



Pushing the Limits of Food Allergen Detection: Allergen Threshold Guidance for Food Manufacturers and Processors

Introduction: 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%.¹ In the United States alone, food allergies impact tens of millions with 1 in 10 adults and 1 in 3 children affected.²

Although definitive studies are lacking and methodologies vary, it is generally agreed that the most common foods responsible for eliciting allergic reactions include milk, egg, wheat, fish, and nuts.¹

- Worldwide it is estimated that approximately 2.5% of newborns are diagnosed as allergic to cow's milk.
- The EuroPrevall study identified the incidence of hen's egg allergy at 1.23%, whereas the Australian Healthnuts survey indicates a 9% prevalence in that country.
- The global prevalence of wheat allergy is estimated at 0.2% to 1% with children experiencing a higher prevalence than adults.³
- The reported prevalence of fish allergy ranges up to 7% and 10.3% in the case of shellfish.⁴
- The reported prevalence of peanut allergy among children in the United Kingdom, North America, and Australia has doubled in the last decade with a current estimated prevalence of 1.8%, 1.4%, and 3.0% respectively.

Food Allergen Declaration, Global Requirements

In the United States, the FDA *Food Allergen Labeling and Consumer Protection Act*, 21 U.S.C. 2004 (FALCPA)⁵ identified the eight major food allergens which account for more than 90% of allergic reactions. More recently, the *Food Allergy Safety, Treatment, Education, and Research Act* (FASTER Act) described the 9th big allergen, sesame. On-label declaration of sesame becomes mandatory in the United States as of Jan 1, 2023. The government of Canada⁶ follows similar allergen reporting requirements with inclusion of mustard and a specification to identify cereals with gluten.

The European Union Food Information for Consumers Regulation No. 1169/2011 (EU FIC)⁷ requires declaration of 14 major allergens. Beyond the Big 8, European food

manufacturers and processors are required to identify and declare: sesame, celery, mustard, mollusk, lupin, cereals containing gluten and sulfur dioxide (as may be found in wine).

Many other countries defer to the Codex Alimentarius,⁸ the food standards body of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). The Codex General Standards for the Labeling of Prepackaged Foods (4.2.1.4) emphasizes the need to consistently declare the “Big 8” allergens in addition to the specification to identify cereals containing gluten. In light of increasing food sensitivities and specific regional trends, several countries have expanded their mandatory allergen reporting requirements, including China, where previously there were only voluntary disclosure guidelines in place.⁹



Table 1. Global Allergen Declaration Requirements, Partial Listing¹⁰

Country	Egg	Milk	Fin Fish	Shell Fish	Mollusk	Peanut	Tree Nut	Wheat
United States	X	X	X	X		X	X	X
Canada	X	X	X	X	X	X	X	X
European Union	X	X	X	X	X	X	X	X
Australia New Zealand	X	X	X	X	X	X	X	X
Mexico	X	X	X	X		X	X	X
Brazil	X	X	X	X	X	X	X	X
Central America	X	X	X	X	X	X	X	X
China	X	X	X	X	X	X	X	X
India	X	X	X	X	X	X	X	
Japan	X	X		X	X	X	Walnut	X
South Africa	X	X	X	X	X	X	X	X
Country	Cereal with Gluten	Soy	Sesame	Celery	Mustard	Buckwheat	Lupin	Royal Jelly Bee Pollen
United States		X	X					
Canada	X	X	X		X			
European Union	X	X	X	X	X		X	
Australia New Zealand	X	X	X				X	X
Mexico		X	X					
Brazil	X	X	X					
Central America	X	X	X					
China	X	X	X					
India	X	X	X					
Japan	X		X			X		
South Africa		X	X					

Food Allergen Detection Thresholds

It is understood that absolute zero allergen risk is not an achievable goal for food manufacturers and processors. Despite the requirement to detect and declare, only a single allergen has been assigned a formal detection threshold. Detection values ≤ 20 ppm (mg/kg) can be labeled as gluten-free in the United States¹¹ and the European Union.¹² But the lack of definitive threshold guidance for each of the allergens of interest creates an efficacy challenge for food safety programs.

*The discipline of food safety is hard to define... each individual's definition comes from their own experience.*¹³

The balance of this document will provide an overview of allergen testing program goals, methods for assessing the appropriate allergen detection threshold, and the value of partnering with a dedicated team of scientists to incorporate best-fit analytical methods to support an optimal food allergen testing program.

Food Safety Program Allergen Testing Goals

Allergen testing goals are twofold: **Verification** and **Validation**

Verification analysis occurs at the site of manufacture/processing according to the facility's adopted allergen control testing plan. This process includes frequent confirmation (verification) that established cleaning procedures are effectively removing allergen residues and contaminants. The verification analysis is typically conducted with a high sensitivity, qualitative immunochromatographic method (e.g., lateral flow device (LFD)).

Validation analysis may occur at a manufacturer's on-site laboratory, or more frequently, at a third-party reference laboratory. This process demonstrates (validates) the efficacy of the on-site, internally verified testing method. Validation is performed prior to adopting a formal cleaning procedure. It is also performed routinely (quarterly, annually, semi-annually) as an integral component of a food safety program. The validation analysis is commonly conducted with a quantitative enzyme-linked immunoassay (ELISA) or polymerase chain reaction (PCR) method.

In both cases, relevant assay development requires adherence to a set of defined quality standards; the establishment of proper allergen thresholds among them.

Assuring a Safety-First Allergen Testing Program

Because the majority of the significant food allergens have no formal, government-sanctioned threshold guidance, the US FDA describes four methods that may be used to establish test sensitivity (threshold) cut-off values. They are as follows:

1. Analytical Method

Allergen thresholds are determined by the sensitivity of the analytical method used for detection.

2. Safety Assessment Method

Allergen thresholds are determined through analysis of human challenge studies using a defined "uncertainty factor" to calculate the no-observed-adverse-effect level.

3. Risk Assessment Method

Allergen thresholds and risk quantification are based on known or potential adverse health effects resulting from human exposures.

4. Statutorily-Derived Method

Allergen thresholds are based on extrapolated applicable law.

Each approach has its unique challenges and limitations. Relying solely on a commercially available Analytical Method, without insight into assay design and development, may leave a food processor at risk. Alternatively, the Safety-Assessment and Risk-Assessment Methods are expensive and impractical for food processors and manufacturers to pursue. Finally, the Statutorily-Derived Method fails to provide the legal granularity to define reliable allergen thresholds.

The best solution? Partnering with a science-forward team that can provide both the expertise and the peace of mind that comes from global environmental monitoring and allergen detection leadership.

The Hygiena™ Solution: A Quality Analytical Method with Robust Safety & Risk Assessment

Hygiena™ takes a dual approach to establishing allergen thresholds. The initial phase relies on the Analytical Method approach to reliably detect low levels of antigen while avoiding inadvertent cross-reactivity (high sensitivity and specificity). The developed assay is then verified for accuracy compared with the gold standard, reference laboratory methodologies. The second phase relies on third-party Safety Assessment and Risk Assessment analyses based on available clinical data.



AlerTox® Sticks

In this case, Hygiena™ refers to the scientific expert panel (VSEP) guidance behind Australia's Voluntary Incidental Trace Allergen Labeling (VITAL). VITAL'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. Evaluation of 3,400 clinical data points using the Stacked Model Averaging program was used to produce Eliciting Dose (ED) curves for each allergen. Adopted by the food industry in Australia and beyond, Hygiena™ references the VITAL minimum ED₀₅ guidelines to determine essential threshold limits for its allergen test menu. This standard sets an appropriate sensitivity cut-off to ensure the health and safety of 95% of the population.

With these quality standards in mind, Hygiena™ offers a line of LFD and ELISA assays ideal for the food industry. AlerTox® and GlutenTox® Sticks utilize lateral flow technology to identify and quantify food allergens, while AlerTox® and GlutenTox® ELISA tests validate low level allergen presence at or below the VITAL minimal eliciting dose. (Table 2).



Table 2. Big 9 Allergen Testing Thresholds, Hygiena™

Big 9 Food Allergens	Analytical Methods-Based Approach to Allergen Threshold Determination		Cumulative Minimal Eliciting Dose for 95% of the population ¹⁴
	AlerTox®/GlutenTox® LOD ^a , ppm Verification (LFD) ^b	Hygiena™ ELISA/GlutenTox® LOD ^a /LOQ ^c , ppm Validation (ELISA) ^d	2019 VSEP/VITAL Cumulative MED ₀₅ (mg/kg) ^e
Gluten	≥ 1.0	0.3	20.0 ^f
Egg	1.25	0.5/0.4	2.4
Milk	2.5	0.05/0.5	3.1
Peanut	1.0	0.3/1.0	3.9
Tree Nut	from 2.3 ^g	from 0.1/1.0 ^g	Varied ^h
Soy	10.0	0.016/0.05	10.0
Fin Fish	5.0	1.4/4.0	14.1
Shell Fish/Crustacean	10.0	0.001/0.02	429.0
Sesame	3.0	0.2/2.0	4.2

^aLimit of Detection (sensitivity threshold)

^bLateral Flow Device

^cLimit of Quantification

^dEnzyme Linked Immuno Assay

^eppm = mg/kg

^fRegulatory defined threshold for gluten (FDA, EU, etc)

^gAssay thresholds vary for each specific type of tree nut

^hMED₀₅ thresholds vary for each specific type of tree nut

Hygiena™ also offers the foodproof® line of DNA-based, real-time PCR allergen testing suitable for all food matrices. With exceptional sensitivity and available quantification, foodproof® assays are readily compatible with commonly utilized RT PCR instrumentation.

Conclusion

Since food safety is paramount to population health, optimal allergen detection is a primary goal for food manufacturers. However, establishing rational allergen thresholds in the absence of clear regulatory guidelines can be a significant challenge. Fortunately, partnership with a science-first leader in environmental hygiene monitoring and allergen detection like Hygiena™ can provide a sound and safety-centric approach to ensuring compliance, safety, and brand integrity.

About Hygiena™ - The One Health Approach to Food Safety and Protection

As a recognized global leader in environmental monitoring, Hygiena™ offers a broad range of high-quality, simple-to-use testing solutions. From rapid microbial detection and identification to cleanliness and allergen monitoring tools, Hygiena™ delivers comprehensive food safety technology backed by world-class scientists and applications developers, knowledgeable and localized 24/7 support, proactive innovation, and deep regulatory expertise to help maintain facility compliance.

To learn more, visit [Hygiena.com/allergens](https://www.hygiena.com/allergens).

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