Analysis for food allergens – An Overview

Food allergy

Food allergy is the result of an abnormal immune response towards harmless food antigens.

1. Initial allergen exposure. T cell response leads to the induction of IgE production towards allergens by B cells.

2. IgE in turn bound mast cell

3. When the allergen is re-encountered, IgE can be cross-linked, leading to degranulation of mast cells.

4. The mediators released by the mast cells lead to the typical allergic symptoms, such as itching or sneezing.

《2004年食物及藥物（成分組合及標籤）（修訂）規例》
Food and Drugs (Composition and Labelling) (Amendment) Regulation (The Amendment Regulation)

Declaration of Food Allergens

One of the new requirements is declaration on the food labels the presence of the eight most common allergy causing substances.

a. cereals containing gluten; 含有麩質的穀類（即小麥、黑麥、大麥、燕麥、裂穀小麥、它們的混合變種及它們的製品）

b. crustacean and crustacean products;甲殼類動物及甲殼類動物製品

c. eggs and egg products;蛋類及蛋類製品

d. fish and fish products;魚類及魚類製品

e. peanuts, soybeans and their products;花生、大豆及它們的製品

f. milk and milk products (including lactose);奶類及奶類製品（包括乳糖）

g. tree nuts and nut products 木本堅果及堅果製品 and

h. food containing sulphite (with concentration of 10 parts per million or more).

亞硫酸鹽〔如食物由濃度達到或超過百萬分之十（即每公斤10毫克）

Food Allergen Analysis

Consideration:

Matrix and allergen involved

Method chosen should be robust, reliable, repeatable, sensitive and specific.

Laboratory analysis:

- Enzyme-Linked Immunosorbent Assay (ELISA) laboratory kits 酶聯免疫吸附測定
- Polymerase Chain Reaction (PCR) 聚合酶鏈反應
- Liquid Chromatography Mass Spectrometry (LC-MS)液相色譜質譜法

ELISA (Enzyme Linked Immunosorbent Assay) test kits

- ✓ Commonly used for routine food allergen detection
- ✓ These test kits are available for detecting many of the common food allergen proteins.
- ✓ ELISA test kits generally focus on specific ‘marker’ proteins.
- ✓ They should be specific (minimal false positives)
- ✓ Quantitative (provide an allergen concentration)
- ✓ Sensitive (able to detect very low (ppm) levels of the protein.

👎 No single ELISA kit available that will detect all the relevant allergen in a single assay.

Polymerase Chain Reaction (PCR)

PCR amplify and detect the DNA of an allergen.

- ✓ They are specific, sensitive, and qualitative, can verify or clarify an ELISA result
- ✓ can detect potentially allergenic products for which no ELISA test is currently available.
- ✓ can be useful for food products containing hydrolysed proteins.
- ✓ Can be used to detect more than one allergen at a time.

- 👎 Some processing methods can destroy detectable DNA, causing false negative results.
- 👎 DNA methods are not suitable for detection of allergens in food with high protein and low levels of DNA e.g. egg and milk.

Mass Spectrometry (MS)

MS identifies peptide markers from the allergic protein

- ✓ Specificity, separate closely related proteins.
- ✓ May still be able to detect denatured protein.
- ✓ Directly analyze multiple allergens in a single analysis.

- 👎 assumptions made in MS when calculating protein quantity from peptide signal intensity
- 👎 allergenic protein and peptide targets are not standardized and a high level of expertise is required to develop targeted MS assays

Standard Method Performance Requirements (SMPRs)

ELISA-based methods

AOAC SMPR® 2018.003 Quantitation of Milk in the Context of Food Manufacturing

AOAC SMPR® 2018.012 Quantitation of Peanut in the Context of Food Manufacturing

Parameter	Minimum acceptance criteria for target matrix	
Analytical range, ppm[a]	Lower limit	≤10
	Upper limit	≥20[b]
LOQ, ppm[b]	≤10	
LOD, ppm[b]	≤10	
Recovery, %[c]	50–150	
RSD_r, %	≤20	
RSD_R, %	≤30	

[a] ppm in nonfat dried milk.
[b] *See* "Choice of LOD/LOQ for Quantitation of Milk by ELISA-Based Methods" for rationale for setting lower limit of range.
[c] Use incurred samples as per Appendix M. Incurred materials can be obtained from MoniQA Association.

- Validation data in all claimed matrices
- correspond with either a regulatory or a health-driven threshold limit

Standard Method Performance Requirements (SMPRs)

ELISA-based methods

Table 2. Food commodities that should be included in cross-reactivity testing for ELISA methods targeting milk

Almond	Barley	Brazil nut	Beef	Buckwheat
Cashew	Chick peas	Cocoa	Corn meal	Crustacean/prawn
Egg	Fish	Hazelnut	Lecithin	Lima bean
Oats	Peas	Peanut	Pecan	Pine nut
Pistachio	Poppy seed	Pumpkin seed	Rice–white and brown	Rye
Sesame seed	Soy bean	Split peas	Sunflower seed	Walnut
Wheat				

Table 3. Matrixes of interest for ELISA methods targeting egg and milk

Egg	Milk
Chicken	Cookies, baked goods
Ice cream	Dark chocolate
Pasta	Drink mixes (ex. alcoholic beverage premix)
Salad dressing	Orange juice
Soy milk	Infant formula
Wine	Wine

Mass spectrometry-based methods

AOAC SMPR ® 2016.002 Detection and Quantitation of Selected Food Allergens

Table 1. Method performance requirements

	Target allergen			
Parameter	Whole egg	Milk	Peanut	Hazelnut
Analytical range, ppm	10–1000	10–1000	10–1000	10–1000
MQL[a], ppm[b]	≤5	≤10	≤10	≤10
MDL[a], ppm[b]	≤1.65	≤3	≤3	≤3
Recovery, %	60–120	60–120	60–120	60–120
RSD_r, %	≤20	≤20	≤20	≤20
RSD_R, %	≤30	≤30	≤30	≤30

[a] Definitions for MQL and MDL provided in section 4.

[b] Reported as ppm of the target allergen in food commodity, i.e., 25 ppm of "whole egg" in cookies.

Table 2. Priority allergen/matrix combinations

Whole egg	Cookies Bread Dough Salad dressing Wine
Milk	Cookies, baked goods Infant formula Wine Dark chocolate (optional matrix for methods that claim a chocolate matrix)
Peanut	Cookies Ice cream Breakfast cereal Milk chocolate (optional matrix for methods that claim a chocolate matrix)
Hazelnut	Cookies Ice cream Breakfast cereal Milk chocolate (optional matrix for methods that claim a chocolate matrix)

Journal of AOAC International Vol. 103, No. 2, 2020 Detection and Quantitation of Selected Food Allergens by Liquid Chromatography with Tandem Mass Spectrometry: First Action 2017.17

Received First Action Official Method classification

⇨initiate a two-year assessment

⇨ **Final Action Method** status

Table 1. Uniqueness of the marker peptides determined from database search using the NCBI Protein BLAST query

Allergen	Protein	Peptide sequence	BLAST search results	Peptide ID
Whole egg	Gal d 2	GGLEPINFQTAADQAR	Gallus gallus (chicken)	Ew1
	Gal d 3	YFGYTGALR	Gallus gallus (chicken), Coturnix japonica (Japanese quail), Meleagris gallopavo (Turkey)	Ew2
Whole milk	Bos d 9 (α-s1-casein)	YLGYLEQLLR	Bos taurus (cattle/cow), Capra hircus (domestic goat), Ovis aries (sheep), Bubalus bubalis (water buffalo)	M1
		EDVPSER	Bos taurus (cattle/cow), Capra hircus (domestic goat), Ovis aries (sheep), Bubalus bubalis (water buffalo)	M2
Peanut	Ara h 3	WLGLSAEYGNLYR	Arachis hypogaea (peanut),	P1
Hazelnut	Cor a 9	ADIYTEQVGR	Corylus avellana (hazelnut)	H1

Example: Detection of peanut allergen by ELISA

Quantitative analysis of peanut residues in food products

1.Empty well with coated antibody

2. Incubate with **antigen**

3. Incubate with antibody-enzyme conjugate

4. Add substrate and observe colour change

Results:

– Range of quantitation 2.5-25ppm peanut

Conclusion:

Commonly used method for routine food analysis

Example: Detection of Peanut Protein (Ara h1) by MS

– proteins were extracted from matrix in alkaline medium
– Reduction with DTT cleave S-S bond and alkylation with IAA
– Extracts were incubated overnight with trypsin (37°C)
– Stop reaction by Formic acid
– Digested sample was analysed/characterised using LC-MS/MS

Example: Detection of Peanut Protein (Ara h1) by MS

Biomarkers (peptides) are used to identify the presence of Ara h1 in food matrices

- DLAFPGSGEQVEK
- VLLEENAGGEQEER
- IFLAGDKDNVIDQIEK

Results:
– mixture of peptides identified as Ara h1 specific
– 3 most abundant peptides (MW: 1375.65, 1571.73 and 1816.95) were found to be unique for Ara h1 (unique sequences)

- concentration of Ara h1 protein (mg peanut/g sample)

Conclusion:
This method has broad applicability as a confirmatory test for ELISA

Regulatory Limit/ Risk-base limit

Analytical targets should be set at or below the action level in order for the method to be suitable for the purpose.

Health-driven threshold / Risk-base limit

Initiative of the Allergen Bureau in Australia and New Zealand developed a scientific approach
-- The Voluntary Incidental Trace Allergen Labeling (VITAL)

(1) using reference doses for allergen risk characterization, a reference dose is defined as the milligram protein level (total protein from an allergenic food) below which only the most sensitive individuals (between 1 and 5% depending on the quality of the data set available) in the allergic population are likely to experience an adverse reaction.

Action Levels

- Action Levels are found in the interactive VITAL Action Levels Grid
- Calculated from **Reference Dose** and **Reference Amount/Serving Size**

Transition between Action Levels*
= Reference Dose x (1000 / Reference Amount/ Serving Size)
*with the exception of gluten containing cereals where it is either this formula or 20ppm, whichever is smaller

Regulatory Limit

Only a few jurisdictions such as Japan have set a regulatory limit of 10 ppm protein for all their priority allergens.

📖 https://farrp.unl.edu/IRChart

Reference

https://www.cfs.gov.hk/english/whatsnew/whatsnew_act/List_of_Samples_with_Discrepancy_for_NL.html

http://allergenbureau.net/aoac-international-gives-thumbs-mass-spec-allergen-screening-method/

http://www.aoaceurope.com/

https://farrp.unl.edu/IRChart