

CODEX ALIMENTARIUS COMMISSION



Food and Agriculture
Organization of the
United Nations



World Health
Organization

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Agenda Item 8

CX/MAS 25/44/11

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ORIGINAL LANGUAGE ONLY

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

44th Session

Virtual

5 – 8 May and 14 May 2025

METHODS OF ANALYSIS FOR PRECAUTIONARY ALLERGEN LABELLING

(Report of the Electronic Working Group (EWG) prepared by the United States of America, chair of the EWG)

Introduction

1. The 47th session of the Codex Committee on Food Labeling (CCFL47) requested advice from CCMAS on standardized analytical methods and sampling used for determining allergenic protein in foods ([CX/MAS 23/42/2 Add.1](#)). Specifically, CCFL47 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 priority allergens agreed by CCFL and adopted in the revision of the *General Standard for the Labelling of Prepackaged Foods* (CXS 1-1985) are as follows:
 - Cereals containing gluten (wheat and other *Triticum* species, rye and other *Secale* species, barley and other *Hordeum* species)
 - Crustacea
 - Eggs
 - Fish
 - Peanuts
 - Milk
 - Sesame

- Specific tree nuts (Almond, Cashew, Hazelnut, Pecan, Pistachio, Walnut)
3. CCMAS42 established an EWG to develop a discussion paper which would discuss the best practices for the selection of validated analytical methods and for the validation of such methods. CCMAS42 agreed that the EWG would not address the question on sampling plans, noting that sampling plans are covered by the *General Guidelines on Sampling* (CXG 50-2004).
 4. The discussion paper was presented at CCMAS43 ([CX/MAS 24/43/9](#)). CCMAS43 noted the general support to continue work in the EWG, and that the methods in the Appendix I to CX/MAS 24/43/9 (which also included confirmatory methods) were a good starting point for evaluation against the CEN performance characteristics and the AOAC validation guidelines.
 5. CCMAS43 noted that the discussion paper did not fully answer the questions from CCFL47 and agreed to re-establish the EWG, chaired by the United States of America, and co-chaired by the United Kingdom, working in English to:
 - request Members to submit validation data of the methods listed in Appendix I of CX/MAS 24/43/9
 - evaluate the submitted validation studies through published method validation guidelines from AOAC and CEN performance requirements
 - submit a list of methods that meet either one or both of the AOAC validation guidelines and CEN performance requirements

EWG Process and Discussion

6. Members and Observers were invited to join the EWG in June 2024. Submitted methods from CX/MAS 24/43/9 were provided to EWG members, and a “Preventative Allergen Method Validation Information Form” was distributed to EWG members via the Codex forum. Comments were received from 14 Members and Observers. The information form can be found in Appendix I.
7. The following important information on methods was requested: method title, analysis principle, target analyte, conversion factor from analysis result to mass of total protein from the allergenic food, LOQ or analytical measurement range, validation status (i.e. single lab, collaboratively studied method, performance tested method, validation quality assurance including whether a reference material was used, whether the target allergen was spiked before or after processing, and the matrices and concentrations that were included in the validation study. In addition, the information form collected the method performance during the validation study including repeatability (RSD_r), the reproducibility (RSD_R), and % recovery.
8. More than 100 sets of method validation data were submitted for evaluation against the following method development, validation and performance guidelines:
 - AOAC Appendix M
 - EN 17855 (ELISA)
 - EN 17644 (LC-MS)
 - EN 17254 (ELISA Gluten)
 - EN 15634 (PCR)
9. The methods and validation status are found in Appendix II. In many cases, validations were performed by different Members on a single method. Appendix II retains those separate validation results. Efforts were made to consolidate the method validation and performance data; however, the extensive data provided by Members and Observers is quite large and is therefore made available in Microsoft Excel on the Codex forum for ease of use and also linked in Appendix II.
10. No methods were submitted for pecan or pistachio.
11. There was general agreement within CCMAS43 and the EWG that methods using incurred spiking protocols (i.e. allergens added to matrix prior to processing), collaboratively studied and performance tested methods, and those methods maintained by Standard Development Organizations were most suitable for reference back to CCFL.
12. It was noted by EWG members that both the AOAC Appendix M guidelines and the EN performance standards were published in 2024. Many methods were developed and validated before these guidelines were published and therefore may have been validated according to a different scheme. However, the results of these methods are not invalidated, and additional validation data could be obtained where needed.
13. Appendix II includes many methods currently in use, but the list is not exhaustive, and future methods will likely become available that also meet the performance requirements. The list in Appendix II should be treated as

“currently available methods” but a future method that meets the performance requirements published by AOAC and EN should also be considered as acceptable. Upon completing its review of the methods in Appendix II, CCMAS may consider including in its response to CCFL advice that future methods with acceptable performance will likely augment the current list.

14. The EWG Chairs has initiated a review of the methods in Appendix II but given the extensive amount of validation data provided, CCMAS may need more time to examine the validation data further before submitting a response to CCFL. For this reason, CCMAS44 may wish to consider re-establishing the EWG to complete the review of the validation data and develop a recommended response from CCMAS to the next session of CCFL (CCFL49).
15. Method submissions that contained validation data that did not meet the AOAC and EN performance requirements are highlighted in yellow with notes indicating the reason.
16. The list of participants in the EWG are included in Appendix III.

Recommendation

17. CCMAS44 is invited to:
 - i. Inform CCFL of the recently published method validation and performance guidelines by AOAC and the relevant EN standards;
 - ii. Review the included methods in Appendix II, and consider whether the methods meet the validation and performance guidelines from AOAC and the relevant EN standards; and
 - iii. Determine if the methods meet the CCFL threshold levels in *Risk Assessment of Food Allergens Part 2: Review and Establish Threshold Levels in Foods for the Priority Allergens*, and for which matrices.

Preventative Allergen Method Validation Information Form

Submitter Name:

This form will be used to collect information on the suitability and validation status of food allergen quantification methods.^{1,2}

Method Title	
Principle	
Analyte	
Conversion factor from analyte to protein	
LOQ and/or range (mg protein/kg food) ^a	
Additional Information	

Validation Status (check all that apply)

Single lab validation

SLV Citation

Multi lab validation

MLV Citation

Performance Tested Method

PTM Citation

Validation Detail

1. Was a certified reference material used?

Yes

If yes, list CRM _____

No

2. Were foods tested with incurred analytes (spiked prior to any processing) during the validation?

Yes

No

Method Performance

[illegible]

1. AOAC Appendix M Guidance on Food Allergen Immunoassay Validation.
<https://doi.org/10.1093/9780197610145.005.013>
2. EN 15842, EN 17855:2024 (ELISA), EN 15634-1-2019 (DNA), EN 17644-2022 (LC-MS)
3. mass of total protein from allergenic food per mass of food or environmental sample (e.g., mg total peanut protein per kg food, mg total milk protein per kg food)

Appendix II**The Preventative Allergen Methods and Validation Data**

Due to the size of the table of methods and their validation data, Appendix II is presented as an linked Microsoft Excel table [here](#).

Appendix III

List of Participants

Chair

Patrick Gray

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United States of America

Co-Chair

Oliver Severn

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United Kingdom

Name	Country	Organization	Status
Gloria Castillo Vargas	Perú	Instituto Nacional de Calidad - INACAL	Member Country
Jean Christophe Kremer	Belgium	International Special Dietary Foods Industries	Observer Organization
Food Industry Asia	Singapore	Food Industry Asia	Observer Organization
Mariam Barsoum Onsy	Egypt	Egyptian Organization for Standardization & Quality	Member Country
Richard COGHLAN	Australia	National Measurement Institute - Australia	Member Country
Thea Rawn	Canada	Health Canada	Member Country
Samaneh Eghtedari	Iran	INSO	Member Country
Christophe LEPRÉTRE	USA	ICGA - International Chewing Gum Association	Observer Organization
Rungrassamee Mahakhaphong	Thailand	ACFS	Member Country
Melina Flores	Costa Rica	Ministerio de Economía Industria y Comercio	Member Country
Jean-Luc DEBORDE	FRANCE	SCL - Service Commun des Laboratoires	Member Country
Laura Gelezuinas	France	FIVS	Observer Organization
Bhavna Parmar	UK	Food Standards Agency UK	Member Country
Eleonora Alquati	Belgium	ICA - International Confectionery Association	Observer Organization
Siska Pottie	Belgium	IMACE	Observer Organization
Allison Graham	United States	International Confectionery Association (ICA)	Observer Organization

Name	Country	Organization	Status
Youngjun Kim	Republic of Korea	the Ministry of Food and Drug Safety	Member Country
Neil Shepherd	Australia	NATA	Member Country
Peter Di Tommaso	Italy	Food and Agriculture Organization of the UN	Member Country
Krisztina Bakó-Frányó	Hungary	National Food Chain Safety Office	Member Country
Danset Moranga	Kenya	Kenya Bureau of Standards	Member Country
Kang Zhou	Italy	FAO	FAO/WHO
Anabel Mulet Cabero	Netherlands	International Dairy Federation	Observer Organization
Ana Claudia Araujo	Brazil	Anvisa	Member Country
Kittiporn Phuangsuk	Thailand	Ministry of Agriculture and Cooperatives	Member Country
Katerina Mastovska	United States	AOAC	Observer Organization
Vera Pavlicheva	Russia	Rospotrebnadzor	Member Country
Norimasa Tamehiro	Japan	National Institute of Health Science	Member Country
Annie Rubin de Celis	Peru	Comite Nacional de Codex	Member Country
Mohrah Ali Alenazi	Saudi Arabia	SFDA	Member Country
Stephan Walch	Germany	Chemisches und Veterinäruntersuchungsamt Karlsruhe	Member Country
Denise Lee	Canada	Baking Association of Canada	Member Country
Chitrlada Booncharoen	Thailand	National Bureau of Agricultural Commodity and Food	Member Country
Dr. Markus Lacorn	Deutschland	ICC	Observer Organization
Oliver Jack Severn	United Kingdom	Food Standards Agency	Member Country
Dr Eszter Fejesné Tóth	Hungary	National Food Chain Safety Office	Member Country
Nikoletta Farago	Hungary	National Food Chain Safety Office	Member Country
Sara Ares Santos	Spain	FAO/WHO	Member Country