



## Comparison of AllerSnap® and SuperSnap® to ELISA

### Detection of Crustacean in Complex Food Matrices

#### Introduction

Food facilities can verify their allergen cleaning efficiency by running either allergen-specific tests (e.g., ELISA tests) or proxy tests to measure other components in complex foods (e.g., ATP or protein residue) as indicators for cleanliness. Here, we compare three methods for monitoring crustacean contamination using dilutions of 4 complex food matrices.

#### Equipment, Supplies and Reagents

The following tests, instruments and samples were used in this study:

- Complex source of crustaceans – prawn crackers, crab paste, prawn salad sandwiches, prawn cocktail crisps
- Stomacher® bags (Seward)
- Crustacean ELISA Kit [Bio-Check (UK)]\*
- SuperSnap® High Sensitivity ATP Surface Test (Product no. SUS3000) with a Hygiena® luminometer
- AllerSnap® High Sensitivity Protein-Residue Surface Test (Product no. ALS-100)

#### Sample Preparation and Enrichment Method

10 g of each matrix was added to 90 mL of extractant from the ELISA kit. The samples were homogenized in Stomacher bags for 1 minute. The samples were allowed to stand for 20 minutes before the supernatants were carefully removed. The resulting supernatants were the 10% suspensions of prawn crackers, crab paste, prawn salad sandwiches and prawn cocktail crisps.

The 10% suspensions were then serially diluted to 1%, 0.1%, 0.01%, 0.001% and 0.0001% in the ELISA diluent.

Each dilution of each matrix was then used to run 3 tests:

1. ELISA test for true allergen content – ppb of crustacean allergen per dilution:

The ELISAs were run using the manufacturer's instructions. Each plate had a standard curve for crustacean allergen as supplied by the manufacturer.

2. ATP content testing using SuperSnap devices:

The SuperSnap devices were inoculated with 10 µL of each dilution, 2 replicates of each dilution were run and the mean RLU was compared with the true allergen content.

3. Protein content testing using AllerSnap devices:

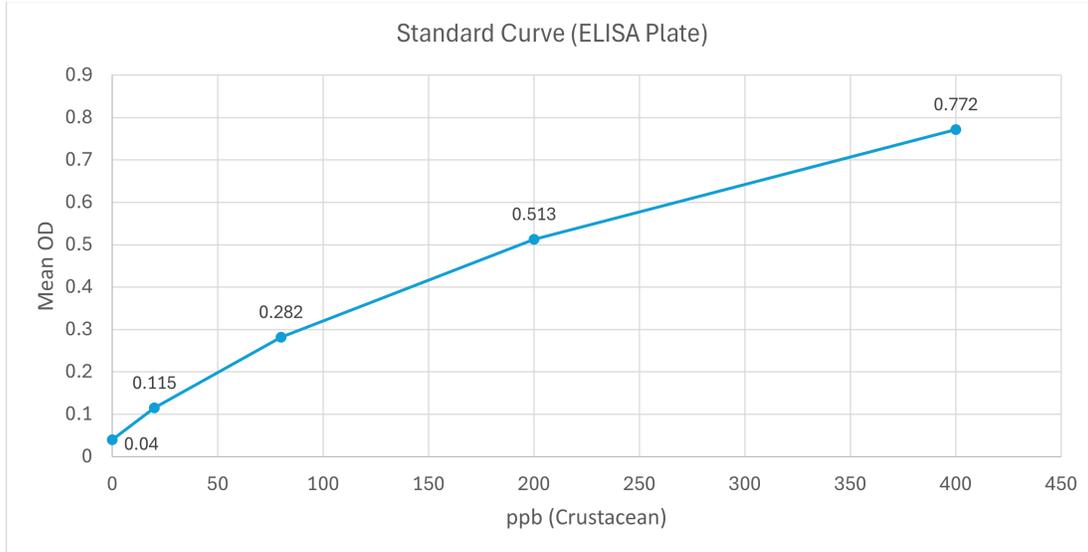
The AllerSnap devices were inoculated with 10 µL of each dilution, and 2 replicates of each dilution were tested. The devices were incubated at 37 °C for 30 minutes before being assessed for color formation.

\* This kit was chosen based on performance and similarity to our AlerTox® ELISA kits, which were still in development at the time of this study.

## Results

### Standard Curve

A representative standard curve from the ELISA kit is shown in Figure 1.



**Figure 1. Standard Curve from an ELISA Plate.**

### Comparisons of Results from SuperSnap and AllerSnap with ELISA Kits

Tables 1 through 4 summarize the results from ELISA, SuperSnap and AllerSnap testing of the 4 sample matrices. Light blue shading highlights the limit of detection (LOD) for the test for the sample matrix used.

**Table 1. Results for Prawn Cracker Dilutions.**

Prawn Cracker Dilutions (%)	Allergen ppb	ELISA, Mean OD	SuperSnap, Mean RLU	AllerSnap	
				Rep 1	Rep 2
10	35,088	1.44	381.5	++++	++++
1	3,508	1.358	39	-/+	-
0.10	350	0.725	4	-	-
0.01	36	0.159	0.5	-	-
0.001	0	0.057	0	-	-
0.0001	0	0.044	0	-	-
Blank	0	0.041	0	-	-

**Table 2. Results for Crab Paste Dilutions.**

Crab Paste Dilutions (%)	Allergen ppb	ELISA, Mean OD	SuperSnap, Mean RLU	AllerSnap	
				Rep 1	Rep 2
10	348,110	1.004	212.5	+++	+++
1	34,811	1.037	2,685.5	-/+	-/+
0.10	3,481	0.947	1,115.5	-	-
0.01	348	0.72	158	-	-
0.001	62	0.206	13.5	-	-
0.0001	0	0.062	1.5	-	-
Blank	0	0.043	1.5	-	-

**Table 3. Results for Prawn Salad Sandwich Dilutions.**

Prawn Salad Sandwich Dilutions (%)	Allergen ppb	ELISA, Mean OD	SuperSnap, Mean RLU	AllerSnap	
				Rep 1	Rep 2
10	377,000	1.318	5,738.5	+	++++
1	37,700	1.262	2,385	-/+	-/+
0.10	3,770	1.174	242.5	-	-
0.01	377	0.772	33	-	-
0.001	25	0.138	3.5	-	-
0.0001	0	0.05	2	-	-
Blank	0	0.046	1.5	-	-

**Table 4. Results for Prawn Cocktail Crisp Dilutions.**

Prawn Cocktail Crisp Dilutions (%)	Allergen ppb	ELISA, Mean OD	SuperSnap, Mean RLU	AllerSnap	
				Rep 1	Rep 2
10	0	0.048	4,570.5	++++	++++
1	0	0.048	3,223.5	-	-
0.10	0	0.040	284	-	-
0.01	0	0.040	36	-	-
0.001	0	0.053	5	-	-
0.0001	0	0.042	2.5	-	-
Blank	0	0.042	2	-	-



## Conclusions

The measurable levels of crustacean allergens in each of the food matrices is different and depends on the testing method.

1. Specific allergen testing using ELISA tests:

The dilution levels measurable by the crustacean-specific allergen tests were 0.01% for prawn crackers, 0.001% for crab paste, 0.001% for seafood sandwiches and no detection for prawn cocktail crisps.

2. ATP determination using the Hygiena SuperSnap device and a Hygiena luminometer:

The dilution levels measurable by SuperSnap devices were 0.01% for prawn crackers, 0.001% for crab paste, 0.01% for prawn salad sandwiches and 0.01% for prawn cocktail crisps.

3. Non-specific protein determination using the Hygiena AllerSnap device:

The dilution levels detectable by AllerSnap devices were 1% for prawn crackers, 1% for crab paste, 1% for prawn salad sandwiches and 10% for prawn cocktail crisps.

## Discussion

The level of cleanliness required for a surface dictates which regime is most appropriate for routine testing.

The cleanest surfaces can be attained by monitoring with ELISA crustacean tests, like the AlerTox ELISA Crustacean kits (data not shown here; Product no. KIT3059).

In cases of complex, high-allergen risk situations, operational needs may require testing for specific allergens at very low levels using antibody-based tests, like ELISAs. However, this method may not be suitable for all verification needs because it takes several hours to complete, requires a plate reader and is more costly.

Because the levels of crustacea-specific allergens that cause reactions in people is not known, the use of proxy tests, such as ATP or non-specific protein, can be an excellent way to maintain control of daily surface cleanliness without sacrificing time, sensitivity or cost.

- In terms of sensitivity, the next best testing regime compared to ELISAs is using the Hygiena SuperSnap device, which provides results in less than 1 minute and to a level that is slightly less sensitive than direct allergen-testing methods.

Note that this study used the EnSURE® Luminometer (discontinued) to measure results from the SuperSnap devices. We recommend using the newer version: the EnSURE® Touch Luminometer, which provides essentially the same results [1].

- The simplest and most cost-effective method is using the Hygiena AllerSnap device, which has a higher allergen-detection level that may be acceptable in some situations.

If testing plans require multiple test types with various schedules and locations, the SureTrend® platform can simplify how you can most efficiently track and trend test results. Learn more at [www.hygienea.com/analytics](http://www.hygienea.com/analytics).

To learn more about allergen testing options [2] and the EnSURE Touch Luminometer, visit [www.hygienea.com](http://www.hygienea.com).

## References

The following technical bulletins are available at [www.hygienea.com/documents](http://www.hygienea.com/documents).

1. EnSURE® Touch takes ATP Monitoring to the Next Level.  
*Comparison study of three Hygienea luminometers.*
2. Rapid allergen test comparison for cleaning verification.  
*Comparison of rapid tests for protein residue, ATP, and specific allergens (lateral flow tests) vs. ELISAs.*