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Table 1. Allergens Tested

Allergen	Source
Der p 1	*Dermatophagoides pteronyssinus,* Dust Mite
Fel d 1	*Felis domesticus,* Cat
Can f 1	*Canis familiaris,* Dog
Bla g 2	*Blatella germanica,* Cockroach
Amb a 1	*Ambrosia artemisiifolia,* Short Ragweed Pollen

Part 2: MARIA Method Interference Test

An interference test was performed to determine if the cleaning product formulations have an effect on Der p 1, Fel d 1, Can f 1, Bla g 2, and Amb a 1 allergen detection in the MARIA system. This was done by preparing a solution containing 1% product and 99% allergen standard mix that was allowed to incubate on a rocking platform for two hours. A control sample was made similarly, using 1% deionized water instead of product. These preparations were analyzed using MARIA, and the concentration of each allergen was plotted as a standard curve. Curves for each of the two products were compared to the control by overlaying them in a Microsoft Excel plot in order to detect potential interferences in the MARIA assay (Figures 1-5). Allergen concentration was expressed in nanograms per milliliter (ng/mL) and plotted against median fluorescence intensity (MFI).

Figure 1. Der p 1 Interference Test

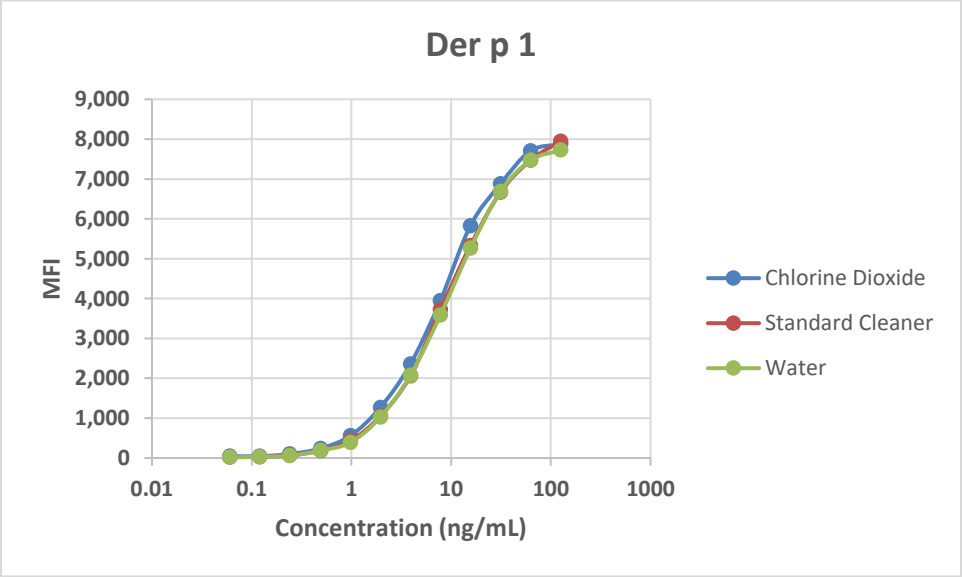

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Figure 2. Fel d 1 Interference Test

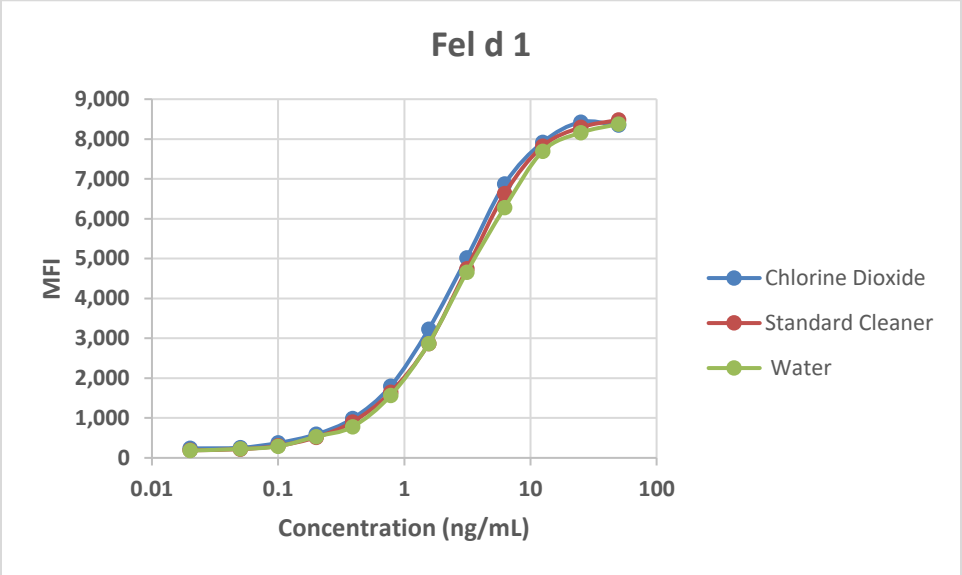

Figure 3. Can f 1 Interference Test

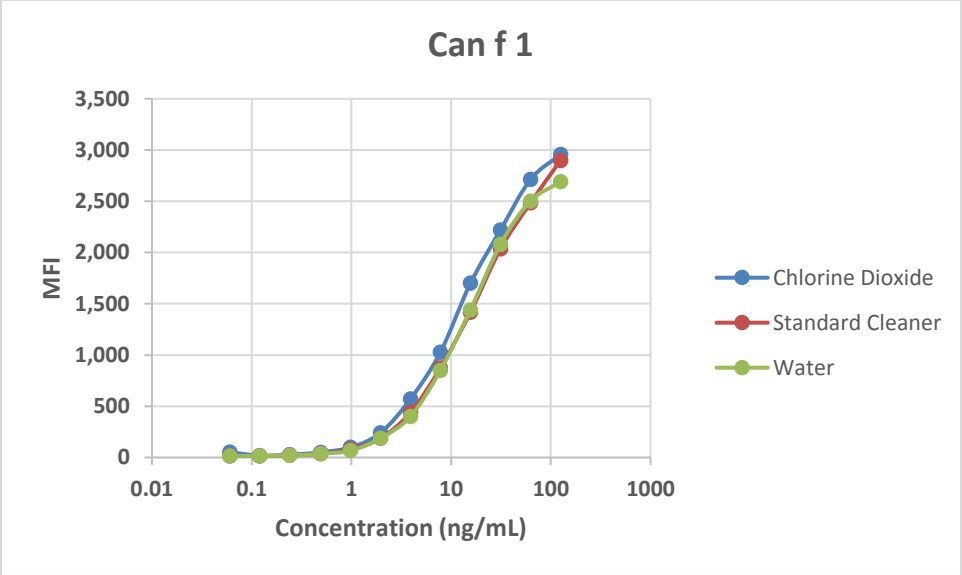

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Figure 4. Bla g 2 Interference Test

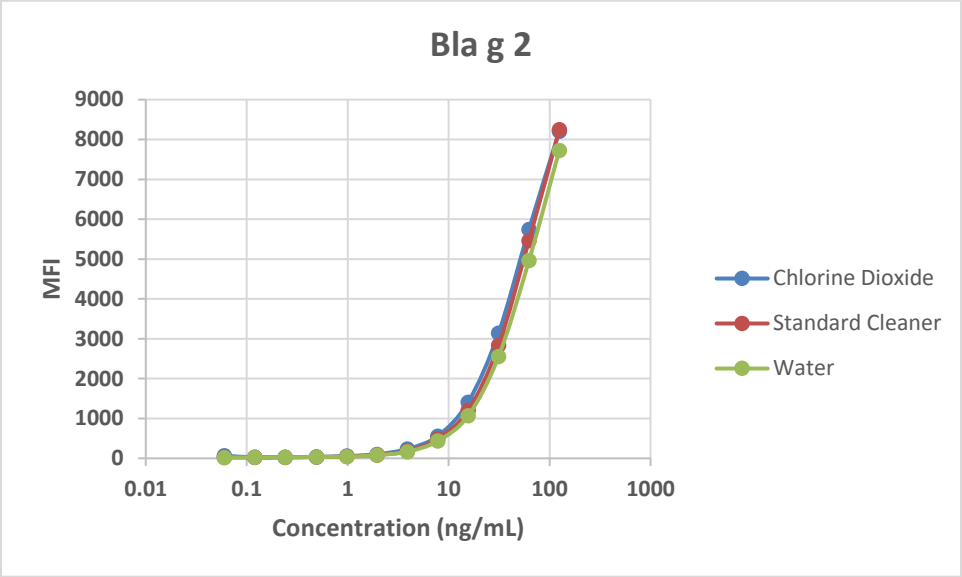
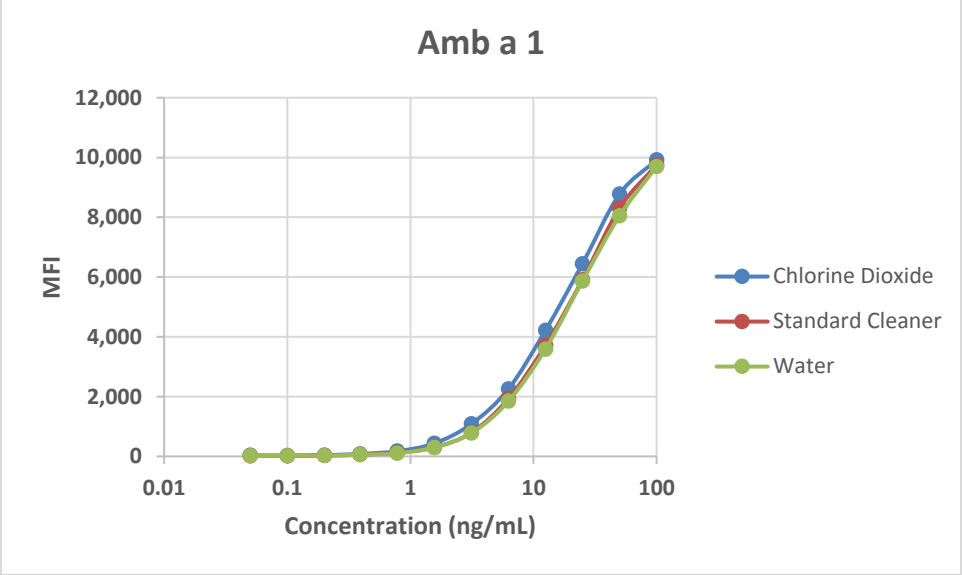


Figure 5. Amb a 1 Interference Test



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Part 3: Evaluation of Allergen Reducing/Denaturing Properties in Solution

The allergen solution prepared in Part 1 was used to evaluate the effect of the Chlorine Dioxide and Standard Cleaning product on five allergens in solution, compared to a PBS control. Three test samples were prepared by mixing 0.5mL of allergen solution with 0.5mL of each of the two products and PBS in separate tubes. The samples were gently agitated on a rocking platform for two hours at room temperature. All samples were analyzed in duplicate and at nine dilutions using MARIA. The average (based on duplicate testing) allergen concentration in each sample was reported in units of ng/mL, and the effect on each allergen was expressed as a percent reduction compared to the PBS control (Tables 2-6).

Table 2. Allergen Reduction/Denaturing Properties in Solution - effect of cleaning products on Der p 1 versus PBS control.

Product	Der p 1, Dust Mite Concentration (ng/mL)	**Percent Reduction**
Chlorine Dioxide	<0.60	**99-100**
Standard Cleaner	1,142.40	**57**
Control	2,664.46	**-**

Table 3. Allergen Reduction/Denaturing Properties in Solution - effect of cleaning products on Fel d 1 versus PBS control.

Product	Fel d 1, Cat Concentration (ng/mL)	**Percent Reduction**
Chlorine Dioxide	<0.20	**99-100**
Standard Cleaner	2,936.35	**0**
Control	2,802.03	**-**

Table 4. Allergen Reduction/Denaturing Properties in Solution - effect of cleaning products on Can f 1 versus PBS control.

Product	Can f 1, Dog Concentration (ng/mL)	**Percent Reduction**
Chlorine Dioxide	<0.60	**99-100**
Standard Cleaner	1,398.25	**5**
Control	1,475.74	**-**

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Table 5. Allergen Reduction/Denaturing Properties in Solution - effect of cleaning products on Bla g 2 versus PBS control.

Product	Bla g 2, Cockroach Concentration (ng/mL)	Percent Reduction
Chlorine Dioxide	<2.40	**99-100**
Standard Cleaner	5,215.03	**0**
Control	4,792.37	**-**

Table 6. Allergen Reduction/Denaturing Properties in Solution - effect of cleaning products on Amb a 1 versus PBS control.

Product	Amb a 1, Ragweed Pollen Concentration (ng/mL)	Percent Reduction
Chlorine Dioxide	<1.00	**99-100**
Standard Cleaner	874.29	**16**
Control	1,036.87	**-**

Comments

The interference test (Part 2) demonstrated that the two products do not interfere with the MARIA method, as indicated by the overlapping curves compared with the control. For Part 3, when the products were mixed with allergen solution, the Chlorine Dioxide product denatured all five allergens, preventing them from being detected in the MARIA system.The Standard Cleaning Product had a moderate effect on Der p 1 dust mite allergen, reducing the measured concentration by 57%, and a minor effect on short ragweed pollen allergen, Amb a 1, reducing the measured concentration by 16%. The Standard Cleaning product had no measurable effect on the other three allergens tested: Fel d 1 (cat), Can f 1 (dog) and Bla g 2 (cockroach). Typically, a percent reduction of 20 or less is not considered to have a denaturing effect on the allergen. A reduction of 21-39 is considered mild, while 40-69 is considered moderate. A 70-100 percent reduction in allergen concentration is significant.

Notes: MARIA is an antibody-based immunoassay method developed by InBio utilizing Luminex xMAP® technology. The method has been validated through an international, multi-center trial, and studies utilizing MARIA have been published in leading scientific journals. InBio's MARIA 8-plex allergen analysis service is ISO/IEC 17025:2015 accredited (PJLA accreditation #76453; certificate #L20-587).
Percent reduction of allergen in the test samples is based on allergen concentration measured by the MARIA assay. Because there is a limit to the assay sensitivity, it is not possible to determine complete (100%) allergen reduction.

Stephanie Filep
Director of Laboratory Services