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Validation of Existing and New Technologies, and How Fitness for Purpose Affects Methods Validation

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Outline

- Why we Validate Methods
- Examples of Internationally Recognized Validation Protocols
- ISO 17025 and How it relates to Methods Validation
- FDA Validation Guidelines
- Example of a FDA Fit for Purpose Validation

Fit for Purpose

- How do you determine if a method is fit for purpose?
  - Validate it for the organism and matrices of concern
  - Verify that a method works in the hands of qualified analysts

Validation

- To assure ourselves that negative results are negative
- To assure ourselves that rapid instrumental methods are equivalent to a reference method: culture and non-culture alike
- To demonstrate that methods are truly fit for purpose
  - No method works for all matrices
    - A method that works well for *Salmonella* in lettuce may not work so well for *Salmonella* in spices
- To be compliant with ISO 17025:2017
  - "General requirements for the competence of testing and calibration laboratories"
  - No accreditation without 17025:2017 compliance!

Methods Validation protocols

- FDA's Microbiological Methods Validation Guidelines
  - http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf
- AOAC's Appendix J
  - http://www.eoma.aoac.org/app_j.pdf
- ISO 16140:2016 parts 1 & 2—Protocol for the validation of alternative (proprietary) methods against a reference method
  - https://www.ansi.org
- ISO 17468:2016—Technical requirements and guidance on establishment or revision of a standardized reference method
  - https://www.ansi.org

Methods Validation Protocols

- There is no distinction between existing and new technologies
- Culture methods, immunological, and molecular methods are treated exactly alike
  - Compared to reference method, if existent, or subjected to spiking experiments to determine LOD_{50}

Methods Validation Protocols

- Reviewers concentrate on
  - Claims—What the method is designed to do?
  - Correctly structured inclusivity panels
  - Correctly structured exclusivity panels
  - Appropriate matrices
  - Robustness
  - Repeatability
  - Interlaboratory reproducibility
  - Comparison to the correct reference method

Reference Methods

- ISO 16140-1:2016 Definition: "internationally recognized and widely accepted method"
- Reference methods are used for regulatory sample analysis
- Reference methods are also called standard methods
- Examples of reference methods in the United States
  - *Bacteriological Analytical Manual* (BAM)
  - USDA's *Microbiology Laboratory Guidebook* (MLG)
  - EPA Water Methods
- Examples of reference methods outside the US
  - For the EU and other Nations, ISO Methods
  - For China, GB Standards

ISO 17025:2017 Validation

7.2.2.1 The laboratory **shall** validate non-standard methods, laboratory-developed methods and standard methods used outside their intended scope or otherwise modified. The validation shall be as extensive as is necessary to meet the needs of the given application or field of application.

d) comparison of results achieved with other validated methods;

e) interlaboratory comparisons;

7.2.2.2 When changes are made to a validated method, the influence of such changes shall be determined and where they are found to affect the original validation, a new method validation **shall** be performed..

ISO 17025:2017 Verification

7.2.1.5 The laboratory **shall** verify that it can properly perform methods before introducing them by ensuring that it can achieve the required performance. Records of the verification **shall** be retained. If the method is revised by the issuing body, verification **shall** be repeated to the extent necessary.

- Proficiency testing
- Training records
- 16140-3 (Verification)—Verifies that a lab is capable doing specific types of analyses

ISO 17025:2017

- Does not refer to ISO 16140:2016 or ISO 17468:2016
- Simply requires the use of validated methods and verification that laboratorians can properly perform methods

FDA's Methods Validation Guidelines

The Science and Research Steering Committee (SRSC), of the Office of Foods and Veterinary Medicine (OFVM), approved guidance to be used for validation of microbiological and chemical methods.

Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods
http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM273418.pdf

Guidelines for the Validation of Chemical Methods for the FDA Foods Program
http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf

Scope

"These criteria apply to all FDA laboratories that develop and participate in the validation of analytical food methods for Agency-wide implementation in a regulatory capacity. This includes all research laboratories, and field labs where analytical methods may be developed or expanded for potential regulatory use. These documents will supersede all other intra-agency documents pertaining to food-related method validation criteria for microbial and chemical analytes. the SRSC will authorize the formation of a Methods Validation Subcommittee (MVS) to serve as the governing body for all method validation concerns."

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Method Validation is Required for…

1. **Submission of a new or original method, *OR,***
2. **Any significant modification of a method that may alter its performance specifications or changes to the fundamental science of an existing method. Categories include:**
   - **Substitutions of reagents/apparatus**
   - **Expansion of the scope of an existing method to include additional analytes.**
   - **Changes in intended use *i.e.* screening or confirmatory.**
   - **Platform extensions or significant parameter changes e.g. adaptation to another real-time PCR thermal cycler.**
   - **Matrix extensions.**
   - **Changes to time/temperature incubation periods, or enrichment media.**
   - **In cases where the sample preparation and/or the extraction procedure/analytical method is modified from the existing test procedure and protocol, *i.e the new method should demonstrate that the modifications do not adversely affect the precision and accuracy or bias of the data obtained.***
   - **Modification of a method's performance range *e.g.* specificity, sensitivity beyond previously validated levels.**

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Levels of Validation

Two levels of performance are defined: emergency and non-emergency (SLV, Independent Lab, MLV). The hierarchy of scrutiny will provide general characteristics on the method's utility and insights for its intended use, the assessed risk, and the food-borne illness potential for an analyte-matrix pairing.

Not all methods will or should be validated to meet the requirements of a full collaborative study.

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Table 1- General Guidelines for the Validation of Qualitative Detection Methods for Microbial Analytes

	Emergency	Non-Emergency Validation Processes		
Criteria	**Emergency Use**	**Single Laboratory Validation Study**	**Independent Laboratory Validation Study**	**Collaborative Validation Study**
Participating Laboratory	Originating Laboratory	Originating Laboratory	Collaborating Laboratory	Collaborating Laboratories
# of target organism (inclusivity)[a]	[f]TBD	50 (unless 50 aren't available)[b,c]	[e]NA	[e]NA
# of non-target organism (exclusivity) [a]	[f]TBD	30 strains[d]	[e]NA	[e]NA
# of laboratories providing usable data	1	1	1	10
# of foods	1or more[g]	1or more[g]	1or more[g]	1or more[g]
# of analyte levels/food matrix	[f]TBD	Two inoculated levels[i] and one uninoculated level	Two inoculated levels[i] and one uninoculated level	3 levels: One inoculated fractional level[i], one at 1 log higher[e] and one uninoculated level
Replicates per food at each level tested	[f]TBD	20 for the fractional level (5 each for the uninoculated and high levels)	20 for the fractional level (5 each for the uninoculated and high levels)	8
Aging of inoculated samples prior to testing	No	Yes[h]	Yes[h]	Yes[h]
Addition of competitor strain[j]	Normal background flora	In 1 food at +1 log>analyte at fractional positive[i] analyte level	In 1 food at +1 log>analyte at fractional positive[i] analyte level	In 1 food at +1 log>analyte at fractional positive[i] analyte level
Reference Method Comparison Requirement[k]	[f]TBD	Yes, if available	Yes, if available	Yes, if available

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Table 2 - General Guidelines for the Validation of Qualitative Detection Methods for Microbial Analytes - Unique Isolation and/or Enrichment Challenges

	Emergency	Non-Emergency Validation Processes		
Criteria	**Emergency Use**	**Single Laboratory Validation Study**	**Independent Laboratory Validation Study**	**Collaborative Validation Study**
Participating Laboratory	Originating Laboratory	Originating Laboratory	Collaborating Laboratory	Collaborating Laboratories
# of target organism (inclusivity)[a]	[f]TBD	[f]TBD	[e]NA	[e]NA
# of non-target organism (exclusivity)[a]	[f]TBD	[f]TBD	[e]NA	[e]NA
# of laboratories providing usable data[b]	1	1	1	5[b]
# of foods	1 or more[g]	1 or more[g]	1 or more[g]	1 or more[g]
# of analyte levels/food matrix	[f]TBD	One inoculated level[i] and one uninoculated level	One inoculated level[i] and one uninoculated level	3 levels: One inoculated level[i], one at 1 log higher[e] and one uninoculated level
Replicates per food at each level tested	[f]TBD	3	3	8[n]
Reference Method Comparison Requirement[k]	[f]TBD	Yes, if available	Yes, if available	Yes, if available

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Methods Modifications

- **Methods can only be modified after validation**
  - **Modifications must be validated**
- **Methods should never be modified without appropriate validation with a single exception**
  - **An Emergency, but you must have some sort of data!**
- **Modifications without validation data call into question negative sample results**

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Validation of a Method for the Detection of *Cyclospora* in Agricultural Water

- **Table 2 applies**
  - **It is a non-culturable organism, so test portion inoculation is an issue—oocysts are difficult to obtain**
  - **It is a complicated method that will only be used in regulatory/specialty labs**

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Participating Laboratory	Originating Laboratory	Originating Laboratory	Collaborating Laboratory	Collaborating Laboratories
# of target organism (inclusivity)[a]	[b]TBD	[b]TBD	[b]NA	[b]NA
# of non-target organism (exclusivity)[a]	[b]TBD	[b]TBD	[b]NA	[b]NA
# of laboratories providing usable data[d]	1	1	1	5[e]
# of foods	1 or more[a]	1 or more[a]	1 or more[a]	1 or more[a]
# of analyte levels/food matrix	[b]TBD	One inoculated level[a] and one uninoculated level	One inoculated level[a] and one uninoculated level	3 levels: One inoculated level[a], one at 1 log higher[a] and one uninoculated level
Replicates per food at each level tested	[b]TBD	3	3	8[e]
Reference Method Comparison Requirement[a]	[b]TBD	Yes, if available	Yes, if available	Yes, if available

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Collaborative Study

- **Eight Labs Participated**
  - **5 FDA**
  - **1 Commercial**
  - **2 State Labs**
- **Labs were qualified prior to the collaborative study by PT**

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Fig 1. MLV Study Round Workflow

Day 1-2	Water collected from local source and tested for background *C. cayetanensis*
Day 2-4	*C. cayetanensis*-free water subdivided into 10 L carboys, seeded and MLV filter samples prepared. Pre-shipment testing performed at Moffett
Day 5	Pre-shipment testing completed and shipment documents cleared
Day 6	One set of eight filters shipped to each MLV participant (eight sets per round)
Day 7/8	The MLV samples are received by the MLV participants and stored until testing begins the following Monday
Days 11-15	The MLV samples are tested by the participants (backflush, concentration, DNA extraction and real time PCR) and results are submitted to the MLV Organizers

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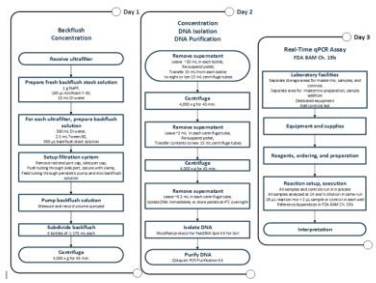

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	Number of positive results/ Number of tests					
LIN	**0 oocysts**	**Specificity for each laboratory**	**6 oocysts**	**Sensitivity for each laboratory**	**100 oocysts**	**Sensitivity for each laboratory**
65	0 / 9	100%	12 / 14	85.71%	9 / 9	100%
11	0 / 9	100%	14 / 14	100%	9 / 9	100%
45	0 / 9	100%	10 / 14	71.43%	9 / 9	100%
88	0 / 9	100%	11 / 14	78.57%	9 / 9	100%
71	0 / 9	100%	14 / 14	100%	9 / 9	100%
98	0 / 9	100%	14 / 14	100%	9 / 9	100%
77*	3 / 9	33.33%	14 / 14	100%	9 / 9	100%
47	0 / 9	100%	11 / 14	78.57%	9 / 9	100%
No. of Positive Results	0		86		63	
Total No. of Results	63		98		63	
Specificity for each seeding level	100%		N/A		N/A	
Sensitivity for each seeding level	N/A		87.8%		100%	

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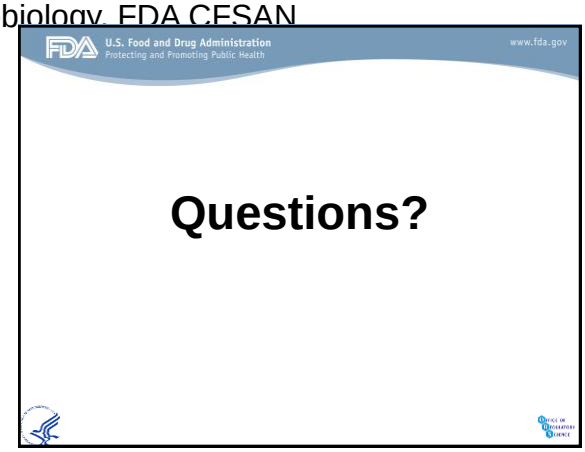
Next Steps

Publication in FDA's *Compendium of Analytical Laboratory Methods for Food and Feed Safety*
https://www.fda.gov/food/laboratory-methods-food/compendium-analytical-laboratory-methods-food-and-feed-safety

Final Publication in the *Bacteriological Analytical Manual*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Questions?