



Allergen Detection in Food Safety

Analysis & Application





Allergen Detection of the Big 9

SSOP Cleaning Verification & Validation

Food allergies affect approximately 2.5% of the general population worldwide, with reported prevalence rates ranging from 1% to 10%.¹

The average cost of an allergen-related food recall is estimated at \$10 million (USD). High sensitivity allergen testing can help avoid recall-related costs and product release delays.

Allergen Cleaning Verification

- Verifies efficacy of cleaning procedures to remove allergen proteins
- Conducted at run or shift completion
- Performed on location by food sanitation staff
- Analyzed by high sensitivity, preferably quantitative, rapid test methods
 - ATP
 - Protein Residue Testing
 - Lateral Flow

Allergen Detection Method Validation

- Validates efficacy of allergen cleaning verification method
- Conducted at SSOP adoption and at least annually thereafter
- Performed by reference laboratory (internal or third-party)
- Analyzed by high sensitivity, quantitative reference methods
 - Enzyme Linked ImmunoSorbent Assay (ELISA)
 - Polymerase Chain Reaction (PCR)
 - Flow Cytometry (Reference Lab)

¹ <https://www.worldallergy.org/education-and-programs/education/allergic-disease-resource-center/professionals/food-allergy> Accessed April 18, 2022.

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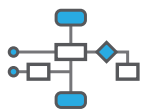


1 | Food Allergies Worldwide

Recognized as a growing problem in most countries, food allergy affects approximately 2.5% of the general population worldwide, with reported prevalence rates ranging from 1% to 10%. In the United States alone, food allergies impact tens of millions with 1 in 10 adults and 1 in 3 children affected.

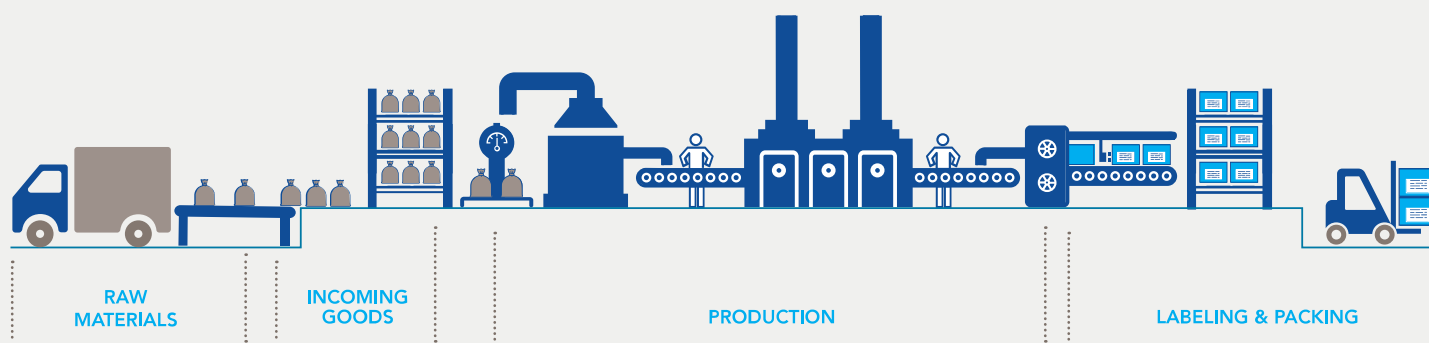


- 2.5% of newborns have cow's milk allergy (~1.0% in adults (physician confirmed))
- Up to 9% of the global population is allergic to hen's eggs
- Wheat allergy affects 1% of the adult population with higher rates among children
- The prevalence of seafood allergy ranges from 7% to 10%
- Peanut allergy has doubled in recent years, reaching 3% in some regions



2 | The Allergen Control Plan (ACP)

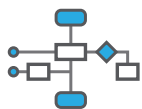
The foundation of any successful allergen control plan lies with a knowledgeable team consisting of professionals within the organization who understand ingredient sourcing, workflow, processing, packaging, storage and most importantly, the importance of identifying and controlling allergen risk.



As a key component of an effective Hazard Analysis and Critical Control Points (HACCP) program, the allergen control team should conduct a facility risk assessment including the development of an allergen flow diagram (allergen map) to trace allergenic ingredients throughout the facility from raw materials to finished products.

Although absolute zero allergen risk is not a practical nor an achievable goal in the food industry, a reliable allergen testing program plays a critical role, reducing risk in the process through:

- Testing raw materials to confirm the purity of the contents
- Verification of the efficacy of cleaning procedures via rapid tests
- Validation of the accuracy of the rapid tests via reference laboratory methods
- Identification and control of potential cross-contamination
- Confirmation of product label accuracy
- Assurance of food safety



Allergen Verification and Validation

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. Verification is typically conducted with a high sensitivity, qualitative/semi-quantitative method such as a high-sensitivity ATP detection, general protein test or lateral flow assay. The latter method, lateral flow assays, can detect specific allergens such as milk proteins or tree nuts.

Validation may occur at a manufacturer's on-site laboratory, or more frequently, at a third-party reference laboratory. This process demonstrates efficacy of the on-site, internally verified testing method. Validation is performed prior to adopting a formal cleaning procedure. It is also performed routinely as an integral component of a food safety program and when changes are made to manufacturing lines or processes. Validation is commonly conducted with a quantitative enzyme-linked immunoassay (ELISA), polymerase chain reaction (PCR), or flow cytometry (FC).

Establish **Sanitation Standard Operating Procedure** (SSOP):

- Define scope
- Define responsibilities
- Define cleaning instructions and frequency



Establish Cleaning

Verification Procedure:

- Define intention and scope of verification process
- Define sampling procedure and analytical method
- Define final acceptance (verification) criteria

Establish Cleaning **Validation** Procedure:

- Define intention and scope of validation process
- Define sampling procedure and analytical method
- Define final acceptance (validation) criteria



Hygiena® Allergen Limits of Detection

Hygiena takes a comprehensive approach to establishing allergen thresholds. The initial phase defers to the Analytical Method (test type) to maximize detection of significant antigen levels while avoiding inadvertent cross-reactivity (high sensitivity and specificity). The developed assay is then verified for accuracy compared with gold standard, reference laboratory methodologies.

Next, Safety Assessment and Risk Assessment analyses, based on available clinical data, is conducted.



To achieve this, Hygiena refers to the scientific expert panel (VSEP) guidance behind Australia's Voluntary Incidental Trace Allergen Labeling (VITAL). ¹VITAL's 2019 allergen threshold guidance was developed from published and unpublished data of low-dose oral food challenges in the United States, Australia, and the European Union.

This comprehensive approach defines the test sensitivity cut-off (limit of detection) to ensure the health and safety of ≥95% of the population.

¹ <https://vital.allergenbureau.net/vital-science/>

3 | Safety First Allergen Testing

Despite the importance of an effective allergen testing program, most recognized food allergens have no formal detection threshold limits or sanctioned regulatory guidance.

This lack of regulation puts the responsibility for selecting test sensitivity and testing frequency with the allergen control team. Aligning with a high-quality test manufacturer partner, therefore, becomes a helpful component of ACP success.

Allergen Testing Frequency

The appropriate frequency of allergen testing is specific to each facility and designed to detect product-relevant allergens.

The allergen control team, armed with their internal risk assessment, will make the appropriate recommendations which are reflected in the facility's standard operating procedures (SOPs).

Allergen Test Sensitivity (Limits of Detection)

US FDA describes four methods that may be used to establish useful sensitivity thresholds for allergen detection in the food industry.

- I. **Analytical Method**
Allergen threshold is determined by the sensitivity of the analytical method utilized.
- II. **Safety Assessment Method**
Allergen threshold is determined via human challenge studies to calculate the "no observed adverse effect" cut-off.
- III. **Risk Assessment Method**
Allergen threshold is determined based on known or hypothesized adverse health effects.
- IV. **Statutorily Derived Method**
Allergen threshold is based on applicable regulatory guidance/ local laws.

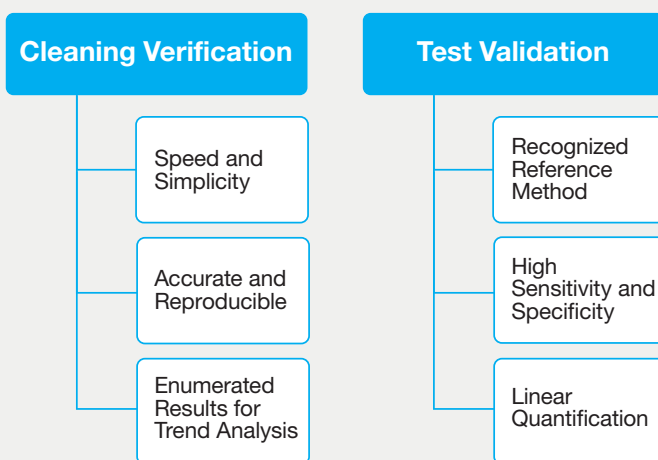
Because it can be difficult to put this summary guidance into practice toward an optimal allergen control program, partnership with a quality focused, safety-first test manufacturer will provide defensible data driven sensitivity limits for each specific allergen.



4 | Allergen Detection Methods

There are several test methods available for allergen detection and quantification. Optimal selection should be made with attention to the purpose of testing (for example, cleaning verification versus test validation) as well as the accuracy of the discrete test.

Key Factors in Allergen Test Selection



Cleaning Verification

In the case of on-site, cleaning verification, selection of a rapid method that is simple to perform, consistently accurate across matrices and reliably reproducible with enumerated results offers the best-fit solution.



Rapid testing is a critical component for workflow optimization and timely product release. Methods including ATP, total protein, and lateral flow are well suited for the purpose.



Test simplicity is also a crucial factor as it allows virtually any technician to reliably perform the assay with standard training.



Accuracy is perhaps the most obvious requirement, but food matrices can be challenging. Therefore, it is important to confirm relevant matrix performance with the test manufacturer.



Consistent reproducibility (repeatability or precision) is not only a critical component of test accuracy, it also supports informed trend monitoring and analyses.



Semi-quantitative, enumerated results provide insight into cleaning efficacy as well as process control over time. This information allows for timely response and correction before a failure impacts workflow and delays product release.



The value of enumeration is best utilized as a component of a holistic data management system designed to consolidate reporting and identify trends for early intervention and informed decision making.



Cleaning Verification Methods: Allergen Detection and Cross-Contamination Prevention

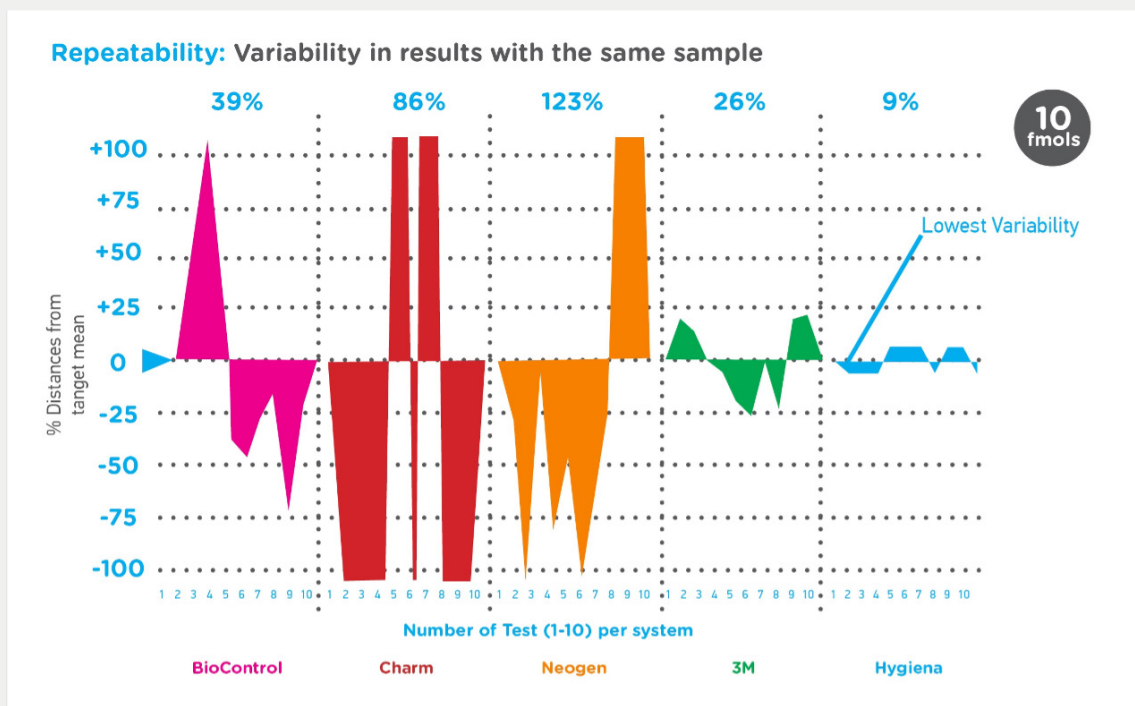
Adenosine Triphosphate (ATP) Bioluminescence

Accuracy, ease of use, time-to-results, and enumerated results have made adenosine triphosphate (ATP) bioluminescence a widely adopted industry standard for cleaning verification and allergen cross-contamination prevention.



When evaluating an ATP method, it is important to evaluate both the performance of the luminometer and the bioluminescence chemistry involved. A quality test manufacturer will be able to share both internal validation data and comparative performance data as well as offering a comprehensive data management tool to consolidate reporting and simplify trend analyses.

Comparative ATP System Performance¹



¹ Food. Analysis & Control. Issue 3. 2009.



Comparative Biochemistry Performance: Sensitivity, Reproducibility and Linearity

| ATP System | Blank RLU output with zero ATP sample | Sensitivity fmols ATP | Assay Reproducibility %CV | Assay Linearity® |
|---|---|--------------------------|---------------------------------|---------------------|
| BioControl MVP with lightening swab | 283 | 1.1 | 39% | 0.982 |
| 3M™ UniLite™ NG with CleanTrace™ Swab | 4.3 | 1.3 | 26% | 0.988 |
| Charm novaLUM with PocketSwab® Plus | 0 | 10.0 | 86% | 0.949 |
| Hygiena SuperSnap® | 0 | 0.17 | 9% | 0.987 |

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. However, protein residue detection is non-specific (all organic protein is detected), so where specific allergen protein detection is desired, a lateral flow method is preferred.

A common protein detection test utilizes the Biuret reaction where, under alkaline conditions, copper ions (Cu^{2+}) form a complex with protein peptide bonds resulting in reduced copper Cu^+ .



The Cu^+ specific bicinchoninic acid (BCA) reagent then forms a purple chromogen complex which is read visually.

When evaluating a protein residue test method, it is important to understand matrix compatibility as well as sensitivity limits: too high a threshold risks false-negative results while too low a threshold results in unwarranted recleaning, workflow interruption and product release delays.

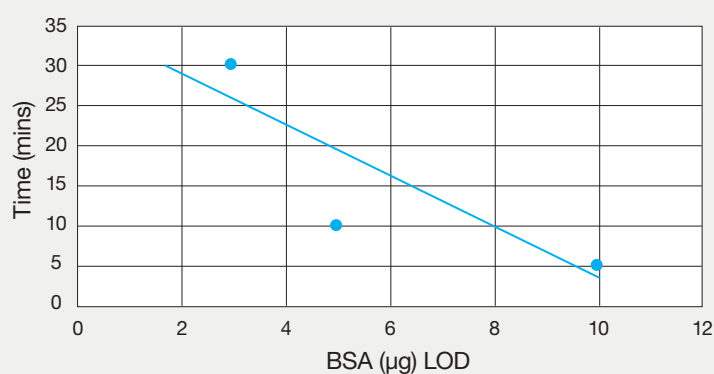


Hygiena Allersnap® Sensitivity

- 3.0 µg at 30 minutes
- 5.0 µg at 5 - 10 minutes
- 10.0 µg at 5 minutes

BSA protein detection sensitivity (37 °C)

Allersnap Protein LOD v Incubation Time



Allersnap device



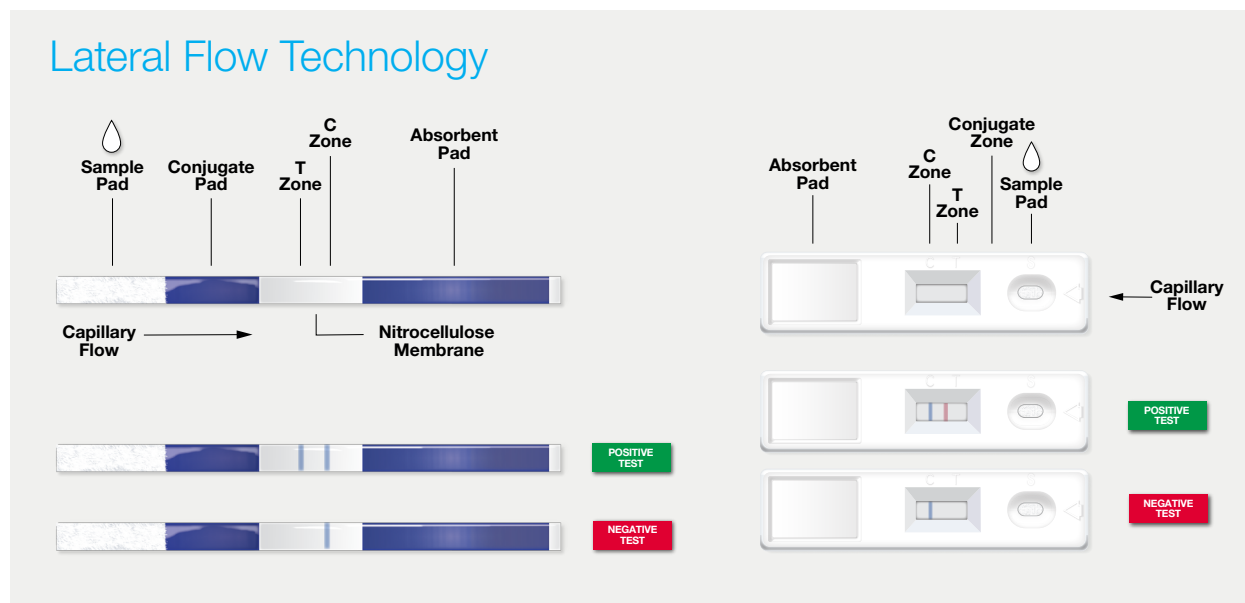
Allersnap Validated Matrices





Immunochromatographic Lateral Flow

Lateral flow or immunochromatographic assays are available as strips/sticks or in cassette format. The principle of the test involves sample application and progression of the sample via capillary action to the reaction zone where, if the target allergen protein is present, it will bind to the specific test antibody. Binding of the antibody results in a visible line indicative of a positive result (presence of allergen).



When evaluating a lateral flow method, the manufacturer will be able to detail the performance specificity of the test to ensure accurate detection while avoiding false-positive results. Adequate and appropriate sensitivity is also an important consideration prior to HACCP SOP test adoption.

Hygiena Lateral Flow Sensitivity Thresholds

| Big 9 Allergens | AlerTox®/GlutenTox® Lateral Flow (ppm) | VITAL Reference Cumulative MED ₀₅ (mg/kg) |
|-----------------------|---|---|
| Gluten | ≥1.0 | 20.0 |
| Egg | 1.25 | 2.4 |
| Milk | 2.5 | 3.1 |
| Peanut | 1.0 | 3.9 |
| Tree Nut | from 2.3* | Varied* |
| Soy Plus | 1.5 | 10.0 |
| Fin Fish | 5.0 | 14.1 |
| Shell Fish/Crustacean | 10.0 | 429.0 |
| Sesame | 3.0 | 4.2 |

*Sensitivity threshold varies according to type of nut



Test Validation

Allergen test method validation may occur at an on-site laboratory, or more frequently, at a third-party reference laboratory.

The goal of validation is to demonstrate the efficacy of the on-site testing method. Validation is performed prior to adopting a formal cleaning verification procedure, when process changes are made, and on a defined schedule according to the HACCP plan.

Key considerations for a reliable validation method:



To ensure accuracy, validation of the selected cleaning verification test must reference an established laboratory method.



High sensitivity and specificity are features of a robust validation test providing for detection of significant allergen levels while avoiding false positives.



The ability to accurately quantify allergen levels requires a linear assay.

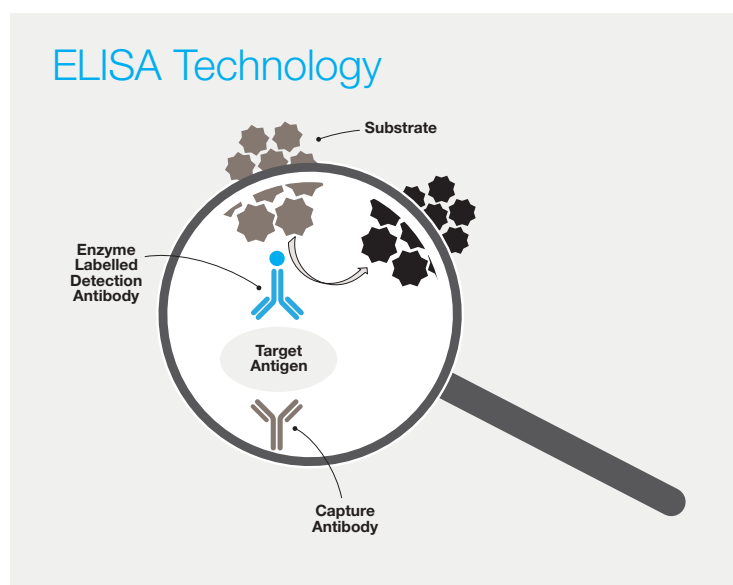
Enzyme-Linked Immunosorbent Assays (ELISA)

ELISA is a commonly used method for food allergen detection, quantification, and test method validation. The test procedure begins with adding a diluted test sample to an antibody-coated microwell (microtiter plate). If the targeted antigen (allergen protein) is present it will bind to the antibody. After a wash step, a second antibody linked to an enzyme (conjugate) is added. The conjugate will bind to the captured antigen if present. Following an additional wash, a substrate is added. In the presence of bound conjugate, a colored reaction becomes visible, the intensity of which is directly proportional to

the amount of antigen (allergen protein) in the sample.

ELISA tests can be performed manually with a reader, or with standard microtiter plate automation equipment.

When evaluating an ELISA test, the test manufacturer will be able to provide you with a method and system validation report to demonstrate test performance.



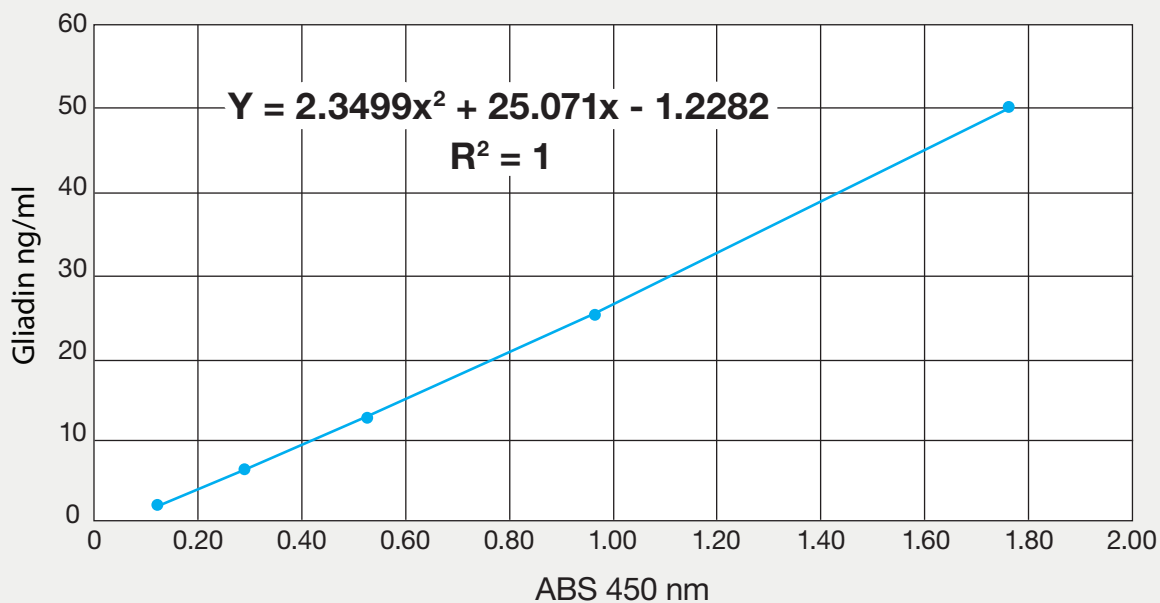


Hygiena ELISA Test Sensitivity Limits

| Big 9 Allergens | AlerTox®/GlutenTox® Rapid ELISA (ppm) | VITAL Reference Cumulative MED ₀₅ (mg/kg) |
|-----------------------|---|---|
| Gluten | 0.3 | 20.0 |
| Egg | 0.5 | 2.4 |
| Milk | 0.05 | 3.1 |
| Peanut | 0.3 | 3.9 |
| Tree Nut | from 0.1* | Varied* |
| Soy | 0.016 | 10.0 |
| Fin Fish | 1.4 | 14.1 |
| Shell Fish/Crustacean | 0.001 | 429.0 |
| Sesame | 0.2 | 4.2 |

*Sensitivity threshold varies according to type of nut

Hygiena GlutenTox Linearity

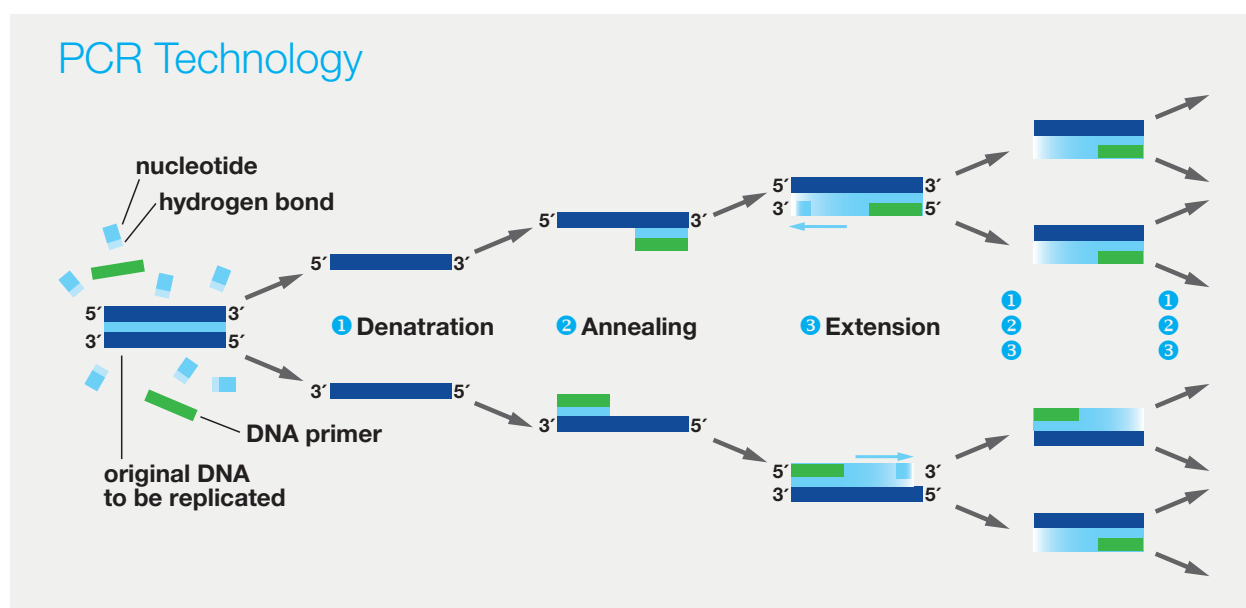


Note: ELISA assay linearity will vary somewhat dependent on allergen and matrix.



Polymerase Chain Reaction (PCR)

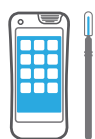
A more complex but fully automatable method, PCR testing detects the DNA of an allergen rather than the protein itself. As a result, PCR methods are more sensitive than ELISAs. This can be particularly beneficial in the food industry for testing cooked products because while DNA generally remains intact, allergen proteins can denature in heat (or cross-react with other proteins, i.e., celery).



When evaluating a PCR test, the test manufacturer will be able to provide you with a method and system validation report to demonstrate test performance.

Hygiena foodproof® Allergen PCR Testing





5 | Selecting an Optimal Allergen Testing Partner

In summary, selection of an optimal allergen test manufacturer with deep industry expertise and a true partnership philosophy is a vital component of an effective allergen control plan.

A few of the most important considerations for selecting the right partner include:

- A comprehensive and innovative allergen menu across test methods (ATP, Protein Detection, Lateral Flow, ELISA, PCR)
- Detailed test performance data to demonstrate accuracy, appropriate sensitivity limits, precision, linearity and enumeration
- Deep food industry knowledge and focus
- Multiple food matrix test validation data and custom validation services
- Modular test automation options to meet current demand and expand with growth
- Integrated results reporting and trend analysis to support HACCP goals
- Knowledgeable and responsive technical support
- Reliability of supply (limited impact from backorder situations)

As a global leader in holistic food safety solutions, Hygiena is dedicated to providing the exceptional test performance and support required for HACCP excellence and public health protection. To learn more visit: www.hygiena.com

| PRODUCT RANGE Allergen Monitoring and Detection Solutions | | | | | |
|---|---|--|--|--|---|
| Protein Residue Test | Lateral Flow Tests | | | ELISA | PCR |
| General Protein | AlerTox® Sticks | GlutenTox® Pro | GlutenTox® Sticks Plus | AlerTox® ELISA | foodproof® |
| AlerSnap® High-sensitivity broad spectrum protein 5 - 30 minutes | Trace allergen contamination in food, liquid, and surfaces Allergens that can be detected with AlerTox Sticks: Almond Casein Crustacean Egg Fish Hazelnut β - Lactoglobulin Mustard Seed Peanut Sesame Seed Soy Total Milk Walnut 10 minutes | AOAC-certified gluten detection harnessing the power of the G12 antibody 10 minutes Hygiena® Cube Readable and trackable quantitative or Pass/Fail results Allerflow® Gluten High-sensitivity gluten specific 10 minutes | Detection limit 3 ppm (< 20 ppm is considered "gluten-free") 10 minutes Hygiena® Cube Readable and trackable quantitative or Pass/Fail results | AlerTox ELISA Almond AlerTox ELISA BLG AlerTox ELISA Casein AlerTox ELISA Cashew AlerTox ELISA Coconut AlerTox ELISA Crustacean AlerTox ELISA Egg AlerTox ELISA Fish AlerTox ELISA Hazelnut AlerTox ELISA Lupine AlerTox ELISA Lysozyme AlerTox ELISA Macadamia AlerTox ELISA Milk AlerTox ELISA Mustard AlerTox ELISA Ovalbumin AlerTox ELISA Peanut AlerTox ELISA Pistachio AlerTox ELISA Sesame AlerTox ELISA Soy AlerTox ELISA Walnut 60 minutes GlutenTox® ELISA Gluten Tox® ELISA Rapid G12 GlutenTox® ELISA Competitive G12 90 minutes | Celery Gluten Hazelnut Peanut Soya Walnut 180 minutes |

Choose Tests Tailored to Your Needs