Gluten Quantitative Method Validation Guidelines

AOAC Gluten & Food Allergens Program

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GFCO

Started in 2005

1700+ Companies

2000+ Brands

60,000+ Products

History of GFA

- Began with thought leader meetings 2020
- Identified gaps in gluten and food allergens
- Separate gluten and allergen working groups were formed

Gaps

- Reference materials
  - Wehling & Scherf 2020* (USP, https://store.usp.org/product/1294839)
- No non-ELISA confirmatory method
- No specific guidance for gluten methods other than ELISA
  - Koerner et al 2013**
- More specific guidance needed for development and validation of antibody-based methods

*_Journal of AOAC INTERNATIONAL_, Volume 103, Issue 1, January-February 2020, Pages 210-215, https://doi.org/10.5740/jaoacint.19-0081

**_Journal of AOAC International_, Volume 96, No. 5, 2013, Pages 1033-1040, https://doi.org/10.5740/jaoacint.13-043

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Gluten Working Group Goals

1. Validation Guidance Document(s)
   - Qualitative, quantitative
   - “Binding assays” (ELISA, LFD other), as well as LC-MS
2. End-User Guidance Document
   - Matrix extension
   - Method suitability
   - Sampling
3. SMPR for General Antibody-Based Methods
4. SMPR for LC-MS Method

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Qualitative Validation Guidance

- Focused on LFD
- Consensus reached on:
  - Spike levels for selectivity and interference studies, and what to do about unexpected results
  - Spike/incursion levels for the matrix studies, and what to do if a fractional level isn't found
  - Number of replicates for each spiked/incurred matrix sample, with acceptance criteria for POD
  - Spike level and number of replicates for the robustness study, and the statistical analysis of that data

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Qualitative Validation Guidance

- Consensus reached on:
  - Addition of a hook effect study
  - Additional details, and acceptance criteria, for lot-to-lot consistency and stability studies
  - Agreement that incurred materials are required
  - Agreement that each gluten source (wheat, rye, barley (oats)) must be tested in the validation
  - Independent study procedure
  - Collaborative study procedure

Qualitative Validation Guidance

- Consensus reached on:
  - Matrix extension procedures for PTM and OMA
  - **Definition of “claimed detection capability” or CDC**
  - The use of matrix categories for gluten
  - Sample preparation
    - bulk spikes, test portion spikes, incurred samples – and when each can be used
    - Surfaces, CIP, rinsates

Quantitative Validation Guidance

- Covers
  - Single Lab Validation (SLV)
  - Independent Laboratory Study
  - Collaborative Study
  - Annexes for
    - Selectivity Panels (Cross-reactivity, interference and breadth)
    - Test material preparation
    - Matrix categories
    - Statistical methods

Quantitative Validation Guidance

- Study Material Criteria
  - Test materials for SLV may be prepared in-house
  - All test materials for independent lab and collab study should be prepared independently
    - At least one incurred material must be prepared independently

- Calibration Fit Study
  - Multiple replicates of each calibration standard
  - Calculate residuals for each replicate based on instrument response (OD)
  - Plot residuals vs concentration
  - Residuals should have a random distribution and be centered on zero
  - Residuals should be <15% of the measured response, up to 20% at the lowest standard

Quantitative Validation Guidance

- Selectivity Study
  - Breadth: *Triticum compactum*, durum, einkorn, emmer, Khorasan, spelt and triticale in addition to common wheat (*aestivum*), rye, barley, oats
  - Cross-reactivity, and interference at 3x the LOQ or 20 ppm

- Matrix Study
  - Repeatability
  - Intermediate precision
  - LOD
  - LOQ
  - Recovery
  - A blank and three positives, one at or below 20 ppm

Quantitative Validation Guidance

- Matrix Studies - Gluten Source Rotation

Table 1. Rotation of gluten sources across claimed matrices for methods claiming to detect wheat, rye, and barley. The rotation of single gluten sources would continue for six matrices and greater.

	Number of matrices claimed				
	1	2	3	4	5
Matrix A	Wheat Barley Rye	Wheat Barley Rye	Wheat Barley Rye	Wheat Barley Rye	Wheat Barley Rye
Matrix B		Wheat	Wheat	Wheat	Wheat
Matrix C			Barley	Barley	Barley
Matrix D				Rye	Rye
Matrix E					Wheat

Quantitative Validation Guidance

- Matrix Categories
  - Five matrices across each processing type
  - All samples must be incurred
  - Fermented/hydrolyzed must be validated per enzyme/chemical/organism and matrix (no category grouping)

	RAW/ MINIMALLY PROCESSED	PROCESSED, BAKED	FRIED	PRESSURE/EXTRUSION	DEHYDRATED
Cereal Grains	Whole or milled Sorghum, soybeans, corn, millet, teff, rice, fonio, oats; baking mixes	Bread, cakes, cookies, tortillas, fresh pasta, bakery products, confectionaries, crackers, bagels, muffins, grain-based protein bars	Breaders / Batters for fish sticks and chicken nuggets, tortilla chips, donuts	Breakfast cereals, Puffs /pellets	Bread crumbs, Dried Pasta

Quantitative Validation Guidance

- Matrix Studies - Intermediate Precision Designs

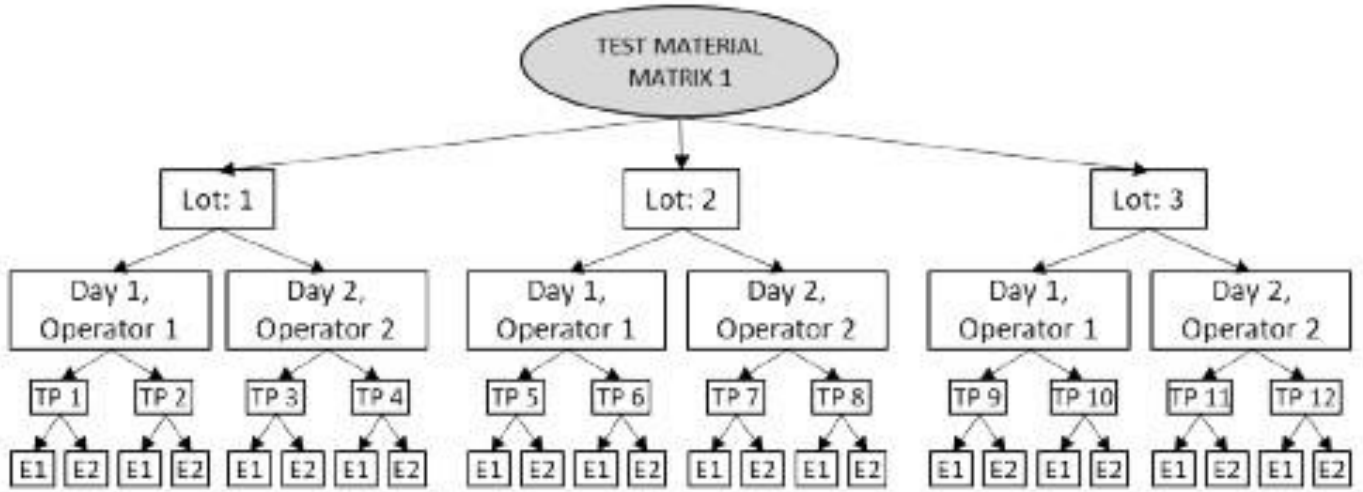

Figure 4. Design 2b. Lot: test kit lot, TP: test portion, E: ELISA measurement. Design 2b can be used to estimate intermediate precision, repeatability, ELISA variance, and lot-to-lot product consistency.

- LOD, LOQ and Recovery can also be calculated from the Intermediate Precision data (LOD and LOQ calculations provided in Annex D)

Quantitative Validation Guidance

- Robustness Study
  - Design and statistical analysis provided in Annex D
- Product Stability and Consistency
  - Can be done as part of intermediate precision, or separately

Quantitative Validation Guidance

Required Method Information

- **(a)** A statement of the expected context(s) of use, expected matrices and expected analytical goals of the method.
- **(b)** Specific qualifications or training required to perform the method.
- **(c)** An applicability statement describing the method's target analyte, measurand, matrices within scope, and important limitations.
- **(d)** If the method is intended to conform to an existing SMPR document, the SMPR citation must be provided.
- **(e)** Step-by-step instructions for test portion preparation and performance of the method are required. Pictorial examples are encouraged.
- **(f)** The reporting unit for all methods should be in mg/kg of gluten, although other reporting units may also be included (e.g., mg/kg of gliadin) with conversion factors.
- **(g)** In addition to the information described in this document, method submissions must provide any additional details mandated by relevant SMPRs.

- In the validation study report, method developers must provide:
- **(a)** Information on which gluten fractions from each claimed gluten source (e.g., gliadins from wheat, hordeins from barley) the antibody/antibodies detect. Information on specific proteins or epitopes may also be provided if available.
- **(b)** Information on calibrants:
- *(1)* Identification of the calibrant for the method
- *(2)* How the calibrant was prepared
- *(3)* How the concentration value of the calibrant was assigned
- *(4)* Whether the calibrant made from raw or processed material
- *(5)* Whether the calibrant was extracted or purified, and the method
- *(6)* Whether the calibrant is provided in extraction or dilution buffer
- *(7)* How the concentration of the calibrant is expressed
- *(8)* Whether the calibrant is commercially available.
- **(c)** Complete information on the gluten sources (genus and species), matrices, and procedures used to prepare validation test materials

Quantitative Validation Guidance

- Independent Laboratory Study
  - Each gluten source in at least one matrix for every five matrices evaluated in the Method Developer Study
  - At least one environmental surface/Clean-In Place (CIP) solution for every five claimed
    - Choose surface over CIP
- Collaborative Study
  - Reproducibility
  - LOD
  - LOQ
  - Minimum of 8 laboratories
  - Each gluten source in at least one incurred matrix per category, or one per every five matrices claimed
- Matrix Extension

Quantitative Validation Guidance

- Document is currently open for public comments

https://www.aoac.org/news/call-for-public-comments-gluten-quantitative-method-validation-guidance-document/

Next Steps

- New Working group on End User Guidance for Gluten and Food Allergen Methods
- Launched October 15, 2024, will be meeting bi-weekly
- Looking for food manufacturers, laboratories and other stakeholders
- Starting with method verification and matrix extensions; also plan to cover sampling and data interpretation
- Sign up here to join the working group:

https://app.smartsheet.com/b/form/63ee0a9fa0a14603a1135827e5a194f5

Thank You!!

Please reach out with any questions:

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