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**METHODS OF ANALYSIS FOR PRECAUTIONARY ALLERGEN LABELLING**

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

**INTRODUCTION**

1. The 47th session of the Codex Committee on Food Labelling (CCFL47, 2023) 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 analytical 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. Priority allergens and the finalized action levels are listed in Table 11 of [Risk Assessment of Food Allergens Part 2: Review and Establish Threshold Levels in Foods for the Priority Allergens](#).
  - 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. CCMAS42 (2023) agreed to establish an EWG chaired by the USA and co-chaired by the United Kingdom (UK) to develop a discussion paper which would discuss best practices for the selection of validated methods and for the validation of such methods. CCMAS43 (2024) also 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). CCFL was informed of this decision. A discussion paper was presented at CCMAS43 ([CX/MAS 24/43/9](#)).
3. CCMAS43 noted general support to continue work in the EWG and that the methods compiled by the EWG following CCMAS42 were a good starting point for evaluation against CEN performance requirements and AOAC validation guidelines. The EWG was re-established to request Members to submit validation data for the methods, evaluate the submitted validation studies against the AOAC and CEN frameworks, and submit a list of methods that meet either one or both of the AOAC validation guidelines and/or CEN performance requirements. A discussion paper was presented at CCMAS44 ([CX/MAS 25/44/11](#)).
4. CCMAS44 (2025), noting that the output of the EWG was not suitable for referral to CCFL, agreed to re-establish an EWG chaired by USA and co-chaired by UK, working in English to:
  - finalize review of the methods in CX/MAS 25/44/11 against the available validation guidelines and performance requirements;
  - simplify the presentation of methods and their validation status included in CX/MAS 25/44/11 Appendix II;

- develop a draft response for consideration by CCMAS45 to CCFL49; and
- prepare and submit the report of the EWG to the Codex Secretariat at least three months before CCMAS45.

## BACKGROUND

5. 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)

### *Previous EWG Work*

6. The EWG work was done in a two-step process. First, members were asked to submit the methods in use in their countries for each allergen. The list of the submitted methods are in [CX/MAS 24/43/9](#). CCMAS43 further suggested that the submitted methods and their associated validation data be evaluated against established guidelines. More than 100 sets of method validation data were submitted for evaluation against the following method development, validation and performance guidelines:
    1. AOAC Appendix M
    2. EN 17855 (ELISA)
    3. EN 17644 (LC-MS)
    4. EN 17254 (ELISA Gluten)
    5. EN 15634 (PCR)
  7. The following information on the relevant 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
    - method performance during the validation study including repeatability (RSD<sub>r</sub>), the reproducibility (RSD<sub>R</sub>), and % recovery.
  8. The submitted methods along with their validation statuses were compiled and presented at CCMAS44.
  9. The methods and validation status are found in [CX/MAS 25/44/11](#) Appendix II. In many cases, validations were performed by different Members on a single method. CX/MAS 25/44/11 Appendix II retains those separate validation results.
- CCMAS44**
10. At CCMAS44 and during the virtual working group (VWG) preceding CCMAS44, consistent with discussions held during the course of previous EWGs, CCMAS members raised the following concerns regarding the response to CCFL:

- Most methods submitted to the EWG rely on proprietary methods, typically in the form of Enzyme-Linked Immunosorbent Assay (ELISA) kits. The *Codex Procedural Manual* specifies that “a proprietary method should not be endorsed if a suitable non-proprietary method of analysis is available” and that “preference should be given to adopting appropriate method criteria rather than endorsing a specific proprietary method of analysis.”<sup>1</sup> Although the EWG is not endorsing any method through this work, the same principles should apply to the recommendations from CCMAS to CCFL.
- Most proprietary methods are not distributed globally and the lack of availability of certain regions to access these methods would be restrictive to trade.
- Some Member method submissions included qualitative methods. However, some Members indicated that these methods are not suitable to determine whether a commodity would meet the threshold levels. Qualitative methods were not included in the tables of recommended methods.
- The threshold levels in Table 11 of [Risk Assessment of Food Allergens Part 2: Review and Establish Threshold Levels in Foods for the Priority Allergens](#) vary by approximately two orders of magnitude. Whether or not a method will be suitable at the relevant threshold concentration is dependent on the food intake amount (RfA) and the allergen. For example, some methods included in the results of this EWG are appropriate at certain RfA but not others. The testing laboratory should confirm that the method's measurement range spans the threshold action level.
- The performance (accuracy, precision, recovery, etc.) of allergen analytical methods is heavily dependent upon the food matrix and food production processing. For example, egg white proteins exposed to high temperatures will undergo denaturation leading to reduced solubility and antibody recognition. This leads to an underestimation of egg proteins in thermally processed foods from certain ELISA kits. It is critical that trading partners understand the limitations of ELISA to detect allergens in processed foods and ensure their choice of method is suitable.
- Some ELISA kits have undergone critical changes since the time when validation studies were performed. For example, some manufacturers have changed extraction buffers to less hazardous reagents, and the associated performance of these kits may have changed as a result. Users must ensure that the chosen ELISA method or kit can meet the intended needs.
- The reporting units in many ELISA kits are not in the same units as the threshold levels used by CCFL. In many cases there is a conversion factor required to convert the test reporting units into mg total allergen protein per kg food. Conversion factors reported during the EWG process varied, even among the same ELISA kits which shows that consistent reporting can be difficult.
- Proprietary ELISA kits have typically included cross reactivity studies in their manufacturer validations. However, it is up to the laboratory users to identify the cross reactivities and choose ELISA kits that will not produce false positives on the food matrix being tested. The manufacturer selectivity study is a resource but not a guarantee against cross reactivity.
- The tables reflect methods submitted by EWG members and are not exhaustive. Future methods will likely become available that also meet either the CEN performance requirements and/or the AOAC validation guidelines. The methods included in the CCMAS response to CCFL should not preclude future methods from being developed and used in trade.
- Collaborative studies are used to estimate expected method performance in practice, particularly precision and recovery. Similarly, independent laboratory studies (e.g. performance tested methods) can show how the method performs on an unknown sample. However, simply because a method has been collaboratively studied does not necessarily indicate that it performs superior to methods that have only been validated by the manufacturer or validated in a single laboratory.
- The completeness of validation data varied significantly among the submitted methods. Some assays had extensive multi-laboratory validation available, while others provided only limited in-house data or none publicly at all. This variability in validation rigor and transparency should be considered by the trading partners and may be addressed in the future considering the recently published AOAC and EN guidelines.

## EWG CONSULTATION 1

11. The EWG chairs took into consideration the discussions before and during CCMAS44 and prepared two tables (available in Appendix II of this document) for a response to CCFL. Table 1 included methods that were either collaboratively studied or performance tested methods. These methods have shown acceptable performance

<sup>1</sup> *Codex Procedural Manual*. 30<sup>th</sup> edition. Section 2.13: Provisions on the use of proprietary methods in Codex standards. Pg. 70.

on blinded food samples. Table 2 included methods that were validated either at the manufacturer, in a single laboratory, or in-house.

12. In addition, the EWG chairs developed a draft response to CCFL to accompany these tables and fulfill the request from CCFL47 (Appendix I). In the draft response, the EWG chairs proposed emphasizing that the methods contained in the tables are not being recommended for endorsement by CCMAS; rather, they are currently in use and a method would only be considered suitable to support allergen labelling when it has been demonstrated to be fit for purpose for the action level or reference dose, and matrix in question. The draft response outlined the limitations of the methods listed in Appendix II, per the above-mentioned concerns, and in doing so sought to respond to CCFL's request for CCMAS' comment on methods that can detect and quantify UAP relative to the levels established by the FAO/WHO expert consultation.

#### *Summary of EWG comments*

13. EWG members were invited to review the draft response to CCFL as well as the accompanying tables and provide their comments using the Codex Online Forum. Comments were received from one Member organization, four Members, and two Observer organizations.

#### *General comments*

14. There was general support for the approach outlined in the consultation paper providing a draft response with the tables containing the methods that meet the validation criteria. Amendments were incorporated where possible.
15. One member expressed their view that CCMAS should instead provide method performance criteria (MPC) and list examples of applicable methods that meet the criteria while also providing Tables 1 and 2 as suitable analytical methods for determining UAP in foods. One observer's comments supported this view, and another member suggested MPC could be developed in the future. These comments summarized that MPCs define the measurable quality requirements a method must meet, rather than identifying specific brands; should include quantitative targets for key performance indicators such as detection limits (at or below the FAO/WHO action levels), repeatability and reproducibility ranges, recovery rates, and validation using incurred matrices where feasible; and could be structured by food matrix category. In considering these views, the EWG Chair notes that the discussion paper and response to CCFL references the AOAC and EN guidelines which already contain performance metrics against which the methods have been evaluated. Development of performance criteria by CCMAS may be duplicative or contradictory to the references to AOAC and EN guidelines agreed upon by CCMAS and would not fulfill the request by CCFL as submitted to CCMAS. Further, the EWG Chair notes that the *Codex Procedural Manual* states that the numeric performance criteria are not applicable to ELISA methods (note 1 following para. 177 in the *Procedural Manual*). If CCFL wishes to establish MPC, then it may be feasible to mirror those set in the AOAC and EN guidelines which are already referenced in this consultation paper.
16. It was noted that methods for pecan and pistachio were not submitted; another member offered to provide additional method information for pecan and pistachio if required in the future. The current EWG terms of reference (TORs) do not allow new methods to be added at this time, but additional information could be provided to CCFL should CCFL request it upon reviewing the response from CCMAS.
17. One member recommended that CCMAS indicate which methods can quantify concentrations corresponding to a 1 kg intake and which apply only to lower ranges. Reference doses range from 200 for crustacean down to 1.0 mg total protein from allergenic food per kg food. To address this, a note has been added to the draft response indicating that the analytical range of the method should span the relevant action level in Table 11 of Risk Assessment of Food Allergens Part 2 when deciding if the method is fit for purpose.
18. There was a recommendation that CCMAS extend its task to cover the regional allergens identified in the FAO/WHO expert consultation report. The original matter referred to CCMAS from CCFL and the TORs defined the allergens listed in Table 11 of Risk Assessment of Food Allergens Part 2: Review and Establish Threshold Levels in Foods for the Priority Allergens. CCMAS may expand the allergen method review to regional allergens in the future, if requested by another Committee.
19. There was a request for the response to include the methods, even if not endorsed, in an informative appendix. The Chairs note that the methods are included as Appendix II for informative purposes to CCFL and further information on the methods and their validation status is found in CX/MAS 25/44/11. This could also be referenced for CCFL if more in-depth validation information was needed. There were a few comments indicating that the expression of analytical ranges in Tables 1 and 2 should be standardized for clarity and consistency. PPM and PPB should be changed to mg/kg and µg/kg, respectively. This has been incorporated in the updated Appendix II.
20. An additional comment noted that caution should be exercised and reiterated with respect to interlaboratory comparability and method reproducibility. Results from proficiency testing programs have shown high variability

among analytical methods and commercial kits, even when applied to the same matrix. It was recommended that the CCMAS response clearly state that the mere listing of methods does not imply their endorsement or equivalence in analytical performance. This has been noted by the Chairs.

#### *Specific comments*

21. Specific edits to the draft response were incorporated in Appendix I.
22. Clarification of the statement “Laboratory users must identify the cross reactivities” was requested, noting that it may be more appropriate for users to review the manufacturers’ validation to identify if cross reactivity exists and undertake additional cross reactivity validation if needed. Edits to this effect were incorporated.
23. Several comments expressed a need to ensure consistency in the “Analytical Range/Limits (mg/kg)” column of the tables, to indicate whether the units are in allergenic food, allergenic protein, etc. The entries were updated accordingly.

#### **EWG CONSULTATION 2**

24. EWG members were again invited to review the second version of the draft response to CCFL as well as the accompanying tables and provide their comments using the Codex Online Forum. Comments were received from five Members, and two Observer organizations.

#### *General Comments*

25. Comments from the EWG members and observers were generally supportive of the revised document, with focus on the caveats and ensuring the methods are fit for purpose. The most significant concern of EWG members was on the numerous considerations required to determine if a method was suitable to test for UAP. The updated draft response captures these concerns and caveats.
26. Some views on developing MPCs were reiterated. Comments suggested that additional information will ensure the fitness for purpose of methods and allow Codex to adopt appropriate method criteria while avoiding the endorsement of specific proprietary methods of analysis. A Member urged caution that the current list in the draft response to CCFL, without continuous updating, would become obsolete over time. One member stated “Method Performance Criteria, structured by action level and relevant food matrix, could provide a transparent and technologically neutral framework for assessing whether analytical methods are fit for purpose in support of precautionary allergen labelling, without relying on a fixed list of methods. In this context, it should be noted that the Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens, as well as the recent Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens on reference dose(s) for cereals containing gluten or gluten, both recommended the establishment of MPC to address known limitations in allergen analytical methodology.”
27. Overall, there were mixed recommendations on whether CCMAS should prepare numeric performance criteria for the allergen methods, but, as addressed above, this was outside the TORs of the current EWG, would not fulfill the current request from CCFL, and noting the general support for the approach outlined over several sessions of CCMAS, the EWG Chairs have proceeded in line with the EWG’s TORs. Nevertheless, a suggestion to this effect was captured in the conclusions below for CCMAS to consider.
28. One comment requested that the draft response not be forwarded to either CCMAS or to CCFL at this stage. It was the EWG Chair’s decision to complete the draft response according to the TORs and based on general support for the approach and present the work to CCMAS for discussion.

#### **CONCLUSIONS**

29. The EWG completed the tasks identified in its TORs. Specifically the EWG:
  - finalized the review of the methods in CX/MAS 25/44/11 against the available validation guidelines and performance requirements;
  - simplified the presentation of methods and their validation status included in CX/MAS 25/44/11 Appendix II; and
  - developed a draft response for consideration by CCMAS45 to CCFL49.

#### **RECOMMENDATION**

30. CCMAS45 is invited to:
  - i. consider the draft response to CCFL in Appendix I, including the 2 method tables in Appendix II, with a view to forward them to CCFL; and
  - ii. consider whether to inform CCFL (and any other relevant committees) that it would be possible to develop numeric performance criteria for allergen detection methods.

## APPENDIX I

## DRAFT RESPONSE FROM CCMAS TO THE REQUEST FROM CCFL47

*(for consideration to forward to CCFL for information)*

1. In response to the request from CCFL47 to CCMAS (see [REP23/FL](#)) regarding suitable methods of analysis to support precautionary allergen labelling (PAL), CCMAS compiled methods in use by Codex Members for each priority allergen listed in Table 11 of Risk Assessment of Food Allergens Part 2: Review and Establish Threshold Levels in Foods for the Priority Allergens. These allergens include: wheat, cereals containing gluten (e.g. wheat) plus other gluten containing foods (Triticum species including rye and other Secale species, barley and other Hordeum species and their hybridized strains), crustacea, eggs, fish, milk, peanuts, sesame, and specific tree nuts (almond, cashew, hazelnut, pecan, pistachio, and walnut). No methods were submitted for pecan or pistachio but these could be reviewed again should CCFL require it. In addition to wheat, CCMAS agreed to include cereals containing gluten (e.g. other Triticum species, rye and other Secale species, barley and other Hordeum species and their hybridized strains). CCMAS additionally collated and categorized the method title, analysis principle, target analyte, conversion factors to mass of total protein from the allergenic food, LOQ or analytical measurement range, validation status, validation quality assurance, and method performance data from the validation study. In all, CCMAS collected over 100 sets of method validation data for evaluation against the following method development, validation, and performance guidelines (noting, the most recent guideline version must be utilized in each case):
  - AOAC Appendix M
  - EN 17855 (ELISA)
  - EN 17644 (LC-MS)
  - EN 17254 (ELISA Gluten)
  - EN 15634 (PCR)
2. It is important to note that these AOAC and EN guidelines are not officially endorsed by Codex but serve as important reference against which to evaluate method performance and validation statuses. CCMAS reviewed and agreed to include in its response to CCFL the methods contained in Appendix I (Tables 1 and 2). Table 1 includes methods that were either collaboratively studied or performance tested methods. These methods have shown acceptable performance on blinded food samples. Table 2 includes methods that were validated either at the manufacturer, in a single laboratory, or in-house.
3. The analytical methods in Tables 1 and 2 may be suitable for use in the process of conducting risk assessment for determining if UAP can be controlled below the specified action levels (ALs) for each allergenic food and supporting PAL. The AL will be dependent on the reference amount determined to be relevant in the risk assessment. However, food business operators must demonstrate that the selected method is fit for purpose for the specified AL and matrix in question. In addition, the following caveats apply:
  - The tables reflect methods compiled by CCMAS that meet either the CEN performance requirements and/or the AOAC validation guidelines for at least one commodity—they are not exhaustive, and not all methods are able to measure across all foods at all specified ALs. Future methods will likely become available that can also meet the performance requirements.
  - Currently, only a limited number of collaboratively studied and standardized test methods for allergen determination are available.
  - The performance (accuracy, precision, recovery, etc.) of food allergen analytical methods is heavily dependent upon the food matrix and food production processing (e.g. exposure to high temperature, fermentation, etc.) and can lead to erroneous results. Consistent with FAO/WHO Risk Assessment of Food Allergens Part 2' Section 8.2 paragraph 1 the CCMAS tables 1 and 2 list methods using ELISA, LC MS/ MS and PCR, with a majority of ELISA methods because of their wider use and consequently the larger underlying evidence base, followed to a less extent LC-MS/MS and Quantitative PCR. Although it is preferable for allergen test methods to target protein, in some instances where such test methodology is lacking, alternative methods, such as those based on DNA, may need to be used, nevertheless, conversion of DNA copies to total protein is a potential source of issues for these techniques and constitutes an indirect method for determining the presence of allergenic food.
  - Food business operators must be aware that quantitative testing results produced by different test kits on the same test material may not necessarily agree. They are advised to select a test kit that has an appropriate sensitivity for the specified allergen in the selected food matrix and compiles with the performance requirements in AOAC Appendix M and/or EN 17855 (ELISA).



- With regard to whether the methods are suitable for assessing the risk of UAP in foods, the ALs in Table 11 of the [Risk Assessment of Food Allergens Part 2: Review and Establish Threshold Levels in Foods for the Priority Allergens](#) vary by approximately two orders of magnitude. The suitability of a method at the relevant AL is dependent on the amount of food consumed, reference amount (RfA), and the reference dose (RfD). Some methods included in Tables 1 and 2 are appropriate at certain RfAs but not others. The analytical range of a method (including dilutions as needed to quantify higher concentrations) must span the relevant AL before food business operators and/or trading partners begin testing. If there are instances where the level of UAP approaches the AL, then the precision and accuracy of the method at those concentrations should be understood.
- The LOQ of the method should be lower than the allergen AL because methods tend to not be as reliable at concentrations near the LOQ. A factor of 3 has been proposed to provide a safety margin (e.g. at an AL as low as 1 mg/kg, the method should have a LOQ of 0.33 or lower).
- The reporting units in many ELISA kits are not in the same units as the ALs. In many cases a conversion factor is required to convert the test reporting units into mg total protein from the allergenic food / kg food. CCMAS encountered inconsistent reporting of conversion factors. To avoid confusion and simplify interpretation against the ALs, analytical results should be reported in a standardized unit (mg total protein from the allergenic source / kg of food), but this is not always possible to include in a single table (e.g. for crustacean, tropomyosin conversion to total protein is heavily dependent on crustacean source and there is not a single conversion factor for all crustaceans). Food business operators and trading partners must ensure the test results are in the appropriate reporting units or use a valid conversion factor to calculate the correct reporting units.
- Methods for the determination of gluten in tables 1 and 2 lack explicit association to the specific food sources of gluten (e.g. wheat, barley, rye, etc.). Methods that quantify gluten are aligned with the outputs of the recent FAO/WHO expert consultation on reference doses for gluten: <https://openknowledge.fao.org/handle/20.500.14283/cd7703en>.
- Laboratory users must review kit validation data for cross reactivities (e.g. for allergen analytical methods targeting walnut and cashew, a high degree of cross-reactivity with pecan and pistachio, respectively, has been reported, and depending on the assay kit, LOQ for pecan or pistachio may differ by approximately one order of magnitude from those for the intended target analytes). Users must also choose ELISA kits that will not produce false positives on the food matrix being tested. To facilitate this process, sample submitters must provide comprehensive sample product composition. Laboratory users should also note there are other factors in the samples under analysis which can cause false positives which are not related to cross reactivity (e.g. non-specific binding due to polyphenols, colors, etc.). The manufacturer-provided selectivity study is a resource but does not guarantee against cross reactivity.
- Some ELISA kits have critical changes since the time when validation studies were performed. For example, some manufacturers have changed extraction buffers to less hazardous reagents, and the associated performance of these kits may have changed. Since testing kits are updated on a regular basis, often maintaining the same kit name, it is difficult to relate the literature to the current iteration of the kit. Few kit manuals reference or publish the data relating directly to the development of that kit. If required, kit users can approach kit manufacturers and request whether further details and validation data are available to receive.<sup>3</sup> Users must ensure the method or ELISA kit chosen can meet the intended needs.
- Regarding validation, while collaborative studies estimate method performance in practice and independent laboratory studies (e.g. performance tested methods) can demonstrate how the method performs on an unknown in practice, these do not necessarily indicate that a method performs superiorly to methods that have only been validated by the manufacturer or validated in a single laboratory.
- Most proprietary methods are not distributed globally and the lack of availability of certain regions to access these methods would be restrictive to trade. Nevertheless, the provision of the information in Appendix I may encourage broader supplier distribution.
- While the tables include methods that were submitted, multiple allergen testing kits with manufacturers' in-house validations are available from a range of suppliers and may also be appropriate, but this should be verified (see AOAC and EN guidelines reference above for guidance).
- Qualitative methods submitted to CCMAS were excluded from the tables of recommended methods given the intended use.

4. CCMAS therefore encourages CCFL to consider these limitations with respect to the recommendations in Tables 1 and 2 and to ensure that trading partners and users of the methods are aware of them. Users will need to review and if necessary, to verify method performance for their specific case and should consult the validation guidelines and performance requirements above. In addition to future methods likely becoming available, CCMAS emphasizes that there are many methods that were developed and validated before the AOAC guidelines and CEN performance requirements were published—the results of those methods are not invalidated, and users can obtain additional validation data where needed.
5. For CCFL's information, most of the methods submitted to the EWG rely on proprietary methods, typically in the form of Enzyme-Linked Immunosorbent Assay (ELISA) kits. The Codex Procedural Manual specifies that “a proprietary method should not be endorsed if a suitable non-proprietary method of analysis is available” and that “preference should be given to adopting appropriate method criteria rather than endorsing a specific proprietary method of analysis.”<sup>2</sup>

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<sup>2</sup> *Codex Procedural Manual*. 30<sup>th</sup> edition. Section 2.13: Provisions on the use of proprietary methods in Codex standards. Pg. 70.

<sup>3</sup> FSA-UK (2023) Review of allergen analytical testing methodologies: Allergen detection methods: Unbiased literature search , <https://www.food.gov.uk/research/review-of-allergen-analytical-testingmethodologies-allergen-detection-methods-unbiased-literature-search>



## APPENDIX II

## METHODS OF ANALYSIS ON PRECAUTIONARY ALLERGEN LABELLING

*(for consideration to forward to CCFL for information)*

Table 1: Methods of analysis in support of precautionary allergen labeling with published, multi-laboratory validation studies or performance tested methods.

Allergen	Method	Principle	Catalog or website	Analytical Range / Limits (mg/kg)	Validation Citation
<b>Crustacea</b>	Shimadzu FA test EIA-crustacea II	ELISA	08624	0.31 – 20 mg crustacean protein/kg	J AOAC Int., 101(3), 798-804 (2018); J AOAC Int., 91, 123-129 (2008)
<b>Crustacea</b>	Crustacean kit II "Maruha Nichiro"	ELISA	55362	LOQ: 0.66 mg crustacean protein/kg (Catalog range 0.8 – 20 mg crustacean protein/kg)	J AOAC Int., 101, 798-804 (2018)
<b>Egg</b>	FASTKIT ELISA Ver.III EGG	ELISA	NPH-999100430EX	0.31 – 20 mg egg protein/kg	Food Safety 9.4 (2021): 101-116
<b>Egg</b>	Allergeneye ELISA II Egg Prima	ELISA	077834	1 – 20 mg egg protein/kg	Food Safety 9.4 (2021): 101-116
<b>Egg</b>	Morinaga BioSciences Egg (Ovalbumin) ELISA Kit II	ELISA	M2111	0.31 – 20 mg/kg egg protein	J AOAC Int., 89(6), 1600-1608 (2019); <a href="https://doi.org/10.1093/9780197610145.003.2985">https://doi.org/10.1093/9780197610145.003.2985</a>
<b>Gluten</b>	AOAC PTM 081202: ALLER-TEK® Gluten ELISA	ELISA	ELISA Technologies	LOQ: 5 mg gluten /kg	AOAC PTM 081202
<b>Gluten</b>	AOAC PTM 061201:Veratox® for Gliadin R5	ELISA	Neogen	LOQ: 5 mg gluten /kg	AOAC PTM 061201
<b>Gluten</b>	AOAC PTM 052005: SENSISpec INgezim Gluten R5	ELISA	Gold Standard Diagnostics	LOQ: 3 – 4 mg gluten /kg	AOAC PTM 052005
<b>Gluten</b>	AOAC PTM 042301: GlutenTox ELISA Rapid G12	ELISA	Hygiena	LOQ: 1.2 mg gluten /kg	AOAC PTM 042301

Allergen	Method	Principle	Catalog or website	Analytical Range / Limits (mg/kg)	Validation Citation
<b>Gluten</b>	AOAC PTM 032301: TotalTarget Kit for Gluten	Immunochromatographic test	EnviroLogix	LOQ: 4 mg gluten /kg	AOAC PTM 032301
<b>Gluten</b>	AOAC PTM 011804: Wheat/Gluten ELISA Kit	ELISA	Morinaga BioSciences M2103	LOQ: 0.06 – 0.49 mg gluten /kg	AOAC PTM 011804
<b>Gluten</b>	AOAC 2018.15: RIDASCREEN® Total Gluten	ELISA	R-Biopharm R7041	LOQ: 5 mg gluten /kg	<a href="https://doi.org/10.1093/jaoac/102.5.1535">https://doi.org/10.1093/jaoac/102.5.1535</a>
<b>Gluten</b>	AOAC 2015.05: RIDASCREEN® Gliadin competitive	ELISA	R-Biopharm R7021	LOQ: 10 mg gluten /kg	<a href="https://doi.org/10.5740/jaoacint.CS2015.15">https://doi.org/10.5740/jaoacint.CS2015.15</a>
<b>Gluten</b>	AOAC 2014.03: AgraQuant Gluten G12 ELISA®	ELISA	Romer Labs	LOQ: 4 mg gluten /kg	<a href="https://doi.org/10.5740/jaoacint.14-197">https://doi.org/10.5740/jaoacint.14-197</a>
<b>Gluten</b>	AOAC 2012.01: RIDASCREEN® Gliadin	ELISA	R-Biopharm R7001	LOQ: 5 mg gluten /kg (2.5 mg gliadin /kg)	<a href="https://doi.org/10.1093/jaoacint/qsab148">https://doi.org/10.1093/jaoacint/qsab148</a>
<b>Gluten</b>	FASTKIT ELISA Ver.III WHEAT	ELISA	999100135	0.31 – 20 mg gluten /kg	Food Safety 9.4 (2021): 101-116
<b>Gluten</b>	Morinaga BioSciences Wheat/Gluten (Gliadin) ELISA Kit II	ELISA	M2114	0.31 – 20 mg wheat protein/kg, 0.26 – 17 mg gluten/kg	Food Safety 9.4 (2021): 101-116; AOAC PTM No.011804
<b>Gluten</b>	Allergeneye ELISA II Wheat	ELISA	077847	1 – 20 mg wheat protein/kg	Food Safety 9.4 (2021): 101-116
<b>Milk</b>	AOAC PTM 101501: RIDASCREEN® FAST Milk	ELISA	R-Biopharm R4652	LOQ: 2.5 mg milk protein/kg	AOAC PTM 101501
<b>Milk</b>	FASTKIT ELISA Ver.III MILK	ELISA	999100424	0.31 – 20 mg milk protein/kg	Food Safety 9.4 (2021): 101-116
<b>Milk</b>	Allergeneye ELISA II Milk Prima	ELISA	077836	1 – 20 mg milk protein/kg	Food Safety 9.4 (2021): 101-116

Allergen	Method	Principle	Catalog or website	Analytical Range / Limits (mg/kg)	Validation Citation
<b>Milk</b>	Morinaga BioSciences Total Milk ELISA Kit II	ELISA	M2122	0.31 – 20 mg milk protein/kg	Casein Protein ELISA Kit: J AOAC INT.VOL. 89, NO. 6, (2006)
<b>Peanut</b>	AOAC PTM 112102: RIDASCREEN® Peanut	ELISA	R6811	LOQ: 0.75 mg peanut protein /kg	AOAC PTM 112102
<b>Peanut</b>	FASTKIT ELISA Ver.III PEANUT	ELISA	999100141	0.31 – 20 mg peanut protein/kg	Food Safety 9.4 (2021): 101-116
<b>Peanut</b>	Allergeneye ELISA II Peanut Prima	ELISA	077860	1 – 20 mg peanut protein /kg	Food Safety 9.4 (2021): 101-116
<b>Peanut</b>	Morinaga BioSciences Peanut ELISA Kit II	ELISA	M2116	0.31 – 20 mg peanut protein/kg	Food Safety 9.4 (2021): 101-116.
<b>Walnut</b>	FASTKIT ELISA Ver.III WALNUT	ELISA	999500165	0.31 – 20 mg walnut protein/kg	
<b>Walnut</b>	FA test EIA-Walnut	ELISA	08637	0.31 – 20 mg walnut protein/kg	
<b>Walnut</b>	Morinaga BioSciences Walnut ELISA Kit II	ELISA	M2124	0.31 – 20 mg walnut protein/kg	

Table 2: Methods of analysis currently available in support of precautionary allergen labeling but lacking multi-laboratory validation studies.

Allergen	Method	Principle	Catalog or website	Analytical Range / Limits (mg/kg)	Validation Citation
<b>Almond</b>	RIDASCREEN R FAST Mandel/Almond (R609)	ELISA	R609	4 – 30 mg/kg almond	Manufacturer validation report not available
<b>Cashew</b>	RIDASCREEN® FAST Cashew R6872	ELISA	R6872	2.5 – 20 mg cashew /kg	Member reported in-house validation only
<b>Cashew</b>	BioFront Technologies - MonoTrace Cashew ELISA kit	ELISA	CA2-EK-96	LOQ = 1 mg cashew (whole) /kg, range = 1 – 40 mg cashew (whole) /kg; LOQ = 0.17 mg cashew protein, range 0.17 – 7 mg cashew protein/kg	Manufacturer validation report includes cake, cookies, chocolate, ice cream, powdered infant soy formula, yogurt, milk & spices.
<b>Cashew</b>	Neogen Cashew Protein ELISA Kit	ELISA	E96CHW	Range of Quantitation: 0.90 – 24.00 mg/kg cashew protein	Unpublished in-house validation only.
<b>Cashew</b>	SENSISpec ELISA Cashew	ELISA	HU0030004	2 mg Cashew (whole) /kg, or 0.34 mg Cashew protein /kg	Manufacturer validation report for cookie, cornflakes, ice cream and dark chocolate.
<b>Crustacea</b>	AgraQuant Crustacea ELISA test kit (10002076)	ELISA	10002076	LOQ is equivalent to 0.7 mg/kg of shrimp protein	Unpublished in-house validation only.
<b>Crustacea</b>	ELISA Systems Crustacean Tropomyosin Residue Assay	ELISA	ESCRURD-48	0.05 – 0.5 mg/kg Crustacean Tropomyosin	ELISA Systems Validation Report Crustacean Tropomyosin Oct 2020
<b>Egg</b>	AOAC 2017.17: Detection and Quantitation of Selected Food Allergens: LC-MS/MS	LC-MS/MS		LOQ: 3 mg/kg (1.44 mg total egg protein/kg food)	<a href="https://doi.org/10.5740/jaoacint.19-0112">https://doi.org/10.5740/jaoacint.19-0112</a>

Allergen	Method	Principle	Catalog or website	Analytical Range / Limits (mg/kg)	Validation Citation
<b>Egg</b>	RIDASCREEN FAST Ei/Egg	ELISA	R6402	0.25 mg/kg – 25 mg/kg total protein	Manufacturer validation report Sept. 2017 available online
<b>Egg</b>	Romer Labs AgraQuant Egg White ELISA	ELISA	10002026	0.4 and 10 mg egg white protein/kg	Manufacturer validation
<b>Egg</b>	ELISA Systems Processed Egg Residue Detection Kit	ELISA	ESEGGPR-48	0.48 – 4.8 mg/kg Total Egg Protein	ELISA Systems Validation Report Processed Egg May 2021
<b>Egg</b>	RIDASCREEN FAST Lysozym	ELISA	R6452	0.25 mg/kg – 2.0 mg Lysozyme / kg – food; 0.05 mg/kg – 0.4 mg Lysozyme / kg – wine	r-Biopharm, RIDASCREEN FAST Lysozym Product Information 02/2016
<b>Fish</b>	GOLD STANDARD DIAGNOSTICS FISH ELISA	ELISA	FIS-E01/E04	LOQ: 4.0 mg/kg cod	Manufacturer validation
<b>Fish</b>	AgraQuant Fish ELISA test	ELISA	10002083	4 – 100 mg/kg cod	Manufacturer validation
<b>Gluten</b>	SureFood® ALLERGEN Gluten	PCR	S1053, S3606	LOQ = 1 mg gluten containing cereals / kg food; Range = 1mg – 400 mg gluten-containing cereals / kg food	CONGEN Biotechnologie GmbH May 2019
<b>Hazelnut</b>	AOAC 2017.17: Detection and Quantitation of Selected Food Allergens: LC-MS/MS	LC-MS/MS		LOQ: 10 mg/kg (1.503 mg hazelnut protein/kg food)	<a href="https://doi.org/10.5740/jaoacint.19-0112">https://doi.org/10.5740/jaoacint.19-0112</a>
<b>Hazelnut</b>	RIDASCREEN FAST Hazelnut	ELISA	R-Biopharm R6802	2.5 – 20 mg/kg (hazelnut)	Manufacturer validation

Allergen	Method	Principle	Catalog or website	Analytical Range / Limits (mg/kg)	Validation Citation
<b>Hazelnut</b>	ELISA Systems Hazelnut Residue Detection Kit	ELISA	ESHRD-48	0.5 – 5.0 mg/kg Hazelnut Protein	ELISA Systems Validation Report Hazelnut December 2020 v2
<b>Hazelnut</b>	Hazelnut ELISA Kit II MloBS	ELISA	Morinaga BioSciences M2119	0.16 – 10 mg hazelnut protein / kg	Manufacturer validation
<b>Milk</b>	AOAC 2017.17: Detection and Quantitation of Selected Food Allergens: LC-MS/MS	LC-MS/MS		LOQ: 10 mg/kg (2.564 mg total milk protein/kg food)	<a href="https://doi.org/10.5740/jaoacint.19-0112">https://doi.org/10.5740/jaoacint.19-0112</a>
<b>Milk</b>	Veratox for total milk allergen	ELISA	Neogen 8470	LOQ: 2.5 total milk protein / kg	Manufacturer validation
<b>Milk</b>	RIDASCREEN FAST $\beta$ -lactoglobulin	ELISA	R-Biopharm R4912	LOQ: 0.5 mg $\beta$ -lactoglobulin / kg	Manufacturer validation
<b>Milk</b>	AgraQuant R MILK ELISA	ELISA	RomerLabs 10002080	Range: 0.4 mg/kg – 10 mg/kg food/ 2.0 – 50.0 mg/kg meat products	Manufacturer validation
<b>Milk</b>	AgraQuant Beta-Lactoglobulin ELISA	ELISA	RomerLabs 10002034	Range: 10 – 400 mg $\beta$ -lactoglobulin / kg	Manufacturer validation
<b>Milk</b>	ELISA Systems Casein Residue Detection Kit	ELISA	ESCASPRD-48	0.35 – 3.5 mg/kg Total Milk Protein	ELISA Systems Validation Report Casein September 2024
<b>Milk</b>	ELISA Systems $\beta$ -Lactoglobulin (BLG) Detection Kit	ELISA	ESMRDBLG-48	1.0 – 10 mg/kg Total Milk Protein	ELISA Systems Validation Report BLG Nov 2022
<b>Milk</b>	SENSIspec ELISA total milk protein	ELISA	HU0030014	0.4 – 10 mg/kg milk protein	Manufacturer validation



Allergen	Method	Principle	Catalog or website	Analytical Range / Limits (mg/kg)	Validation Citation
<b>Peanut</b>	AOAC 2017.17: Detection and Quantitation of Selected Food Allergens: LC-MS/MS	LC-MS/MS		LOQ:10 mg/kg (2.22 mg total peanut protein/mg food) in cookies, 3 mg/kg (0.666 mg total peanut protein/mg food) in breakfast cereals	<a href="https://doi.org/10.5740/jaoacint.19-0112">https://doi.org/10.5740/jaoacint.19-0112</a>
<b>Peanut</b>	Morinaga BioSciences High Sensitive Peanut ELISA Kit II	ELISA	M2120	0.2 – 12.8 mg peanut protein/kg	Manufacturer validation
<b>Sesame</b>	RIDASCREEN FAST SESAME	ELISA	R7202	2.5 – 20 mg/kg (Sesame)	Manufacturer validation
<b>Sesame</b>	ELISA Systems Sesame Seed Protein Residue Assay	ELISA	ESSESE-48	0.25 – 2.5 mg/kg sesame seed protein	ELISA Systems Validation Report Sesame Dec 2022
<b>Walnut</b>	SENSISpec ELISA WALNUT	ELISA	HU0030024	LOQ 0.3 mg protein/kg food; RANGE: 0.3 – 3.0 mg protein/kg food	Gold Standard Diagnostic QP-19REP-99 Version 03EN
<b>Walnut</b>	BIOFRONT MONOTRACE WALNUT	ELISA	WJ4-EK-96	LOQ: 2 mg walnut / kg	Manufacturer validation
<b>Walnut</b>	AgraQuant R Walnut	ELISA	10002030	Range 2 – 60 mg walnut / kg	Manufacturer validation
<b>Walnut</b>	Neogen BioKits Walnut Assay Kit	ELISA	902085J	2.4 – 120 mg / kg walnut	

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