


Overview and Comparison of Global Method Validation Schemes

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Organized by IAFP's Applied Laboratory Methods Professional Development Group


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Patrick Bird

- Microbiology R&D Supervisor for Q Laboratories
- Managed dozens of validations for AOAC PTM, AOAC OMA, AFNOR and MicroVAL submission
- Active member of the ISO WG3 for method validation
- Serves on the AOAC Research Institute Board of Directors
- Co-project leader for the AOAC ISPAM working group on Microbiological Quantitative Statistical Analysis


Christopher Haney

- Senior Scientist on Clear Labs' Microbiology Team
- Manages validation pipeline for novel NGS based methods for pathogen detection
- Experience as a Microbiologist at U.S. Food and Drug Administration, GenMark Diagnostics, and Roka Biosciences, where he managed the validation laboratory

Method Validation : Independent Laboratory Perspective

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Objective

- Overview of References/Certification Bodies
- Study Design:
  - AOAC PTM
  - AOAC OMA
  - ISO 16410-2
- Harmonization

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Certification Overview

- Appendix J: *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces* (2012); or ISO 16140:2003.
  - *Performance Tested Methods*SM (PTM)
  - *Official Methods of Analysis*SM (OMA)
- Appendix D: AOAC INTERNATIONAL *Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis*

AOAC RESEARCH INSTITUTE

AOAC INTERNATIONAL

Overview of Reference/Certification Bodies

- ISO 16140 Series
- ISO 16140-1 (2016): Microbiology of the food chain — Method validation — Part 1: Vocabulary
- ISO 16140-2 (2016): Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
- ISO/DIS 16140-6 (2018): Microbiology of the food chain — Method validation — Part 6: Protocol for the validation of alternative (proprietary) methods for Microbial Confirmation and Typing Procedures
- Certification Bodies: **MicroVal, AFNOR, NordVal**

MicroVal
European validation and certification organisation

BY AFNOR CERTIFICATION
NF VALIDATION
EN ISO 16140

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Study Design – AOAC

AOAC Validation

Study Type	PTM Certification	First Action OMA
Method Developer	√	√
Independent	√	√
Collaborative		√
Robustness	√	
Product Consistency	√	
Product Stability	√	
Instrument Variation	√	

- AOAC Research Institute uses guidelines and references developed by AOAC INTERNATIONAL and AOAC volunteer subject matter experts for its testing protocols and data evaluation

- Ruggedness/Robustness, Product performance consistency, product performance stability and instrument performance variation are all certification requirements; technical criteria are developed within the Research Institute with guidance from AOAC volunteers.

AOAC Validation

Method Developer Responsibilities and SLV

- Inclusivity
- Exclusivity
- Matrix Study
  - Claim-dependent
  - POD and dPOD
- Robustness (PTM only)
- Stability (PTM only)
- Instrument Variation (PTM Only)
- Lot-to-Lot Variation (PTM only)

Independent Laboratory Responsibilities

- Matrix Study
  - 1 Food per 5 Foods Validated
  - 1 Surface per 5 Surfaces Validated

Collaborative Study

- Matrix Study
  - ≥10 labs for qualitative methods
    - POD across collaborators
  - ≥8 labs for quantitative methods
  - ≥ 1 Food

Inclusivity/Exclusivity

- Evaluated using pure isolates; no food matrices

Inclusivity (Range of target analytes detected by method)

- 50 Target Strains (100 for *Salmonella* spp.)
  - Will define scope of method
    (ex. Non-lactose fermenting *Salmonella,* 6 common *Listeria* spp.)

Exclusivity (Range of non-target analytes excluded)

- 30 Non-target strains
  - Closely related to target strains

Robustness

- Evaluate performance of the assay with small changes in key parameters

Table 3: Robustness parameters
Table 4: Robustness Experimental Design

  - (incubation time/temperature; volumes of lysis buffer, reagents, etc)

Parameter	Low value	Nominal Value	High value
Sample Volume	Low value	Nominal value	High Value
Lysis Time	Low value	Nominal value	High value
Reagent Volume	Low value	Nominal value	High value

  - Conducted using inoculated food matrix at fractional range

- Factorial Design to minimize number of test portions

Treatment Combination	Enrichment Time	1st Lysis Time	2nd Lysis Time
1	45 mL	Low Value	Low Value
2	45 mL	Low Value	High Value
3	45 mL	High Value	Low Value
4	45 mL	High Value	High Value
5	55 mL	Low Value	Low Value
6	55 mL	Low Value	High Value
7	55 mL	High Value	Low Value
8	55 mL	High Value	High Value
9	50 mL	Nominal Value	Nominal Value

Stability

- Can be conducted in real-time and/or accelerated study.
- Accelerated data can provide immediate information on shelf-life, but must submit data for real-time (ex. provided below)
  - Can be submitted during certificate renewal

Candidate Method	Storage Temperature	Time Points (from the date of production)
Real time	2-8 ± 1°C	1 mo., 2.5 mos., 5 mos., 6 mos
Accelerated	25 ± 2°C	4 days, 9 days, 17 days, 20 days

- **Lot-to-Lot Variation**
  - 3 Lots of Assays
  - Target/non-target strains
  - Can be combined with stability and or instrument variation

- **Instrument Variation**
  - 3 Instruments
  - Target/non-target strains

- Method comparison
  - Choice of matrix
    - The number of matrices chosen determines the claim

Table 1: *Acceptable Multiple Matrix Claims*

Multiple Matrix Claim	Criteria	
	Number of Matrices	**Number of Catergories/Groups**[1]
Broad Range of Foods	15 (3 foods/category)	5 categories
Variety of Foods	≥ 10	5 categories
Selected Foods	≥ 5	2 categories
Food Category/Group	≥ 5	1 category
Environmental Surfaces	7	Not applicable
Selected Surfaces	2-6	Not applicable

Table 2: *Acceptable Environmental Surfaces*

1. Air Filter Material	2. Cast Iron *coated to prevent rusting*	3. Ceramic *glazed earthen material or glass*	4. Plastic *polyethylene, polypropylene, polycarbonate*
5. Rubber	6. Sealed concrete	7. Stainless Steel	

Food Categories		
Raw milk and dairy products	Eggs and derivatives	Dried cereals, fruits, nuts, seeds and vegetables
Heat processed milk and dairy products	Raw and ready-to-cook fish and seafood (unprocessed)	Chocolate, bakery products and confectionary
Raw meat and ready-to-cook meat products (except poultry)	Ready-to-eat, ready-to-reheat fishery products	Multi-component foods or meal Components
Ready-to-eat, ready-to-reheat meat products	Fresh produces and fruits	Pet food and animal feed
Raw poultry and ready-to-cook poultry products	Processed fruits and vegetables	Environmental samples (food or feed production)
Ready-to-eat, ready-to-reheat meat poultry products	Infant formula and infant cereals	Primary production samples (PPS)

- Method comparison
  - Choice of matrix
  - Choice of organism
  - Inoculum level
  - Equilibration period

Table 1: Study Summary

Matrix/ Test Portion	Inoculation Organism	Inoculation Level per Organism	Replicates per method	Inoculating Cells/ Stabilization conditions	Reference Method	Analysis Time Points
Fresh Raw Ground Beef	E. coli O157:H7 ATCC 43895	0 cfu / test portion	5	Fresh culture 4°C, 48-72 h	MLG 5.09, 5B.05, 4.09 and 8.10	22 hours
		0.2–2 cfu/ test portion	20			
		2-5 cfu/ test portion	5			

- Method comparison
  - Choice of matrix
  - Choice of organism
  - Inoculum level
  - Equilibration period
  - **Alternative Confirmations/Reference Method**

In Addition:
Any modification to a method, including extension to a new matrix or target organism requires revalidation/verification

(Note: The degree of verification will
depend on nature/extent of the modifications)

Level 1 – Minor (do not require additional validation work) – software upgrade, etc.

Level 2- Will require validation data (requirement for independent lab depends on modification)

Level 3- Will require validation data from method developer and independent laboratory.

AOAC – OMA Collaborative Study: Qualitative

- Selection of Matrix
- ≥ 10 Collaborators
- 36 Test portions
- Shipment of test portions
- Performance of method/reference method
- Collection of Data/Statistical Analysis
- Submission of Report and Presentation to the ERP

AOAC – OMA Collaborative Study: Qualitative

- Selection of Matrix
- ≥ 8 Collaborators
- 8 Test portions
- Shipment of test portions
- Performance of method/reference method
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Study Design – ISO 16140-2

- Combines aspects of AOAC PTM and AOAC OMA programs
- Entire study must be completed by Expert Lab
- Qualitative:
  - Sensitivity, RLOD, Inclusivity/Exclusivity, ILS
- Quantitative:
  - Trueness, Accuracy, Inclusivity/Exclusivity, ILS

- Other performance requirements (Stability, lot-to-lot) covered under manufacturing standard

- Sensitivity
  - Selection of Categories
    - 3 Types per category
    - 20 individual samples per type
- RLOD
  - 1 Matrix per category validated
- Inclusivity/Exclusivity
  - 50 (100)/30
- ILS
  - ≥ 10 collaborators
  - 24 total test portions

- Relative Trueness
  - Selection of Categories
    - 3 Types per category
    - 5 individual samples per type
- Accuracy
  - 1 Matrix evaluated in duplicate or 2 different matrices per category validated
- Inclusivity/Exclusivity
  - 50 (100)/30
- ILS
  - ≥ 8 collaborators
  - 8 total test portions

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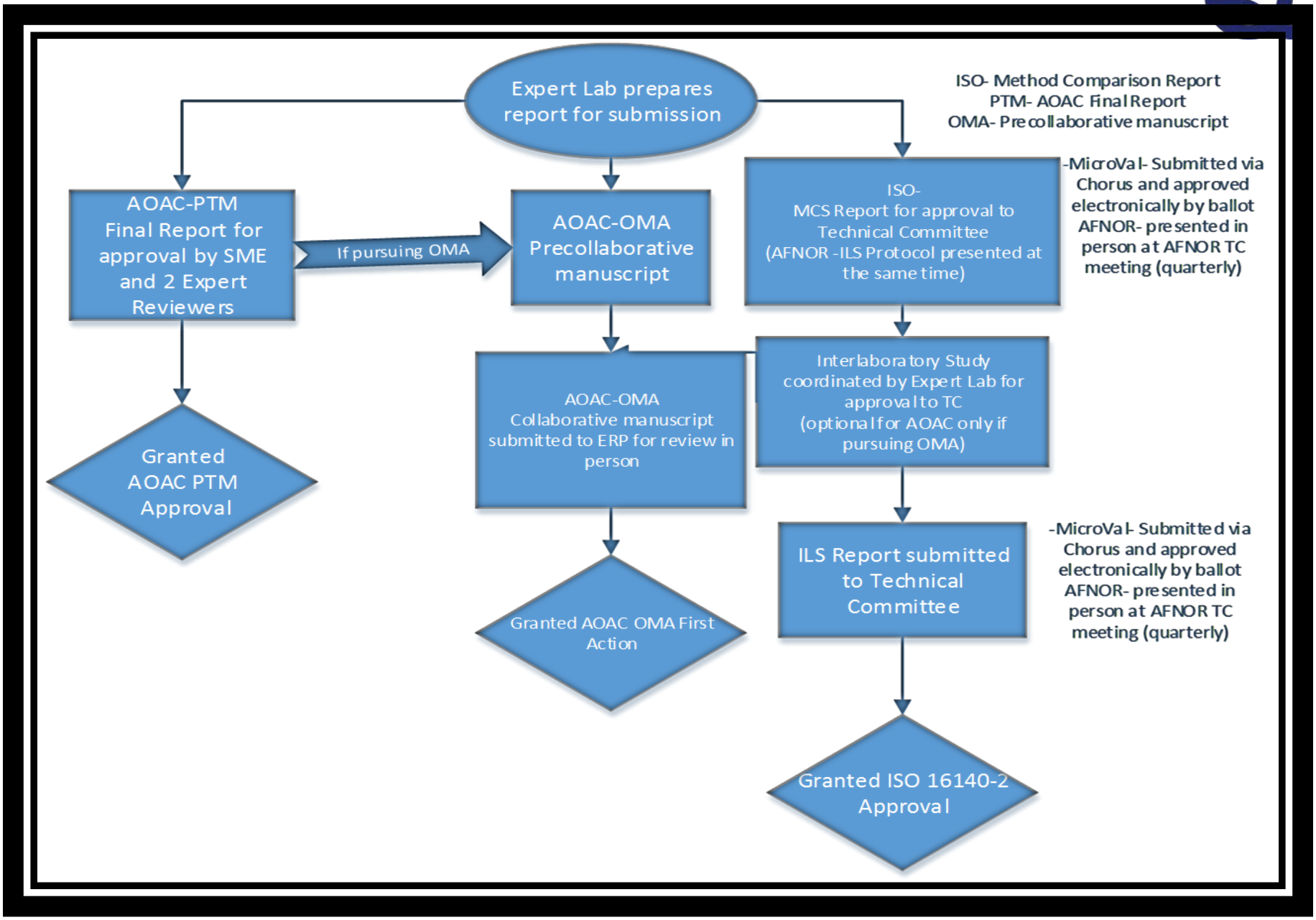
Harmonization

- Programs harmonized with PTM
  - *Official Methods of Analysis*^{SM}
  - Antibiotic drug residues in milk
    - US Food & Drug Administration Center for Veterinary Medicine and the National Conference on Interstate Milk Shipments
  - Health Canada – Bureau of Chemical Safety (Food Allergens)
  - MicroVal
  - AFNOR *(in progress)*
  - NordVal *(in progress)*

- *The goal is to achieve optimal efficiency and avoid duplication of efforts in order to meet regulatory and product safety testing requirements.*

	AOAC Research Institute ***Performance Tested Methods***	**AOAC INTERNATIONAL** *Official Methods*	**MicroVal** **ISO 16140-2:2015**
Types of Methods	Proprietary Methods	Proprietary and Non-commercial	Proprietary and Non-commercial
Reference Methods	AOACI, FDA, USDA, ISO, Health Canada	AOACI, FDA, USDA, ISO, Health Canada	ISO, CEN, Other Reference Methods
Claim	Variety-10 matrices/5 groups Selected-5 matrices/2 groups Group-5 matrices/1 group	Per matrix basis- all matrices in Method Developer Claim	Broad Range of Foods-5 categories Category-3 types Restricted Foods- Specific Categories- 3 types per category **<u>Additional Categories</u>** Primary production, Feed Environmentals
Time to Validation	As little as six months	12 months minimum	Approx. 12 months depending on complexity
Statistical Calculations	Probability of Detection (POD)	Probability of Detection (POD)	RLOD (POD may be analyzed additionally)
Laboratory Accreditation	N/A	N/A	EL is ISO 17025 for reference method
Validated Methods Reviewed	Required yearly	First Action for 2 years Final Action vote by OMB	Required
Methods Published	ILM, Journal of AOAC	Journal of AOAC, Official Methods of Analysis	MicroVal website/Organization dependent

Expectations and planning for dual expert review of final study



Overview and Comparison of Global Method Validation Schemes: Method Developer Perspective

Christopher Haney, Senior Scientist

Clear Labs

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Choosing a Validation Scheme

I. Launch Geography
II. Launch Horizon
III. Cost
IV. Customer Acceptance

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Choosing a Validation Scheme

I. Launch Geography

- *Generally:*
  - ISO 16140 (AFNOR, NordVal, MicroVal) : EU, Asia (Taiwan), Africa (Tunisia)
    - Mandated in EU by EC 2073/2005
    - Country-by-country acceptance outside EU
    - EU members may have additional requirements

The use of alternative analytical methods is acceptable when
the methods are validated against the reference method…in accordance with…ISO standard 16140 or other internationally accepted similar protocols…

- EC 2073/2005; Article 5 § 5

Choosing a Validation Scheme

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    - Validation not mandated for industry use
- *Less Generally:*
  - Country-specific schemes
    - Commonalities with AOAC/ISO 16140
    - *eg.* Health Canada's "Microbiology Food Laboratory Procedure" (MFLP) and "Health Protection Branch" (HMB) certifications

Choosing a Validation Scheme

II. Launch Horizon / Marketing Expediency

- AOAC PTM
  - No application/acceptance windows
  - Study scope customization

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  - 2 Phases First Action; Final Action
  - 2 Year Minimum

Choosing a Validation Scheme

II. Launch Horizon / Marketing Expediency

- AOAC PTM
  - No application/acceptance windows
  - Study scope customization
- AOAC OMA
  - 2 Phases First Action; Final Action
  - 2 Year Minimum
- ISO 16140 (AFNOR)
  - Regimented phases
  - Phase approvals at meetings

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Choosing a Validation Scheme

III. Cost

- Hardware and Scale
  - AOAC PTM: 2 laboratories involved
  - AOAC OMA: ~10 laboratories involved simultaneously
  - ISO 16140: ~10 laboratories involved simultaneously
    - At least 3 countries
    - EU-centric
    - Expert Laboratory

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    - At least 3 countries
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- Study Performance
  - AOAC PTM: can be ~80% in-house, but…
    - In-house expertise: fractional inoculation
    - Laboratory scale: ~100L media + ~26kg matrix per matrix study attempt
    - Strain library: ~$50,000 (*Salmonella enterica*, via ATCC)
  - ISO 16140 Primarily done by Expert Laboratory

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Choosing a Validation Scheme

IV. Customer Acceptance

- In markets without a mandate (*eg.* USA), customers may
  - Prefer ISO 16140
  - Accept PTM
  - Require OMA
  - Reject Certification bodies in lieu of internal methods

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How to Be Ready

I. Consultants
II. Homework
III. Exit R&D
IV. Operations Activated
V. Expectation Management

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I. Consultants – Get one

- Built into AOAC PTM/OMA
- Numerous options for ISO 16140

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II. Homework
- Target markets?
- What do *your* target customers prefer?
- Country/Matrix/Target-specific schemes (*eg.* Nat'l Poultry Improvement Plan; NPIP)

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- Know the result before you start validation

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IV. Operations Activated
- Quality System online
  - ISO 16140 requires an on-site audit
- Product/Method is in a sellable format – bottles, labels, etc in final form
- Multiple production lots on-hand

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How to Be Ready

V. Expectation Management
   - Validation is not an end-point


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Questions?

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