AOAC SMPR® 2018.003

Standard Method Performance Requirements (SMPRs®) for Quantitation of Milk by ELISA-Based Methods

Intended Use: Quantitation of Milk in the Context of Food Manufacturing

1 Purpose

AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels in their evaluation of validation study data for methods being considered for *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*℠, and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

Quantitation of milk in one or more food(s) such as those listed in Table 3 of Appendix M [*Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_m.pdf; *J. AOAC Int.* **93**, 442–450(2010)].

3 Analytical Technique

Enzyme-linked immunosorbent assay (ELISA)-based assays

4 Definitions

Enzyme-linked immunosorbent assay (ELISA).—For the purposes of this document, ELISA is defined as "an analytical procedure characterized by the recognition and binding of specific antigens by antibodies." This definition is not meant to be restrictive and encompasses other related binding-based technologies.

Limit of detection (LOD).—The lowest concentration or mass of analyte in a test sample that can be distinguished from a true blank sample at a specified probability level [Appendix M: *Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_m.pdf; *J. AOAC Int.* **93**, 442–450(2010)].

Limit of quantitation (LOQ).—The lowest level of analyte in a test sample that can be quantified at a specified level of precision [Appendix M: *Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_m.pdf; *J. AOAC Int.* **93**, 442–450(2010)].

Milk.—For the purposes of this SMPR, "milk" refers to nonfat dry milk from pasteurized skim milk. It contains not more than 5% by weight of moisture, and not more than 1.5% by weight of milkfat (*Code of Federal Regulations*, Title 21—Food and Drugs, §131.125; other internationally recognized definitions may be applied), as represented by existing reference materials.

Recovery.—The fraction or percentage of analyte that is recovered when the test sample is analyzed using the entire method.

Repeatability.—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator (in the same laboratory) and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r; ISO 5725-1:1994).

Reproducibility.—Variation arising when identical test materials are analyzed in different laboratories by different operators on different instruments. The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (%RSD_R; ISO 5725-1:1994).

5 Method Performance Requirements

See Table 1.

6 System Suitability Tests and/or Analytical Quality Control

See information on antibodies, cross reactivity, calibrators, and matrices in section "Required Allergen-Specific Information to Be Provided on the ELISA Method" of Appendix M.

Method developers should:

(*1*) Clearly identify component(s) of milk being measured and, specifically, the method performance with regards to the milk component, such as whey (e.g., beta-lactoglobulin) or casein; and the target used to generate immunogens.

(*2*) Provide conversion factor used to equate to nonfat dried milk.

(*3*) Provide applicability statement for intended use and claimed matrices.

7 Reference Material(s)

Nonfat milk powder: MoniQA Association Reference Material SMP-MQA 092014 (MoniQA Association, http://www.moniqa.org/node/910)

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

8 Validation Guidance

Method developers must provide:

(*1*) Data for method performance in all the claimed matrices. Must specify if samples used for validation are incurred or spiked, processed (baked), or unprocessed (raw).

(*2*) Recovery data using incurred samples for all claimed matrices.

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_d.pdf)

Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

Appendix M: *Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices, Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_m.pdf)

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Table 1. Method performance requirements

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

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

Annex I: Choice of LOD/LOQ (for SMPR® 2018.003 Quantitation of Milk by ELISA-Based Methods)

Limit of detection (LOD) and limit of quantification (LOQ) are selected based on user requirements.

The proposed limits in the SMPR constitute minimum requirements for food allergen testing in the targeted matrices as part of a food processing control. Assay users or developers may want to consider assays with a broader performance range, e.g., more sensitive and/or broader range, than the minimum acceptance criteria, as needed by their applications. For example, some users may seek the lower bound of the analytical range to correspond with either a regulatory or a health-driven threshold limit.

Only a few jurisdictions such as Japan have set a regulatory limit of 10 ppm protein for all their priority allergens. Other jurisdictions attempt to rely on risk-based thresholds for the various priority allergens. Nonetheless, recent developments in reference doses (1) have been used by food manufacturers and others as part of risk management approaches that are developed by the food industry sector in Australia and New Zealand (the Allergen Bureau was established in 2005 and is funded by membership from the Australian and New Zealand food industry). Even with this new information, the food safety risk assessment community has not adopted a validated food allergen reference or benchmark doses, which can be applied consistently by food regulators and food manufacturers in allergen-related health risk assessments and the management of precautionary allergen labeling.

The Voluntary Incidental Trace Allergen Labeling (VITAL; 2) initiative of the Allergen Bureau in Australia and New Zealand developed a scientific approach (1) using reference doses for allergen risk characterization, taking into account of clinical food allergen challenge studies. For each priority allergen targeted, a reference dose is defined as the milligram protein level (total protein from an allergenic food) below which only the most sensitive individuals (between 1 and 5% depending on the quality of the data set available) in the allergic population are likely to experience an adverse reaction. For example, in VITAL 2.0 the reference doses are currently set at 0.2 mg protein for peanut, 0.1 mg protein for milk, and 0.03 mg protein for egg (2).

These reference doses may be used to generate action levels for food allergen control, taking into account the serving size of the food in an eating occasion. Analytical targets should be set at or below the action level in order for the method to be suitable for the purpose. For example, LOQ or action level for milk protein potentially present in a food consumed at a 100 g serving size would be 1 ppm milk protein (0.1 mg protein in 100 g).

References

(1) Taylor et al. (2014) Establishment of reference doses for residues of allergenic foods: report of the vital expert panel, food and chemical toxicology, *Food Chem. Toxicol.* **63**, 9–17

(2) http://allergenbureau.net/vital/ (accessed on August 25, 2017)