could also have the ability to interfere with the protein-based assays used to measure these allergens. Thus, to evaluate their effects on different allergens, their effects on the assays must first be evaluated. Secondly, when this problem is encountered, methods developed to circumvent this problem must be evaluated. Once this is done, their ability to affect various allergens can be tested. Thus, this study was performed in three different stages.

I. The first stage consisted of testing whether these products could indeed interfere with the assays used to measure allergens. These initial experiments were designed also to measure the extent of this interference when present.

Methods: 50-microliter aliquots were placed into microtiter wells that have previously been coated with the appropriate antibodies for 15 minutes. Following this, the plates were washed and used to assay the allergens the antibodies are directed against. Data are presented at % inhibition of the assays.

II. The second stage of this project was designed to investigate what measures might be used to prevent the interference of the allergen assays effects. This step is crucial to determining whether these products have the capability to destroy particular allergens.

Methods: Each composition at various dilutions was mixed with and without 10% BSA and the above experiment repeated. These data are reported as the percent of the allergen measured compared to controls.

III. The third stage of these experiments was to utilize what had been learned in the first two stages to test whether these products had an actual effect on the allergens tested.

Methods: Fine dust was extracted with various dilutions of each composition. These extracts were then diluted with and without 10% BSA for assay. The amount of each allergen destroyed was determined by comparing recovery to that of controls. Only those dilutions that showed neutralization of the composition effects on the assay can be considered significant for the denaturation of allergen. Additional experiments were performed using two purified solutions containing 5 ug/ml of major mite allergens.

Results:

Ia. Inhibition Effect of Compositions on Polyclonal Assay (Dust Mite Assay):

- Dustroy AA was found to inhibit the assay. This disappeared at the 1/16 dilution. (Maximum Inhibition = 75%)
- Dustroy EX was found to inhibit the assay. This disappeared at the 1/32 dilution. (Maximum Inhibition = 94%)
- Dustroy EX2 was found to have no assay inhibition effects.
- AllerCare was found to inhibit the assay. This disappeared at the ½ dilution. (Maximum Inhibition = 53%)
- Allersearch ADS was found to inhibit the assay. This disappeared at the ½ dilution. (Maximum Inhibition = 41%)

Thus, the effects on the polyclonal assays can be ordered from most to least as:

- Dustroy EX
- Dustroy AA
- AllerCare
- Allersearch ADS
- Dustroy EX2

These data are presented in Table Ia and accompanying graphs.

Ib. Inhibition Effect of Compositions on Monoclonal Assay (Der f 1):

- Dustroy AA was found to inhibit the assay. This disappeared at the 1/4 dilution. (Maximum Inhibition = 92%)
- Dustroy EX was found to inhibit the assay significantly at the undiluted concentration only. (Maximum Inhibition = 95%)
- Dustroy EX2 was found to have no assay inhibition effects.
- AllerCare was found to slightly inhibit the assay at the undiluted concentration. (Maximum Inhibition = 27%)
- Allersearch ADS was found to significantly inhibit the assay up to the 1/2 dilution. (Maximum Inhibition = 85%)
- Dustroy aa was found to inhibit the assay significantly up to the 1/8 strength. (Maximum Inhibition = 96%)

Thus, effects on the monoclonal assays can be ordered from most to least as:

- Dustroy aa
- Dustroy AA
- Allersearch ADS
- Dustroy EX
- AllerCare
- Dustroy EX2

These data are presented in Table Ib and accompanying graphs.

Notes:

1. Assay inhibition effects were greater with the polyclonal assay than the monoclonal assay.
2. Dustroy aa was the most potent inhibitor of the monoclonal assay.

IIa. Inhibition of Composition's Effect on Polyclonal Assay Using 10% BSA:

- Dustroy AA – 10% BSA was not able to inhibit composition's effects on the assay at the neat concentration. Reduction in assay inhibition was noted as follows: 46% at the 1/2 dilution, 30% at the 1/4 dilution and completely at the 1/8 dilution. Thus, results of assay for allergen can be verified at the 1/8 dilution.
- Dustroy EX – 10% BSA was not able to inhibit composition's effects on the assay at the neat concentration. Addition of 10% BSA resulted in inhibition of the 1/2 dilution down to 8%.
- 67% at the 1/2 dilution to 8%. Thus, results of assay for allergen can be verified at the 1/2 dilution.
- Dustroy EX2 – There was no inhibition of the composition's effect on the assay and again no effect on the assay was seen with 10% BSA. This serves as a control to demonstrate that the process of adding 10% BSA had no significant effect on the assay.
- AllerCare – 10% BSA was not able to inhibit composition's effects on the assay at the neat concentration and showed some enhancement of the inhibition at the 1/2 dilution. There was no effect of this composition at the 1/4 dilution or without 10% BSA. Thus, results of assay for allergen can be verified at the 1/4 dilution.
- Allersearch ADS – 10% BSA was able to inhibit composition's effects on the assay by 14% at the neat concentration. Reduction in assay inhibition was complete at the 1/2 dilution. Thus, results of assay for allergen can be verified at the 1/2 dilution.

These experiments also verified the amount of inhibition by these compositions on the polyclonal assays as noted in Stage I above.

These data are presented in Table IIa and accompanying graphs.

IIb. Inhibition of Composition's Effect on Monoclonal Assay Using 10% BSA:

- Dustroy AA – 10% BSA was able to completely inhibit composition's effects on the assay at the neat concentration. Reduction in assay inhibition was noted as follows: 100% at the neat concentration and1/2 dilution. Thus, results of assay for allergen can be verified at the neat concentration.
- Dustroy EX – 10% BSA was able to completely inhibit composition's effects on the assay at the neat concentration. Thus, results of assay for allergen can be verified using the neat concentration.
- Dustroy EX2 – There was no inhibition of the composition's effect on the assay and again no effect on the assay was seen with 10% BSA. This serves as a control to demonstrate that the process of adding 10% BSA had no significant effect on the assay.

- AllerCare – 10% BSA was able to inhibit composition's effects on the assay by 100% at the neat concentration. Thus, results of assay for allergen can be verified at the neat concentration.
- Allersearch ADS – 10% BSA was able to inhibit composition's effect on the assay 100% at the neat concentration. Thus, results of assay for allergen can be verified at the neat concentration.

The extent of the inhibition effects on the assay were similar to the first experiments in Stage I.

These data are presented in Table IIb and accompanying graphs.

III. Assessment of Composition's Effect on Various Allergens as Measured by Polyclonal & Monoclonal Assays:

Effects on Allergens using Polyclonal Assays:

Note: Results of the polyclonal assays were somewhat variable. They were also more sensitive to the effects of the composition. The composition effects were more difficult to neutralize with the polyclonal assays.

Effects on Allergens using the monoclonal assays:

Dustroy AA:

- Fel d1 (Cat): Destroyed 80% of the allergen at ¼ dilution, whereas the effect on the assay at this dilution was 30% inhibition. There was no effect at the 1/8 dilution.
- Can f1 (Dog): Destroyed 70% of the allergen at ¼ dilution, whereas the effect on the assay at this dilution was 30% inhibition. Destroyed 42% at 1/8 dilution.
- Der p1 & Der f1 (Dust Mite): Total allergen was 30% less at ¼ dilution, but the assay was expected to be 30% lower at this dilution. Thus no effect was seen. There was no effect at the 1/8 dilution, which is in agreement with the ¼ dilution results.
- Bla g1 (Cockroach): There was no effect at the ¼ or 1/8 dilution.

Dustroy EX:

- All Allergens: There was no effect at the 1/8 dilution. There was however, notably diminished cat and mite allergen as compared to the others at the ¼ dilution. Since the protein in the dust that was extracted may have protected the assay somewhat, this can be taken as evidence that Dustroy EX has some effect on cat and mite allergens.

Dustroy EX2:

- All Allergens: There was no effect at any dilution.

AllerCare:

- Fel d 1 (Cat): Destroyed 100% of the allergen at the neat concentration. There was no effect at any other dilution.
- Can f 1 (Dog): Destroyed 100% of the allergen at the neat concentration. Destroyed 37% at 1/2, and 10% at 1/4 dilutions. There was no effect at the 1/8 dilution.
- Der p 1 (Dust Mite): Destroyed approximately 94% of the allergen at the neat concentration. Destroyed 59% at the 1/2 dilution. There was no effect at the 1/4 and 1/8 dilutions.
- Der f 1 (Dust Mite): Destroyed 100% of the allergen at the neat concentration. There was no effect at any other dilution.

Allersearch ADS:

- Fel d 1 (Cat): Destroyed approximately 99% of the allergen at the neat concentration. Destroyed 95% at 1/2, 76% at 1/4, and 39% at 1/8 dilutions.
- Can f 1 (Dog): Destroyed 100% of the allergen at the neat concentration and 1/2 dilution. Destroyed 96% at 1/4, and 88% at 1/8 dilutions.
- Der p 1 (Dust Mite): Destroyed 100% of the allergen at the neat concentration and 1/2 dilution. Destroyed 93% at 1/4, and 74% at 1/8 dilutions.
- Der f 1 (Dust Mite): Destroyed 100% of the allergen at the neat concentration and 1/2 dilution. Destroyed 95% at 1/4, and 87% at 1/8 dilutions.

Note: All of the above experimental points were considered valid except those with the Allersearch ADS product. This is because with the exception of the Allersearch ADS they were all neutralized by 10% BSA. The Allersearch ADS product was neutralized approximately 41% at the 1/4 dilution and 100% at the 1/8 dilution. Thus, only those points at the 1/8 and above can be considered valid.

These data are reviewed in Table IIIa and accompanying graphs.

IIIb. Comparison of Effects of Dustroy aa and Allersearch ADS on Der f 1 and Der p 1 (Dust Mite) Allergens in Solution

Because of problems with foaming with fine dust and some of the compositions, it was determined to examine the effects of these two products on allergen in solution. Two experiments were performed, one with Der f 1 allergen and one with Der p 1 allergen. Equal amounts of dilutions of each composition were added to solutions of antigen diluted to 5 ug/ml for 15 minutes at room temperature. For assay, each sample was then diluted into the assay range using 10% BSA-PBS. This resulted in dilutions of 1/250 to 1/1250 to bring the allergen concentration within assay range. This is far above the dilutions where assay interference was seen .

Dustroy aa:

- Der p 1 (Dust Mite): Destroyed 100% of the allergen at the 1/2, 1/4, and 1/8 dilutions. Destroyed 95% at 1/16, 85% at 1/32, and 70% at 1/64 dilutions.

Allersearch ADS

- Der p 1 (Dust Mite): Destroyed 34% of the allergen at the 1/2 dilution. There was no effect at any other dilution.

Dustroy aa:

- Der f 1 (Dust Mite): Destroyed 100% of the allergen at the 1/4 dilution. Destroyed 80% at 1/8, 64% at 1/32, 19% at 1/64, and 0% at 1/128 dilutions. (Note: the 1/16 dilution data is not presented due to a bad experimental point.)

Allersearch ADS

- Der f 1 (Dust Mite): There was no effect at any dilution from 1/4 to 1/128.

These data are presented in Table IIIb.

Summary of Findings:

Of the products examined, Dustroy AA, Dustroy EX, AllerCare and Allersearch ADS have the capability to inhibit a polyclonal microtiter assay. Dustroy EX2 did not.

The order of their potency in this regard was:

- Dustroy EX
- Dustroy AA
- AllerCare
- Allersearch ADS

Dustroy aa composition was made available after the effects on the polyclonal assay were examined. Thus, no data on its ability to interfer with a polyclonal assay is available.

Of the products examined, Dustroy EX, Dustroy AA, AllerCare, Allersearch ADS and Dustroy aa have the capability to inhibit monoclonal assays. Dustroy EX2 did not.

The order of the potency in this regard was:

- Dustroy aa
- Dustroy AA
- Allersearch ADS
- Dustroy EX
- AllerCare

The effects of these products were different depending upon whether the assays were polyclonal or monoclonal assays.

The addition of 10% BSA to all the compositions showed protective effects on both the polyclonal and monoclonal assays. These differed according to the dilution of the composition used. The 10%BSA inhibited all compositions at the neat concentration except for Allersearch ADS on the monoclonal assay.

Dustroy AA was shown to have denaturing effects on Fel d 1, Can f 1, Der p 1 and Der f 1 in dust samples containing these allergens in monoclonal assays.

Dustroy EX was shown to have denaturing effects on Fel d 1 only in dust samples containing these allergens.

Dustroy EX2 was shown to have no denaturing effects on allergens in dust samples.

AllerCare was shown to have denaturing effects on Fel d 1 and Der f 1 only at the neat concentration in dust samples containing these allergens. This product showed effects on Can f 1 and Der p 1 at the neat and 1/2 dilution only.

Allersearch ADS was shown to have denaturing effects on Fel d 1, Can f 1, Der p 1 and Der f 1 in dust samples containing these allergens.

Dustroy aa was shown to have very effective denaturing effects on Der p 1 and Der f 1 in liquid samples containing these allergens.

Allersearch ADS was not effective in denaturing major mite allergens in liquid samples.

The major allergen from Dermatophagoides p. seems to more easily denatured than the major allergen from Dermatophagoides f.

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