Comparison of Hygiena® AllerSnap® Total Protein, SuperSnap® ATP, AlerTox® and GlutenTox® Sticks (Lateral Flow) Rapid Tests for Allergen Cleaning Verification

An internal evaluation was conducted to evaluate the utility of three rapid allergen test methods for cleaning verification. Assay sensitivity (limits of detection) were compared using three different surface testing assays, and compared to food testing assays using ELISA and the reference doses of food allergens VITAL cumulative ED_{01}, (eliciting dose in 1% of those allergic) to ensure safety of the allergenic population. The results indicate that appropriate rapid test selection is a function of multiple factors including facility design, workflow, product type/s, and SOP stringency.

Introduction: The Role of Allergen Detection in Cleaning Verification

Recognized as a growing problem in most countries, food allergies affect approximately 2.5% of the general population worldwide, with reported prevalence rates ranging from 1% to 10%. Therefore, verifying effective cleaning processes to reduce allergen exposure and cross-contamination risk is a critical component of HACCP standard operating procedures.

Effective allergen cleaning verification occurs on-site according to the allergen control testing plan. This process includes frequent confirmation that established cleaning procedures are effectively removing allergen residues and contaminants. Each facility's allergen control team will assess the optimal rapid test method according to facility design and workflow needs, constituent product ingredients, as well as test method and performance. Verification is typically conducted with an adequately sensitive, visually interpreted, rapid test which may include protein residue, ATP, and/or immunochromatographic lateral flow methods.

Rapid Method Options

Protein Residue Detection

Detection of protein residues, including allergen proteins, is a quick and reliable method for verifying cleaning efficacy and mitigating cross-contamination risk. When evaluating a protein residue test, sensitivity threshold should be considered with reference to facility needs and product line. Because protein residue detection tests are non-specific, any residual organic protein above the limit of detection (LOD) will result in a positive test indicating the necessity for recleaning. In cases where specific allergen protein detection is required or desirable, a lateral flow method is preferred.

Adenosine Triphosphate (ATP) Bioluminescence

Accuracy, ease of use, time-to-results and quantified, objective measures have made ATP detection a widely adopted industry standard for cleaning verification and allergen cross-contamination prevention. Although generally more sensitive than protein residue methods, these tests detect total ATP and do not differentiate specific allergen proteins. So where specific allergen protein detection is indicated, lateral flow is a better option.

Immunochromatographic Lateral Flow

Lateral flow rapid tests are available as strips/sticks or in cassette format. Binding of a test-specific antibody results in a visible line indicative of a positive result (presence of allergen). These tests detect a single allergen protein and offer a higher degree of sensitivity for specific allergen risk mitigation and adherence to more stringent SOPs.

Method: Sample Preparation

Samples were prepared for analysis using the indicated procedures below.

Surface Testing:

- Allergens were diluted in pyrogen-free water
- Various amounts were applied to surfaces and allowed to air dry
- Dry surfaces were swabbed and tested according to the AlerTox®/GlutenTox® Sticks protocols (approximate 16 cm^2 surface area)

Foodstuff Samples:

- Each food type was suspended in water
- Homogenize and add extraction buffer, following the ELISA protocol for each specific antigen
- Centrifuge and analyze according to the specific ELISA procedure
- The food homogenate supernatant was used in a dilution series to determine the amount of the specific allergen protein

Individual Test Performance

Rapid tests were performed according to manufacturer instructions. A brief summary of each is described in Table 1 below.

Table 1. Rapid Test Summaries

Test Method	Sample Type	Detection Target	Incubation Temp	Incubation Time	Results Reporting
AllerSnap®	Surface Swab	Total Protein	37 °C	30 minutes	Semi-Quantitative
SuperSnap®	Surface Swab	Total ATP	15-25 °C	30 seconds	Semi-Quantitative
AlerTox/GlutenTox LF	Surface Swab	Specific Allergen Protein	15-25 °C	10 minutes	Qualitative
AlerTox/GlutenTox Rapid ELISA	Food Sample	Specific Allergen Protein	15-25 °C	60 minutes	OD_{450nm} Quantitative

Surface Testing Options

Qualitative

Semi-Quantitative

Food Testing Options

AlerTox ELISA | GlutenTox ELISA

Results: Allergen Protein Sensitivity Comparison by Test Method

Table 2. Comparative Rapid Test LOD

	Surface			Foodstuff	
Big 9 Allergens	**Total Protein Residue Rapid Test, AllerSnap (ppm) (µg/mL)**	**Total ATP High Sensitivity Rapid Test, SuperSnap (ppm) (µg/mL)**	**Allergen Specific Rapid LF Test, AlerTox/GlutenTox Lateral Flow (µg or ng/cm²)**	**Reference|Validation Method, AlerTox/ GlutenTox Rapid ELISA (ppm)**	**VITAL LOD Reference Dose ED_{01}* (mg protein)**
Gluten	630	63	10 ng/cm²	0.3	0.7
Egg	89	42	1 µg/16 cm²	0.5	0.2
Milk (Total)	623	16	1 µg/16 cm²	0.05	0.2
Peanut	52	52	4 µg/16 cm²	0.3	0.2
Tree Nut**	from 1.0	from 1.0	2 µg/16 cm² (walnut)	from 0.1**	Varied** (0.03 - 0.1)
Soy *(Plus)*#	100	20	0.15 µg/16 cm²	0.016	0.5
Fin Fish	100	Not Tested	50 µg/16 cm²	1.4	1.3
Shell Fish/Crustacean	36	25	50 µg/16 cm²	0.001	25
Sesame	59	7	3.5 µg/16 cm²	0.2	0.1

* https://vital.allergenbureau.net, https://vital.allergenbureau.net/wp-content/uploads/2021/03/VSEP-2019-Summary-Recommendations_FINAL_Sept2019.pdf
ED_{01} is dose which elicicts allergic symptoms in 1% of the allergic population

**LOD varies dependent on nut type

Value with AlerTox Sticks Soy Plus kit (previous kit was 50 µg/16 cm²)

Discussion: Allergen Limits of Detection

Sensitivity is one of the primary considerations when selecting a cleaning verification assay. A LOD set too high can lead to false-negative results, whereas a LOD set too low can trigger an unnecessary and time-consuming reclean leading to potential product release delays. As such, it is important to select the best-fit solution specific to the facility and product line.

To support diverse food safety needs, Hygiena offers a line of rapid allergen tests with a range of sensitivities derived from individual test technologies while referencing US FDA recommended Analytical, Safety, and Risk Assessment methods.

Conclusion

This analysis provides a useful summary of comparative allergen detection across test methods. Insight gained allows the allergen control team to identify the best fit-for-purpose method. For example, in a facility where shellfish is the sole allergen risk, the overall cleaning verification tests, AllerSnap (total protein) and SuperSnap (total ATP), provide sufficient sensitivity to support comprehensive food safety. In cases where there is a need to identify a suspected cross-contamination, use of AllerSnap or SuperSnap may also provide sufficient sensitivity to confirm or rule-out exposure. Whereas in the case of complex, high allergen risk situations, utilization of AlerTox/GlutenTox lateral flow sticks or ELISA tests for reliable identification of specific allergens at very low levels, would better fulfill operational needs.

In summary, selection of the appropriate rapid test should consider test sensitivity threshold (LOD), ease of use, and SOP specifications as indicated by the specific facility, product line, and allergen control team recommendations.

For more information about Hygiena's test menu visit: www.hygiena.com/allergen.