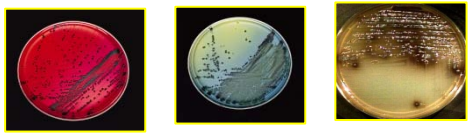
Validation of Screening Tests AOAC Methods Validation Criteria

Thomas Hammack

Chief
Microbial Methods Development Branch
Division of Microbiology
Office of Regulatory Science
Center for Food Safety and Applied Nutrition
Thomas.Hammack@fda.hhs.gov

Equivalent?

Does  or  =



AOAC Validation Programs

- **Official Methods Program**
- **Research Institute**
  - **Performance Tested Methods℠**

Program Comparison

Methods Program	Objective
Official	Multi-laboratory characterization of method performance
Performance Tested	Verification of performance claims of proprietary test kits

Program Comparison

- **Performance Tested Method**
  - An independent lab verifies the performance of the method
  - 1 Food is tested in common between originating and independent labs for every 5 validated foods/surfaces
- **Official Methods Program**
  - 8 – 10 labs verify the performance of the method
  - 1 Food is tested by collaborating labs for each food category claimed
  - It is proposed to change this requirement to 1 food per sample preparation procedure

AOAC Official Methods Program

- Approval of the precollaborative study protocol
- Precollaborative study (or harmonized RI study)
- Approval of collaborative study protocol
- Collaborative study
- Review of the collaborative study report
- Recommendation for First Action
- Recommendation for Final Action

AOAC Research Institute Performance Tested Methods

- Manufacturer obtains application package
- Manufacturer submits previously generated data
- Two Expert Reviewers selected
- General Referee and Expert Reviewers approve protocol for testing
- Independent laboratory uses protocol for additional testing (1 food per every 5 claimed foods)
- Data reviewed by Expert Reviewers and General Referee
- Performance Tested status is granted

Validation Parameters

- Applicability
- Inclusivity
- Exclusivity
- Aging of the Matrix
- Comparison with reference method—if available
- Fractionally positive results
- Naturally Contaminated Foods
- Contaminated Controls
- Cultural Confirmation of Results
- Competitive Microflora

Applicability

- **Applicability = Fit for Purpose**
- **The validation study must fully support the purpose for which the method is designed**

Inclusivity

- **A method must be effective for all variants of the target analyte**
- **Pure cultures must be tested with the method with EXACTLY the same media and under the SAME CONDITIONS as the method specifies for food samples**

Exclusivity

- **Demonstrates that the method will not misidentify non-target microflora as the target analyte**
- **A minimum of 30 strains of competitive microflora, commonly expected to be found in the food or commodity, must be tested with the method**
- **Exclusivity testing is performed with non-selective nutritious media**

Aging of The Matrix

- **Shelf Stable Foods** **2 weeks**
- **Frozen Foods** **2 weeks**
- **Perishable Foods** **48 – 72 h**

Reference Procedure

- **Alternative methods are compared to a reference culture method**
- **ISO, FDA/BAM and USDA/MLG are examples of reference methods**

Fractionally positive results

- **Acceptable results are neither all positive nor all negative**
- **The proportion of positive samples should approximate 50% of the total number of samples in the set**
  - **25 – 75% range is acceptable**
- **Demonstrates the relative effectiveness of the reference and candidate methods at the LOD of the reference method**

Naturally Contaminated Foods

- **Naturally contaminated foods are preferable to artificially contaminated foods**
- **Levels of analyte can be adjusted upward or downward through temperature abuse or dilution**

Negative Controls

- **Negative controls and artificially contaminated foods are prepared at the same time**
- **If negative controls are contaminated, then the data is rejected since none of the data can be trusted. It is assumed that some of the positive test portions are due to cross-contamination (lab error)**

Confirmation of Positive Results

- **There must be a procedure to confirm positive test results**
- **Confirmation procedures always result in a viable isolate which can be subjected to biochemical and serological testing**

Competitive Microflora

- **Should be at least one log higher than the target analyte**
- **Can be single species or pool of several species**
- **Naturally occurring microflora can fulfill this requirement**

Current & Proposed Validation Guidelines

Originating Laboratory Requirements

	Current	Proposed
Inclusivity	50/100[a]	30/100
Exclusivity	30	30
Number of foods	1+	1+
Analyte levels/food	1	2
Replicates/food	20	20
Competitor strain	Yes	Yes
Aging	Yes	Yes
Methods Comparison	Yes	Yes
Statistics	X^2	POD

[a] *Salmonella* serotypes

Current & Proposed Validation Guidelines

Collaborating Laboratory Requirements

		Current	Proposed
Number of Laboratories	(qualitative)	10	10
	(quantitative)	8	8
Number of Foods		1+	1+
Number of strains		1/food	1/food
Analyte levels/food	(qualitative)	3	3
	(quantitative)	4	4
Replicates/food/level	(qualitative)	6	12
	(quantitative)	2	2
Aging		Yes	Yes
Methods comparison		Yes	Yes
Statistics		X^2	POD

How Long Does it Take?

Method	Minimum Length of Process
Official	Two years from submission of precollaborative study data to adoption of method
Performance Tested	Nine months from submission of application to certification

Use of AOAC Validated Methods by FDA

- **Official Methods can be used for the analysis regulatory samples after verification**

- **Research Institute methods can be used for the analysis of regulatory samples after further validation**

Official Methods

- **Verification**
  - **Minimum of 7 samples of each commodity tested in duplicate**
  - **Inoculum level <30 cfu/sample**
  - **Accuracy study with regulatory samples tested by both the alternative and reference methods**

AOAC-RI Methods

- **Validation**
  - **Side-by-side comparisons of the alternative and reference methods with spiked samples**
  - **Accuracy study with regulatory samples tested by both the alternative and reference methods**

Method Modifications

- **Minor (testing of 1 or more foods)**
  - Extending a method to new matrices
  - Changing kit components without changing the fundamentals of the test
    - Change of plastic housing on a lateral flow device
    - Changing from a 96 well format to a strip format
- **Major (revalidation of the method)**
  - Changing primer sequences
  - Changing enrichments
  - Changing time/temperature incubation conditions
  - Changing antibodies

Questions?