



**JOINT FAO/WHO FOOD STANDARDS PROGRAMME  
CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING**

**43rd Session**

**Budapest, Hungary**

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**METHODS OF ANALYSIS FOR PRECAUTIONARY ALLERGEN LABELLING**

*(Prepared by the EWG led by United States of America)*

**BACKGROUND**

1. During the 42nd Session of Codex Committee on Methods of Analysis and Sampling (CCMAS), the Codex Committee on Food Labeling (CCFL) requested advice on standardized analytical methods and sampling used for allergen risk assessment in foods ([CX/MAS 23/42/2-Add.1](#)). CCFL requested CCMAS to:

- recommend suitable analytical methods and guidance on their validation and applications including sampling plans for determining allergens in foods, in particular:
  - The methods should detect and quantify unintended allergen presence (UAP) in foods from cross contact with detection and quantification limits (LOD and LOQ) suitable to determine if UAP is above or below the action levels established by the FAO/WHO Expert Consultation for priority allergens for intakes of foods from 10 g to 1000 g.
  - The analytic methods and sampling plans are needed to enable food business operators to do risk assessment to determine if UAP can be controlled below the specified action level for each allergenic food. (Risk Assessment of Food Allergens Part 2: Review and Establish Threshold Levels in Foods for the Priority Allergens). Priority allergens and the finalized action levels are listed in Table 11 of the above report at the following link: <https://www.fao.org/documents/card/en/c/cc2946en>.
  - CCMAS should take into account the recommendations of the FAO/WHO Expert Consultation regarding requirements for analytical methodologies.
  - CCMAS should also recommend suitable analytical methods to be determined if amounts of allergenic food proteins have been removed sufficiently by processing to exempt foods from allergen declaration at action levels above divided by 30.

2. The list of priority allergens in Table 11 of *RISK ASSESSMENT OF FOOD ALLERGENS PART 2: REVIEW AND ESTABLISH THRESHOLD LEVELS IN FOODS FOR THE PRIORITY ALLERGENS* specifies:

- Crustacea
- Fish
- Wheat and Gluten containing grains
- Hazelnut
- Sesame
- Milk
- Egg
- Peanut
- Cashew
- Walnut

3. CCMAS agreed to establish an Electronic Working Group (EWG) to develop a discussion paper which would discuss best practices for the selection of validated analytical methods, and for the validation of such methods. CCMAS agreed that the EWG would not address the question on sampling plans and noted that sampling plans are covered by the *General Guidelines on Sampling* (CXG 50-2004). CCMAS agreed on the following terms of reference to guide the discussion paper:

- Define standardized and harmonized terminology and definitions for allergen testing methods
- Currently available test methods and validation status for the priority allergens listed in CX/FL 23/47/5 Appendix I and noting the validated scope (food matrices, processed food) of these methods.
- Required information for method evaluation and validation, including antibodies used (if ELISA), cross-reactivity, assay applicability, selectivity, stability (ruggedness), calibration procedures, sensitivity, range of quantification, LOD/LOQ, accuracy/trueness, extraction efficiency, precision, robustness, applicability, recovery and practicability, and whether it reports total protein. Validation requirements for the testing of allergens in foods including accuracy/trueness, extraction efficiency, precision, robustness, applicability, recovery and practicability.
- Confirmatory methods for cases of potential analytical cross-reactivity and examples of such possibly including second ELISA confirmation, DNA based detection, and/or mass spectrometry techniques.
- Reference to other 'best practice' guidance documents, include standard development organization (SDO) validation procedures and relevant Codex texts.

#### **EWG Registration and Consultation**

4. EWG registration on the discussion paper was sent out in August 2023. Registrations included 51 registrants. The list of participants who submitted comments is in Appendix II. Comments were received from 10 member countries and 1 SDO on the survey regarding current methods in use. Comments were received from 2 member countries and 2 SDOs on the discussion paper draft. Comments from these submissions resulted in updates to the discussion document.

5. The discussion paper prepared by the EWG is presented in Appendix I and outlines terminology, required information for quantitative method validation, confirmatory methods, currently available test methods, and Codex method typing for ELISA methods.

#### **Recommendation**

6. CCMAS is invited **to consider**:
- i. the proposed terminology and definitions in the discussion paper;
  - ii. whether secondary confirmatory methods fit within the current Codex method typing system, and if they do not, what approach might be best to integrate these into CXS 234; and
  - iii. whether this discussion paper satisfactorily answers the request from CCFL, or if more work is needed.

## Discussion Paper on Methods of Analysis for Precautionary Labeling

### Section 1 – Introduction

It is preferable to use analytical methods that measure the target analyte. In the case of allergenic foods, this is the protein fraction of the food, components of which induce an allergic response. Two main method approaches are the most common – enzyme linked immunosorbent assays (ELISA) and mass spectrometry (MS). Quantitative allergen detection methods are required for determining whether a food commodity meets the allergen action levels, and the result uncertainty should be stated for comparison against action level concentrations.

ELISA tests can be either quantitative or qualitative. In response to CCFL, this discussion paper will focus on quantitative test methods and not discuss qualitative tests such as lateral flow devices.

The following discussion paper attempts to collect information from stakeholders and standards development organizations (SDOs) on the commonly used terms and their definitions, required information for the evaluation of method validation reports, confirmatory methods and their use in allergen detection, the status of currently available test methods, potential Codex method typing concerns, and links to further 'best practice' documents.

### Section 2 – Terminology

Terminology included in the EWG terms of reference is defined below. Citations to the source of the definition are included<sup>1</sup>.

- **cross-reactivity:** a reaction to a material other than the target analyte
- **assay applicability:** the analyte and matrix pair to which the test method is validated
- **selectivity:** the ability of the method to detect only the analyte of interest, even in the presence of potential interferences
- **stability (ruggedness):** ability of the method to maintain consistent results when reasonable deviations from the procedure are made
- **calibration:** a standardization of instrument response to known concentrations, against which the unknown test portion will be compared
- **sensitivity:** the instrumental response as a function of the calibration standard concentration, often expressed as the slope of the calibration curve
- **range of quantification:** a range of concentrations from the limit of quantification (LOQ) to the upper calibration point
- **Limit of Detection:** the lowest concentration in a test sample that can be distinguished from a blank
- **Limit of Quantification:** the lowest concentration in a test sample that can be quantified with a specified uncertainty
- **accuracy/trueness:** the closeness of the test result to the true value
- **extraction efficiency:** the amount of analyte extracted, divided by the true amount present in the test material
- **precision:** closeness of replicate measurements on a single material
- **robustness:** ability of a method to produce true results even after reasonable deviations in the method procedure
- **recovery:** the amount of analyte extracted and measured by a test method, divided by the true amount present in the test material. Recovery differs from extraction efficiency because it accounts for both extraction and quantification divided by the true amount present in the test material.
- **total protein from the allergenic source:** the protein amount in an unfractionated food allergen source material, as measured by a total nitrogen protein method

### Section 3 – Required Information for Quantitative Method Validation

The following information should be included with any food allergen test or method validation data.<sup>2</sup>

1. Antibody information (immunoassay methods)
2. Cross-reactivity / specificity
3. Information on calibrators
4. Information on matrices
5. Limits of Detection and Quantification
6. Ruggedness and lot-to-lot variability of method performance
7. Information on result reporting units and if how to best convert the result to a total protein from the allergenic source
8. Protein accession number (mass spectrometric methods)
9. Peptide sequences (mass spectrometric methods)
10. Multiple reaction monitoring transitions (mass spectrometric methods)
11. Recovery

The analyte measured must be validated in the matrix tested. Incurred test materials are required for the evaluation of precision, sensitivity (instrumental response to a change in concentration), and recovery. Heavily processed foods such as baked goods are more difficult matrices for allergen detection when compared to the raw commodities or minimally processed finished products. It is therefore imperative that test methods be validated in the same matrix category as the tested foods. If matrix categories are to be used during validation, careful thought should be put into how to define these categories. In many instances, the type of categorization that is appropriate for allergen detection is not the same as for other analytes. Results of an interlaboratory study can be used to evaluate test method performance.  $S_r$  and  $RSD_r$  can be used for repeatability, while  $S_R$  and  $RSD_R$  can be used for reproducibility, LOD, and LOQ. 50-150% range for recovery may be acceptable due to complexity of food matrix and processing conditions.

### Section 4 – Confirmatory Methods

The complexity of the world market food supply and the need to distinguish between related foods containing homologous cross-reactive proteins makes single-analyte methods such as commercial ELISA test kits non-ideal for many circumstances. It is the practice of some countries to require a second ELISA kit confirmation, targeting a different epitope on proteins to confirm the presence of a food allergen.

Orthogonal methods such as polymerase chain reaction (PCR) for DNA detection can confirm presence of plant or meat species but does not measure the allergenic fraction or components of the food (i.e., proteins). PCR has less cross reactivity, but also less sensitivity. Mass spectrometry is also used but is still under development as a research technique and is not widely applied to regulatory use. In general, mass spectrometry methods currently lack validation or standardization, but may become increasingly used in the future. AOAC method 2017.17 uses a tryptic digestion of allergen proteins in food matrices and labeled peptide internal standards.<sup>4</sup> Analysis is done by LC-MS/MS of the signature tryptic peptides of whole egg, whole milk, peanut, and hazelnut and was validated in 8 food matrices. AOAC has also published Official Methods of Analysis for Gluten (991.19, 2012.01, 2014.03, 2015.05, 2015.16, 2018.15)

### Section 5 – Currently Available Test Methods and Validation Status

Table 15 of *RISK ASSESSMENT OF FOOD ALLERGENS PART 2: REVIEW AND ESTABLISH THRESHOLD LEVELS IN FOODS FOR THE PRIORITY ALLERGENS* includes test methods for ingredients derived from allergenic foods, matrices, method and LOQ, references and comments. Additionally, a survey to member countries was sent via the Codex forum. Ten member countries and one SDO responded to the survey request. The responses are tabulated in Annex I. Duplicate entries from separate country responses were retained showing where there is some general agreement on method choice. The table shows a broad variety of methods in use among the respondents and was not meant to be a comprehensive table of all test methods available. Most countries use commercial immunoassay test methods, with a substantial but lesser number using PCR and LC-MS/MS methods.

Gluten is the only allergen provision in CXS 234, listed for gluten free foods. It is a Type I ELISA method. Its entry in CXS-234 is below:

Commodity	Provision	Method	Principle	Type
Gluten-Free foods	Gluten	Enzyme-Linked Immunoassay R5 Mendez (ELISA) Method <i>Eur J Gastroenterol Hepatol 2003; 15: 465-474</i>	Immunoassay	I

### Section 6 – Codex Method Typing for ELISA methods

The allergen test result will be defined by the antibody and specific ELISA kit used. By definition, a defining method will be listed as Codex Type I method. ELISA kits normally also contain a conversion factor for the total allergen content. E.g. an analyte of casein or beta lactoglobulin will be converted to total milk protein. Any use of a conversion factor makes the method a defining method.

Many countries use a two kit ELISA approach. In many cases, either two methods are used consistently to both collect quantitative data, or different methods are used based on applicability and scope. In some countries, the first ELISA kit is used to screen for the allergen, followed by quantification by a second ELISA kit. In the case of a two-kit system when two Type I methods are used, the Codex method typing system may not be appropriate. CCMAS might consider whether confirmatory ELISA methods fit within the current Codex method typing system.

### Section 7 – Best Practice Guidance Documents and References

1. Dr. Latimer, George W, Jr. (ed.), 'Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices', in Official Methods of Analysis of AOAC INTERNATIONAL, 22 (New York, 2023; online edn, AOAC Publications, 4 Jan. 2023), <https://doi.org/10.1093/9780197610145.005.013>
2. Michael Abbott, Stephen Hayward, William Ross, Samuel Benrejeb Godefroy, Franz Ulberth, Arjon J Van Hengel, James Roberts, Hiroshi Akiyama, Bert Popping, Jupiter M Yeung, Paul Wehling, Steve L Taylor, Roland Ernest Poms, Philippe Delahaut, Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices, Journal of AOAC INTERNATIONAL, Volume 93, Issue 2, 1 April 2010, Pages 442–450, <https://doi.org/10.1093/jaoac/93.2.442>
3. Elena Cubero-Leon, Hendrik Emons, Gavin O'Connor, Jørgen Nørgaard, Piotr Robouch, Food allergen analysis: Considerations for establishing a reference measurement system to implement EU legislation, Food Chemistry, Volume 424, 2023, 136391, <https://doi.org/10.1016/j.foodchem.2023.136391>.
4. Lee Sun New, Jianru Stahl-Zeng, Andre Schreiber, Mark Cafazzo, Alex Liu, Sharon Brunelle, Hua-Fen Liu, Detection and Quantitation of Selected Food Allergens by Liquid Chromatography with Tandem Mass Spectrometry: First Action 2017.17, Journal of AOAC INTERNATIONAL, Volume 103, Issue 2, March-April 2020, Pages 570–583, <https://doi.org/10.5740/jaoacint.19-0112>

**EU References of standardized methods for detection and quantification of allergenic ingredients in foods**

CEN/TR 16338:2012	Foodstuffs - Detection of food allergens - Template for supplying information about immunological methods and molecular biological methods
CEN/TS 15633-2:2013	Foodstuffs - Detection of food allergens by immunological methods - Part 2: Quantitative determination of hazelnut with an enzyme immunoassay using monoclonal antibodies and bicinchoninic acid-protein detection
CEN/TS 15633-3:2012	Foodstuffs - Detection of food allergens by immunological methods - Part 3: Quantitative determination of hazelnut with an enzyme immunoassay using polyclonal antibodies and Lowry protein detection
EN 15633-1:2019	Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations
EN 15634-1:2019	Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations
EN 15634-2:2019	Foodstuffs - Detection of food allergens by molecular biological methods - Part 2: Celery ( <i>Apium graveolens</i> ) - Detection of a specific DNA sequence in cooked sausages by real-time PCR
EN 15634-3:2023	Foodstuffs - Detection of food allergens by molecular biological methods - Part 3: Hazelnut ( <i>Corylus avellana</i> ) - Qualitative detection of a specific DNA sequence in chocolate by real-time PCR
EN 15634-4:2023	Foodstuffs - Detection of food allergens by molecular biological methods - Part 4: Peanut ( <i>Arachis hypogaea</i> ) - Qualitative detection of a specific DNA sequence in chocolate by real-time PCR
EN 15634-5:2023	Foodstuffs - Detection of food allergens by molecular biological methods - Part 5: Mustard ( <i>Sinapis alba</i> ) and soya ( <i>Glycine max</i> ) - Qualitative detection of a specific DNA sequence in cooked sausages by real-time PCR
EN 15842:2019	Foodstuffs - Detection of food allergens - General considerations and validation of methods
EN 17254:2019	Foodstuffs - Minimum performance requirements for determination of gluten by ELISA
EN 17644:2022	Foodstuffs - Detection of food allergens by liquid chromatography - mass spectrometry (LC-MS) methods - General considerations

**Appendix I**

<b>Allergen</b>	<b>Method Name</b>	<b>Method Description</b>	<b>Analyte</b>	<b>LOQ or Range</b>	<b>Notes</b>
<b>Crustacea</b>	FA test EIA-crustacea II	ELISA	Total crustacea (shrimp and crab) protein	0.31 – 20 µg/g (expressed as total shrimp and crab protein)	Official Japanese method. Shimadzu Cat # 08624
<b>Crustacea</b>	Crustacea kit II " Maruha Nichiro"	ELISA	Crustacean protein	1 – 20 µg/g (expressed as total shrimp and crab protein)	Official Japanese method. Maruha Cat # 55362
<b>Crustacea</b>	Crustacean ELISA Kit II	ELISA	Black tiger tropomyosin	0.31 – 20 µg/g (expressed as total crustacean protein)	Official Japanese method. MloBS Cat # M2118
<b>Crustacea</b>	RIDASCREEN®FAST Crustacean	ELISA	Crustacea tropomyosin	20 – 160 ppm crustacean	R-Biopharm Cat # R7312
<b>Crustacea</b>	AgraQuant® Crustacea ELISA test kit	Sandwich ELISA	Tropomyosin	20-40 ppb tropomyosin 0.1 – 2 ppm crustacean protein	Romer Labs Cat # 10002076
<b>Crustacea</b>	SureFood® ALLERGEN Crustaceans	RT-PCR	Crustacea DNA	Estimated LOD 2.5 mg/kg of crustacean but depends on matrix, food processing	S3612 R-Biopharm
<b>Crustacea</b>	In-House method	RT-PCR	Crustacea DNA	LOD 5 mg/kg food	
<b>Crustacea</b>	ELISA Systems Crustacean Tropomyosin Residue		Shrimp tropomyosin	0.05 – 0.5 mg/kg (ppm)	ELISA Systems Cat # ESCURD-48
<b>Crustacea</b>	In-House method	ELISA	Total crustacean protein	0.5 – 10 ppm crustacean protein	Kit not commercialized. Accreditation ISO17025
<b>Crustacea</b>	Ridascreen FAST Crustacean	ELISA	Traces of crustacean protein	20 – 160 mg / kg (ppm) crustacean	R-Biopharm Cat # R7312
<b>Crustacea</b>	ELISA Systems Crustacean Tropomyosin Residue	ELISA	Tropomyosin	0.5 – 5 ppm tropomyosin	ELISA Systems Cat # ESCURD-48

<b>Allergen</b>	<b>Method Name</b>	<b>Method Description</b>	<b>Analyte</b>	<b>LOQ or Range</b>	<b>Notes</b>
<b>Crustacea</b>	Crustacean ELISA Kit II	ELISA	Black tiger tropomyosin	0.31 – 20 µg/g (expressed as total crustacean protein)	MloBS Cat # M2118
<b>Crustacea</b>	AgraQuant Crustacea	ELISA	Tropomysin	20 ppb tropomyosin (100 ppb crustacea protein)	Romer Labs Cat # 10002076
<b>Fish</b>	AgraQuant Fish ELISA	ELISA	Fish Parvalbumin (Cod)	4—100 mg/kg (depending of fish species)	Romer Labs Article number: 10002083
<b>Fish</b>	AgraQuant Fish ELISA	ELISA	Fish Parvalbumin	4—100 mg/kg (depending of fish species)	Romer Labs Article number: 10002083
<b>Fish</b>	In-House method	RT-PCR	DNA	LOD 2.5 mg/kg	Based on commercial kit MA 70-BM
<b>Fish</b>	SureFood ® ALLERGEN fish	RT-qPCR	DNA	LOD 2.5 mg/kg	S3610 R-Biopharm
<b>Fish</b>	In-House method	RT-PCR	DNA	LOD 5 mg/kg food	
<b>Fish</b>	SureFood ® ALLERGEN fish	RT-qPCR	DNA	LOQ 4 mg/kg (protocol 1)	S3610 R-Biopharm; To be combined with SureFood PREP Advanced kit S1053, protocol 1 or SureFast Mag PREP Food kit F1060
<b>Fish</b>	Biocheck Fish-check Elisa kit	ELISA	Fish Parvalbumin	5-125 ppm raw fish (cod)	Cat. No. R6009/R6010
<b>Fish</b>	AgraQuant Fish	ELISA	Fish	4 ppm cod	Romer Labs Article number: 10002083
<b>Wheat (gluten)</b>	Ridascreen Gliadin	ELISA	Gliadin	5-80 ng/ml gliadin	Cat. No. R7001
<b>Wheat (gluten)</b>	Ridascreen Gliadin competitive	ELISA	Gliadin	10 -270 ppb (ng/ml) gliadin	Cat. No. R7021
<b>Wheat (gluten)</b>	AgraQuant Gluten G12	ELISA	Gliadin	4-200 mg/kg (ppm)	Romer Labs item no. 1000020480



Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
<b>Wheat (gluten)</b>	Ridascreen Total Gluten	ELISA	Gliadin (Prolamins, HMW glutenins, HMW secalins, LMW glutenins)	5-80 mg/kg (ppm) gluten	Cat. No. R7041
<b>Wheat (gluten)</b>	SureFood Allergen 4 plex cereals	PCR	Wheat Rye Barley DNA	≤ 1 mg/kg	Species Labeling. Cat. No. S7006
<b>Wheat (gluten)</b>	SureFood Allergen Gluten	PCR	gluten-containing cereals (such as spelt and khorasan wheat, rye, barley, oats)	≤ 0.4 mg/kg	Species Labeling. Cat. No. S3606
<b>Wheat (gluten)</b>	FASTKIT ELISA Ver. III wheat	ELISA	Total wheat protein	0.31 – 20 µg/g (expressed as total wheat protein)	Cat. # 385-15051(FUJIFILM Wako Pure Chemical Corporation) Cat. # 08763(Shimadzu Diagnostics Corporation)
<b>Wheat (gluten)</b>	Wheat/Gluten (Gliadin) ELISA Kit II	ELISA	Gliadin	0.31 – 20 µg/g (expressed as total wheat protein) , 026 - 17 µg/g (expressed as total gluten)	MloBS Cat. No. M2114
<b>Wheat (gluten)</b>	Allergeneye ELISA II Wheat	ELISA	Gliadin	0.31 – 20 µg/g (expressed as total wheat protein)	Cat. No. 077847
<b>Wheat (gluten)</b>	Wheat/Gluten ELISA Kit	ELISA	Gliadin	0.31 - 20 µg/g (expressed as total wheat protein) , 026 - 17 µg/g (expressed as total gluten )	MloBS Cat. No. M2103
<b>Wheat (gluten)</b>	R-Biopharm RIDASCREEN®	ELISA	Gliadin	5 – 80 ppm gluten	R7001

<b>Allergen</b>	<b>Method Name</b>	<b>Method Description</b>	<b>Analyte</b>	<b>LOQ or Range</b>	<b>Notes</b>
<b>Wheat (gluten)</b>	Wheat/Gluten ELISA Kit	ELISA	Gliadin	0.78 - 50 ng prtn/mL	MloBS M2103
<b>Wheat (gluten)</b>	Kit ELISA RIDASCREEN Gliadin	ELISA	Gliadin	5 – 80 ppm	R-Biopharm R7001
<b>Wheat (gluten)</b>	kit RIDASCREEN Gliadin competitive	ELISA competitive	Hydrolyzed Gluten	10-270 ppm	R-biopharm R7021
<b>Wheat (gluten)</b>	RIDA®QUICK Gliadin	immunochromatographic test	prolamines from wheat, rye and barley	LOD 4.4 mg/kg gluten in raw materials, 6.3 mg/kg gluten in processed food	R-biopharm R7003
<b>Wheat (gluten)</b>	SENSISpec INgezim Gluten R5		prolamines from wheat, rye and barley	LOD 3-200 mg/kg gluten	Cat. 30.GLU.K.2
<b>Wheat (gluten)</b>	Ridascreen® gliadin	ELISA	Gliadin	5-80 ng/ml gliadin	Cat. No. R7001
<b>Wheat (gluten)</b>	kit RIDASCREEN Gliadin competitive	ELISA competitive	Gliadin	10-270 ppm	R-biopharm R7021
<b>Wheat (gluten)</b>	RIDASCREEN® Gliadin	Sandwich ELISA	Gliadin fraction of gluten / Prolamins from wheat, rye, and barley (QQFPF epitope); hydrolyzed gluten	5 – 135 ppm gliadin 10 – 270 ppm gluten	R-biopharm R7021
<b>Wheat (gluten)</b>	SureFood®ALLERGEN 4plex Cereals	RT PCR	DNA from wheat, rye, and barley	< 1 ppm	R-biopharm S7006 (species labeling)
<b>Wheat (gluten)</b>	Ridascreen® gliadin	ELISA	Gliadin	5-80 ng/ml gluten or 2.5 - 40 gliadin	R-biopharm Cat. No. R7001
<b>Wheat (gluten)</b>	Ridascreen Gliadin competitive	ELISA competitive	Peptide fragments of gliadins and corresponding prolamins	10-270 ppm gluten or 5 – 135 ppm gliadin	R-biopharm R7021 (hydrolyzed gluten)

Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
<b>Wheat (gluten)</b>	Ridascreen®Gliadin	ELISA	Total Gliadins	2,5-80 ug/g total gliadin proten. 5-160 ug/g gluten	R-biopharm Cat. No. R7001
<b>Wheat (gluten)</b>	RIDASCREEN® Gliadin	ELISA	Gluten	2.5 ppm Gliadin or 5 ppm Gluten	R-biopharm Cat. No. R7001
<b>Hazelnut</b>	ELISA Systems Hazelnut Residue	ELISA	Hazelnut	0.5 – 5.0 mg/kg (ppm)	ELISA Systems Product Code: ESHRD-48
<b>Hazelnut</b>	Hazelnut ELISA Kit II	ELISA	11S globulin	0.16 - 10 µg/g (expressed as total hazelnut protein)	MloBS Cat No M2119
<b>Hazelnut</b>	Neogen Veratox	ELISA	Hazelnut protein	2.5 – 25 ppm hazelnut	Neogen Cat. No. 8420
<b>Hazelnut</b>	RIDASCREEN® Fast	ELISA	Hazelnut protein	2.5 – 20 ppm hazelnut	R-biopharm Cat. No. R6802
<b>Hazelnut</b>	CEN - EN 15634-3	PCR	Hazelnut DNA	Qualitative	CEN - EN 15634-3
<b>Hazelnut</b>	RIDASCREEN FAST Hazelnut	ELISA	Hazelnut protein	2,5-20 ppm hazelnut	R-biopharm Cat. No R6802
<b>Hazelnut</b>	RIDASCREEN FAST Hazelnut	ELISA	Hazelnut protein	2,5-20 ppm hazelnut	R-biopharm Cat. No R6802
<b>Hazelnut</b>	In-house kit	ELISA	Total hazelnut protein	0.5 – 10 ppm hazelnut protein	In-House method
<b>Hazelnut</b>	In-house	UHPLC-MS/MS	Total hazelnut protein	LOD = 2.5 ppm hazelnut protein	Qualitative method (multi-allergens)
<b>Hazelnut</b>	RIDASCREEN FAST Hazelnut	ELISA	Hazelnut protein	2,5-20 ppm hazelnut	R-biopharm Cat. No R6802
<b>Hazelnut</b>	In-house method (ILVO)	UHPLC-MS/MS	Total hazelnut protin	LOQ = 1.3 ppm total hazelnut protein	Quantitative method (multi-allergens)
<b>Hazelnut</b>	RIDASCREEN FAST Hazelnut	ELISA	Hazelnut protein	2,5-20 ppm hazelnut	R-biopharm Cat. No R6802

Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
Hazelnut	RIDASCREEN FAST Hazelnut	ELISA	Hazelnut protein	2,5-20 ppm hazelnut	R-biopharm Cat. No R6802
Hazelnut	AgraQuant Hazelnut	ELISA	Hazelnut protein	1 ppm hazelnut	Romer Labs 10002008
Hazelnut	Screening Method for Egg, Milk, Peanut, Soy and Hazelnut Allergens in Cereals and cookies by LC-MS/MS (In-house method)	LC-MS/MS	Hazelnut	N/A.	Not a quantification method; LOD 10 ppm hazelnut
Sesame	ELISA Systems Sesame Seed Protein Residue	ELISA	Sesame heat-stable 2S-albumin proteins	0.25 – 2.5 mg/kg (ppm)	Product Code: ESSESE-48
Sesame	Sesame ELISA Kit II	ELISA	11S globulin	0.16 - 10µg/g (expressed as total sesame protein)	MloBS Cat. No. M2121
Sesame	RIDASCREEN®Fast	ELISA	Sesame proteins	2.5-20 ppm sesame	R-biopharm Cat. No. R7202
Sesame	Sesame ELISA kit II	ELISA	11S globulin	0.87-56 ppm sesame	MloBS Cat. No. M2121
Sesame	In-house DNA	RT PCR	Sesame DNA	Qualitative Method	DOI: 10.1007/978-1-0716-3358-8_8
Sesame	RIDASCREEN®FAST Sesame	Sandwich ELISA	Total Sesame protein	2.5 – 20 mg / kg (ppm) sesame	R-biopharm Cat. No. R7202
Sesame	In-house kit	ELISA	Total Sesame protein	0.1-1 ppm sesame protein	In house method
Sesame	RIDASCREEN®FAST Sesame	ELISA	Total Sesame protein	2.5 – 20 mg / kg (ppm) sesame	R-biopharm Cat. No. R7202
Sesame	RIDASCREEN FAST	PE-I1027 sandwich ELISA	White, black and yellow sesame proteins.	LOQ: 2.5 mg of sesame/Kg with a working range between 2.5 and 20.0 mg of sesame/Kg of sample.	R-biopharm Cat. No. R7202
Sesame	In house	RT PCR	Sesame DNA	5 ppm	Non commercial in house method

Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
Milk	Veratox	ELISA	Total Milk Allergen	2.5 – 25 ppm milk protein	Neogen Cat. No. 8470
Milk	Total Milk ELISA Kit II	ELISA	Total Milk protein	0.92 – 58.8 ppm milk protein	MloBS Cat. No. M2102; validated for baked for processed foods, replaced with Cat. No. M2122
Milk	Total Milk	ELISA	Total Milk protein	1-10 ppm NFM	ELISA Systems Cat. No. EC52310
Milk	Beta Lactoglobulin	ELISA	$\beta$ -lactoglobulin	0.1-1 ppm BLG	ELISA Systems Cat. No. EB52110
Milk	RIDASCREEN®FAST Milk	ELISA	Total milk, based on 50% caseins and 50% $\beta$ -Lactoglobulin	2.5-67.5 mg milk protein/ kg (ppm)	r-Biopharm Art. No.: R4652 Validated by producer for sausages, ice cream, chocolate, bakery goods, cake and bread mix, soups, sauces, dressings and beverages (juice, wine, beer).
Milk	RIDASCREEN®FAST Casein	ELISA	Caseins	0.5-13.5 mg casein/kg (ppm) LOQ depends on matrix and extraction	R-Biopharm Art. No.: R4612  Validated by manufacturer for bakery goods, cake and bread mix, non-hydrolyzed milk-based infant formula, ice cream, beverages, chocolate, wine and sausages.
Milk	RIDASCREEN®FAST $\beta$ -Lactoglobulin	ELISA	$\beta$ -lactoglobulin	0.167-4.5 mg $\beta$ -lactoglobulin / kg (ppm)	Art. No.: R4912  Validated by manufacturer for rice crispies, chocolate and sausage.

Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
Milk	casein ELISA Kit II	ELISA	Total Milk protein	0.31 ppm [ $\mu\text{g}$ of milk protein / g of food] Range: 0.31 - 20 ppm	MloBS Cat. No. M2113
Milk	RIDASCREEN® $\beta$ -Lactoglobulin	ELISA	$\beta$ -lactoglobulin	LOD = 0.9 – 2.1 mg/kg (ppm) depending on the matrix LOQ = 5 mg/kg (ppm)	Art. No.: R4901  Validated by manufacturer for hydrolyzed milk products including hypoallergic baby foods.
Milk	FASTKIT ELISA Ver. III milk	ELISA	Total Milk protein	0.31 – 20 $\mu\text{g/g}$ (expressed as total milk protein)	Cat. # 388-15041(FUJIFILM Wako Pure Chemical Corporation) Cat. # 08762(Shimadzu Diagnostics Corporation)
Milk	Total Milk ELISA Kit II	ELISA	Casein & $\beta$ -lactoglobulin	0.31 – 20 $\mu\text{g/g}$ (expressed as total milk protein)	MloBS Cat. No. M2122
Milk	Allergeneye ELISA II Milk	ELISA	$\beta$ -lactoglobulin	0.31 – 20 $\mu\text{g/g}$ (expressed as total milk protein)	Prima Cat. 077836
Milk	Neogen Veratox	ELISA	Total Milk protein	2.5-50 ppm milk protein	Cat. No. 8430
Milk	ELISA Systems Casein	ELISA	Casein	1.0-10 mg/kg expressed as skim milk powder	Product Code: ESCASPRD-48
Milk	ELISA Systems Beta-Lactoglobulin residue	ELISA	$\beta$ -lactoglobulin	0.0-1.0 mg/kg	Product Code: ESMRDBLG-48
Milk	Ridascreen Fast Milk	Sandwich ELISA	Total Milk proteins	2.5-67.5 ppm	r-Biopharm Cat. No. R4652
Milk	Total Milk ELISA	ELISA	Total Milk proteins	0.31-20 ppm total milk protein	MloBS Cat. No. M2122

Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
Milk	Casein ELISA Kit II	Sandwich ELISA	Casein	0.31-20 ppm milk protein	MloBS Cat. No. M2113
Milk	$\beta$ -lactoglobulin ELISA Kit II	Sandwich ELISA	$\beta$ -lactoglobulin	0.31-20 ppm milk protein	MloBS Cat. No. M2112
Milk	In-house kit	ELISA	Total caseins	0.5-20 ppm caseins	In-house method
Milk	In-house kit	ELISA	$\beta$ -lactoglobulin	0.25-5 ppm caseins	In-house method
Milk	In-house kit	UHPLC-MS/MS	Total milk proteins	LOD = 5 ppm milk protein (beta-lactoglobulin) LOD = 0.5 ppm milk protein (casein)	In-house method
Milk	In-house method (ILVO)	UHPLC-MS/MS	Total milk proteins	LOQ = 1 ppm total milk protein	Quantitative method (multi-allergens)
Milk	Ridascreen Fast Milk	ELISA	Caseins, $\beta$ -lactoglobulin from cow's milk and of sheep, goat, and buffalo milk	LOQ 2.5 ppm	r-Biopharm Cat. No. R4652; sheep, goat, buffalo cross reactivity
Milk	Ridascreen Fast Milk	ELISA	Total Milk protein	2.5-67.5 ppm	r-Biopharm Cat. No. R4652
Milk	Ridascreen@Fast Casein	ELISA	Casein	0,5 - 13,5 mg/kg	r-Biopharm Cat. No. R4612
Milk	Ridascreen@Fast $\beta$ -lactoglobulin	ELISA	$\beta$ -lactoglobulin	0,167 - 4,5 mg/kg	r-Biopharm Cat. No. R4902
Milk	Ridascreen@Fast $\beta$ -lactoglobulin	ELISA	$\beta$ -lactoglobulin hydrolyzed protein	5 - 8,1 mg/kg	r-Biopharm Cat. No. R4901
Milk	RIDASCREEN FAST Casein	ELISA	Casein	0.5 – 13.5 ppm casein, or 2.5 – 13.5 ppm depending on extraction and matrix	r-Biopharm Cat. No. R4612
Milk	Ridascreen Fast Milk	ELISA	Total Milk protein	2.5-67.5 ppm	r-Biopharm Cat. No. R4652

Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
<b>Milk</b>	Screening Method for Egg, Milk, Peanut, Soy and Hazelnut Allergens in Cereals and cookies by LC-MS/MS (In-house method)	LC-MS/MS	Milk	N/A	Not a quantification method; LOD 3 ppm whole milk powder
<b>Egg</b>	Veratox Egg	ELISA	Ovalbumin	2.5 – 25 ppm	Neogen Cat. No. 8450
<b>Egg</b>	Egg (Ovalbumin) ELISA Kit II	ELISA	Ovalbumin	0.65 – 41.6 ppm	MioBS Cat. No. M2111
<b>Egg</b>	FASTKIT ELISA Ver. III egg	ELISA	Total egg protein	0.31 – 20 µg/g (expressed as total egg protein )	Cat. # 381-15031(FUJIFILM Wako Pure Chemical Corporation) Cat. # 08761(Shimadzu Diagnostics Corporation)
<b>Egg</b>	Egg (Ovalbumin) ELISA Kit II	ELISA	Ovalbumin	0.31 – 20 µg/g (expressed as total egg protein )	MioBS Cat. No. M2111
<b>Egg</b>	Allergeneye ELISA II Egg	ELISA	Ovalbumin	0.31 – 20 µg/g (expressed as total egg protein )	Prima Cat. No. 077834
<b>Egg</b>	ELISA Systems Processed Egg Residue	ELISA	Ovomucoid	1 – 10 mg/kg (ppm)	ELISA Systems Cat. No. ESEGGPR-48
<b>Egg</b>	Ridascreen FAST Lysozyme	ELISA	Lysozyme	0.25 – 2.5 (ppm)	r-Biopharm Cat No. R6452
<b>Egg</b>	Ridascreen Fast Egg	Sandwich ELISA	Total Egg proteins	0.5 – 13.5 ppm	r-Biopharm R6402
<b>Egg</b>	Morinaga Egg (Ovalbumin) ELISA Kit II	ELISA	Ovalbumin	0.31 - 20 ppm ovalbumin	MioBS Cat No M2111
<b>Egg</b>	Egg (Ovalbumin) ELISA kit II	Sandwich ELISA	Ovalbumin	0.31 – 20 ppm egg protein	MioBS Cat No M2111
<b>Egg</b>	In-house kit	ELISA	Total egg protein	0.25 – 2 ppm egg protein	In house method



Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
<b>Egg</b>	In-house method	UHPLC-MS/MS	Total egg protein	LOD = 3 ppm egg protein (white) LOD = 30 ppm egg protein (yolk)	Qualitative method (multi-allergens)
<b>Egg</b>	In-house method (ILVO)	UHPLC-MS/MS	Total egg protein	LOQ = 6.2 ppm total egg protein	Qualitative method (multi-allergens)
<b>Egg</b>	Ridascreen®Fast Ei/EGG	ELISA	Whole egg (ovalbumin and ovomucoid)	0,5 - 13,5 mg/kg	r-Biopharm Cat. No. R6402
<b>Egg</b>	Ridascreen® Fast Egg	ELISA	Whole egg powder	0,5 - 13,5 mg/kg	r-Biopharm Cat. No. R6402
<b>Egg</b>	Ridascreen® Fast Egg	ELISA	Whole egg powder	0,5 - 13,5 mg/kg	r-Biopharm Cat. No. R6402
<b>Egg</b>	Screening Method for Egg, Milk, Peanut, Soy and Hazelnut Allergens in Cereals and cookies by LC-MS/MS (In-house method)	LC-MS/MS	Egg	N/A	Not a quantification method; LOD 1.65 ppm whole egg powder
<b>Peanut</b>	Veratox Peanut	ELISA	Peanut proteins	2.5 – 25 ppm peanut	Neogen Cat. No. 8430
<b>Peanut</b>	RIDASCREEN® Peanut	ELISA	Peanut proteins	0.75-6 ppm peanut	r-Biopharm Cat. No. R6811. Specific for roasted peanuts and over detects raw or slightly roasted peanuts
<b>Peanut</b>	FASTKIT ELISA Ver. III peanut	ELISA	Total peanut protein	0.31 – 20 µg/g (expressed as total peanut protein )	Cat. # 389-15071(FUJIFILM Wako Pure Chemical Corporation) Cat. # 08765(Shimadzu Diagnostics Corporation)
<b>Peanut</b>	Peanut ELISA Kit II	ELISA	Partially purified peanut protein	0.31 – 20 µg/g (expressed as total peanut protein )	MloBS Cat. No. M2116
<b>Peanut</b>	Allergeneye ELISA II Peanut	ELISA	Ara h1	0.31 – 20 µg/g (expressed as total peanut protein )	Prima Cat. No. 077860

Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
Peanut	High Sensitive Peanut ELISA Kit II	ELISA	Ara h2, Ara h6	0.2 - 12.8 µg/g as NIST peanut butter SRM2387 protein amount	MloBS Cat No M2120
Peanut	SureFood Allergen Peanut	PCR	Peanut DNA	≤ 1 mg/kg	r-Biopharm Cat. No. S3606
Peanut	RIDASCREEN Peanut	ELISA	Peanut	0.75 - 6 mg/kg (ppm) peanut	r-Biopharm Cat. No. R6811
Peanut	Neogen Veratox Peanut	ELISA	Peanut	2.5 - 25 mg/kg (ppm) total peanut	Neogen Cat. No. 8430
Peanut	Detection of peanut DNA	PCR	Peanut DNA	Qualitative	Hird H. et al. (2003) Detection of peanut using RT-PCR. Eur. Food Res. Techn. 217:265-268
Peanut	Veratox® for Peanut Allergen	Sandwich ELISA	Total Peanut	2.5–25 ppm peanut	Neogen Cat. No. 8430
Peanut	In House Method	LC-MS/MS	Peptide markers for peanut protein	10 ppm total peanut	Targeted MS
Peanut	Veratox® VIP for Peanut	Sandwich ELISA	Peanut	0.25 – 5 ppm peanut protein	Neogen Cat. No. 8430M
Peanut	High Sensitive Peanut ELISA Kit II	Sandwich ELISA	Ara h2, Ara h6	0.2 – 12.8 ppm peanut protein	MloBS Cat. No. M2120
Peanut	In-house kit	ELISA	Total peanut protein	0.25 – 2 ppm peanut protein	In house method
Peanut	In-house method	UHPLC-MS/MS	Total peanut protein	LOD = 2.5 ppm peanut protein	Qualitative method (multi-allergens)
Peanut	RIDASCREEN Peanut	ELISA	Peanut	0.75 - 6 mg/kg (ppm) peanut	r-Biopharm Cat. No. R6811. No cross reactivity in 91 commodities
Peanut	In-house method (ILVO)	UHPLC-MS/MS	Total peanut protein	LOD = 3.8 ppm total peanut protein	Qualitative method (multi-allergens)
Peanut	Ridascreen®Peanut	ELISA	Peanut protein	0.75 - 6 mg/kg (ppm) peanut	r-Biopharm Cat. No. R6811.

Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
<b>Peanut</b>	Ridascreen®Peanut	ELISA	Peanut protein	0.75 - 6 mg/kg (ppm) peanut	r-Biopharm Cat. No. R6811.
<b>Peanut</b>	Ridascreen®Peanut	ELISA	Peanut protein	0.75 - 6 mg/kg (ppm) peanut	r-Biopharm Cat. No. R6811.
<b>Peanut</b>	Screening Method for Egg, Milk, Peanut, Soy and Hazelnut Allergens in Cereals and cookies by LC-MS/MS (In-house method)	LC-MS/MS	Peanut	N/A	Not a quantification method; LOD 3 ppm whole peanut
<b>Cashew</b>	Veratox VIP	ELISA	Cashew protein	1.0 – 25 ppm cashew	Neogen Cat. No. 8570
<b>Cashew</b>	MonoTrace	ELISA	Cashew protein	1.0 – 40 ppm cashew	BioFront Cat. No. CA2-EK-48
<b>Cashew</b>	SensiSpec ELISA Cashew	ELISA	Cashew	2 – 60 mg/kg (ppm)	HU0030004/ HU0030028
<b>Cashew</b>	Biofront Cashew	ELISA	Cashew	1 - 40 mg/kg (ppm)	BioFront Cat. No. CA2-EK-48
<b>Cashew</b>	Detection of cashew DNA	PCR	Cashew DNA	Qualitative method	Ehlert et al. Detection of cashew nut in foods by a specific RT-PCR method. Food Anal Methods (2008) 1:136-143
<b>Cashew</b>	RIDASCREEN®FAST Cashew	ELISA	Total cashew	2.5 – 20 ppm cashew	r-Biopharm Cat. No. R6872
<b>Cashew</b>	3M™ Cashew Protein ELISA Kit	ELISA	Cashew protein	0.9 – 24.3 ppm cashew protein	Neogen Cat. No. E96CHW
<b>Cashew</b>	In-house kit	ELISA	Total cashew nut protein	0.5 – 10 ppm cashew nut protein	In house method
<b>Cashew</b>	In-house method	UHPLC-MS/MS	Total cashew nut protein	LOD = 2.5 ppm cashew nut protein	Qualitative method (multi-allergens)
<b>Cashew</b>	RIDASCREEN®FAST Cashew	ELISA	Total cashew	2.5 – 20 ppm cashew	r-Biopharm Cat. No. R6872
<b>Cashew</b>	RIDASCREEN®FAST Cashew	Sandwich ELISA	Total cashew	2.5 – 20 ppm cashew	r-Biopharm Cat. No. R6872. Cross reactive with pistachio
<b>Cashew</b>	SureFood® ALLERGEN Cashew	RT PCR	Cashew DNA	2.5 – 20 ppm cashew	r-Biopharm Cat. No. S3615

Allergen	Method Name	Method Description	Analyte	LOQ or Range	Notes
Walnut	Neogen BioKits Walnut	ELISA	Walnut protein	2.4 – 120 ppm walnut	Neogen Cat. No. 902085J
Walnut	AgraQuant Walnut	ELISA	Total Walnut protein	2 – 60 ppm walnut	Romer Labs Cat. No. COKAL0948
Walnut	FASTKIT ELISA Ver. III walnut	ELISA	Total Walnut protein	0.31 – 20 µg/g (expressed as total walnut protein)	Cat. # 389-20961(FUJIFILM Wako Pure Chemical Corporation) Cat. # 08791(Shimadzu Diagnostics Corporation)
Walnut	Walnut ELISA Kit II	ELISA	2S albumin	0.31 – 20 µg/g (expressed as total walnut protein)	MloBS Cat. No. M2124
Walnut	FA test EIA-Walnut	ELISA	Total Walnut protein	0.31 – 20 µg/g (expressed as total walnut protein )	Shimadzu Cat. No. 08637
Walnut	SensiSpec ELISA Walnut	ELISA	Walnut protein	2 – 60 mg/kg (ppm)	HU0030024/ HU0030048
Walnut	Neogen BioKits Walnut	ELISA	Walnut protein	2.4 -120 mg/kg (ppm)	Neogen Cat. No. 902085J
Walnut	Detection of walnut DNA	PCR	Walnut DNA	Qualitative method	Brezna et al. A novel real-time PCR method for the detection of walnuts in food. Eur Res Technol (2006) 223: 373-377
Walnut	BioKits Walnut Assay Kit	ELISA	Walnut protein	2.4-120 ppm walnut	Neogen Cat. No. 902085J
Walnut	AgraQuant® Walnut ELISA test kit	ELISA	Walnut protein	2 – 60 ppm walnut	Romer Labs Cat No. 10002030
Walnut	AgraQuant® Walnut ELISA test kit	ELISA	Walnut protein	0.28 – 8.4 ppm walnut protein (2 – 60 ppm walnut)	Romer Labs Cat No. COKAL0948
Walnut	In house method	UHPLC-MS/MS	Total walnut protein	LOD = 5.0 ppm walnut protein	Qualitative method (multi-allergens)
Walnut	SureFood® ALLERGEN Walnut	RT PCR	specific walnut (Juglans regia and Juglans nigra) DNA sequences	1 mg / kg using SureFood® PREP Advanced, protocol 1	r-Biopharm Cat. No. S3607

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<b>Allergen</b>	<b>Method Name</b>	<b>Method Description</b>	<b>Analyte</b>	<b>LOQ or Range</b>	<b>Notes</b>
<b>Walnut</b>	BioKits Walnut Assay Kit	ELISA	Walnut protein	2.4-120 ppm walnut	Neogen Cat. No. 902085J

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**Appendix II****List of Participants****Chair**

Patrick Gray  
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**Co-chair**

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Mia Hallgren  
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Melanie Downs  
Kadeleine Kong

Swedish Food Agency  
Health Canada  
EUMS  
Australia - National Measurement Institute  
Japan - Consumer Affairs Agency  
International Dairy Federation  
AOAC - Food Allergens Working Group Chair  
Singapore Food Agency