International Association for Food Protection WEBINAR

Assuring the Right Fit:
Using risk assessment and alternative approaches to ensuring methods are fit for purpose.

Organized by: IAFP's Applied Laboratory Methods PDG

Moderator: Takiyah Ball PhD

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Today's moderator:

International Association for Food Protection® WEBINAR

Takiyah Ball

Takiyah is a Food Safety Microbiologist at Sargento Foods Inc. She received her Ph.D. in Comparative Biomedical Science from NC State University.
Prior to Sargento, Takiyah was an ORISE Fellow in the molecular genetics department at FDA-CFSAN-OARSA. She also spent fifteen years managing the Salmonella and E. coli lab, a part of the National Antimicrobial Resistance Monitoring System (NARMS) at the USDA.

Applied Laboratory Methods Professional Development Group

Webinar Speakers

Takiyah Ball MS, MPH, PhD
Food Safety Microbiologist
Sargento Inc.
Moderator

Gabriela Lopez Velasco PhD
Senior Technical Service Specialist
Neogen Corporation
Speaker

Patrick Bird MS
Senior Manager of Scientific Affairs
bioMérieux
Speaker

Validation and Verification Subgroup Objective:

To provide suggestions for practical, risk-based approaches to address the gap in the scope of validation by focusing on matrix grouping and levels of test method evaluation.

Overview - Assuring the Right Fit

- **Food testing results drive important conclusions about food production and the food itself**
  - Thermal process is correct
  - Pathogen environmental monitoring is working
  - Hygienic conditions are met during food production
  - Product is being stored correctly
  - Ensure raw materials will not bring hazards into the facility
  - The product meets microbial specifications
  - The product is safe and good for commercialization

Food testing is a big responsibility ….

The Dynamics of an Evolving Testing Market

FSMA Preventive Controls

- The owner, operator, or agent in charge of a facility are effectively and significantly preventing the occurrence of identified hazards.

Why is testing increasing?

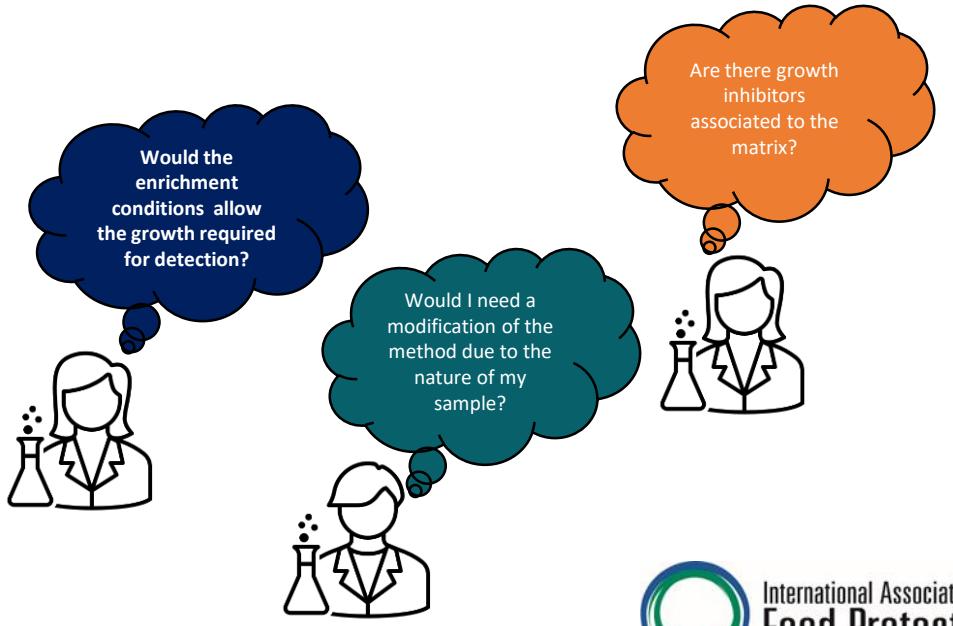
- Regulatory updates
- Globalization of the food supply
- Requirements for shorter product development timelines
- Reformulation of existing products to meet consumer trends

Method Fitness for Purpose

- **A method for testing a food product or a sample collected from the production environment should provide accurate data to the degree needed to make informed decisions for the intended application.**

How do we assure that test results are reliable, and methods are fit for their intended purpose?

- *If a method is validated on a particular matrix, the method is considered to be 'fit for purpose' for that matrix*
- *If the method is not validated for a particular matrix, the laboratory should ensure that the method will render accurate data.*



Source: Food Safety Testing: Understanding Microbiological Method Validation, Verification, and Fitness for Purpose - Eurofins USA

Is the Method Fit for Purpose?

Example 1

Method A
Validated for testing 25 g of a milk-based product

→ Food plant diversifies to a non-dairy product line using plant-based protein ingredients

→ ***Can I keep using method A?***
Do I need to validate?
Do I need to verify?

Example 2

Method B
Is being validated to test for *Salmonella* in 325 g 'a' raw material

→ A single supplier provided the raw material for the past 10 years. An increase in product demand required to receive RM for two additional suppliers

→ ***Can I keep using Method B?***
Do I need to validate?
Do I need to verify?
Do I need to do anything at all?

We often answer these questions based on an educated guess and a logical rationale. Knowing whether the 'matrix' in question *falls within a category of matrices for which the method is validated* helps to answer these questions.

Understanding Validation and Verification

- ISO 17025 requires that laboratories use methods that are both validated and verified.
- **Validation** – Establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled [1]
- **Verification** – Demonstration that a validated method functions in the user's hands according to the method's specification determined in the validation study and is fit for its intended purpose[1]

Validation
Process of demonstrating that the method reliably detects the analyte

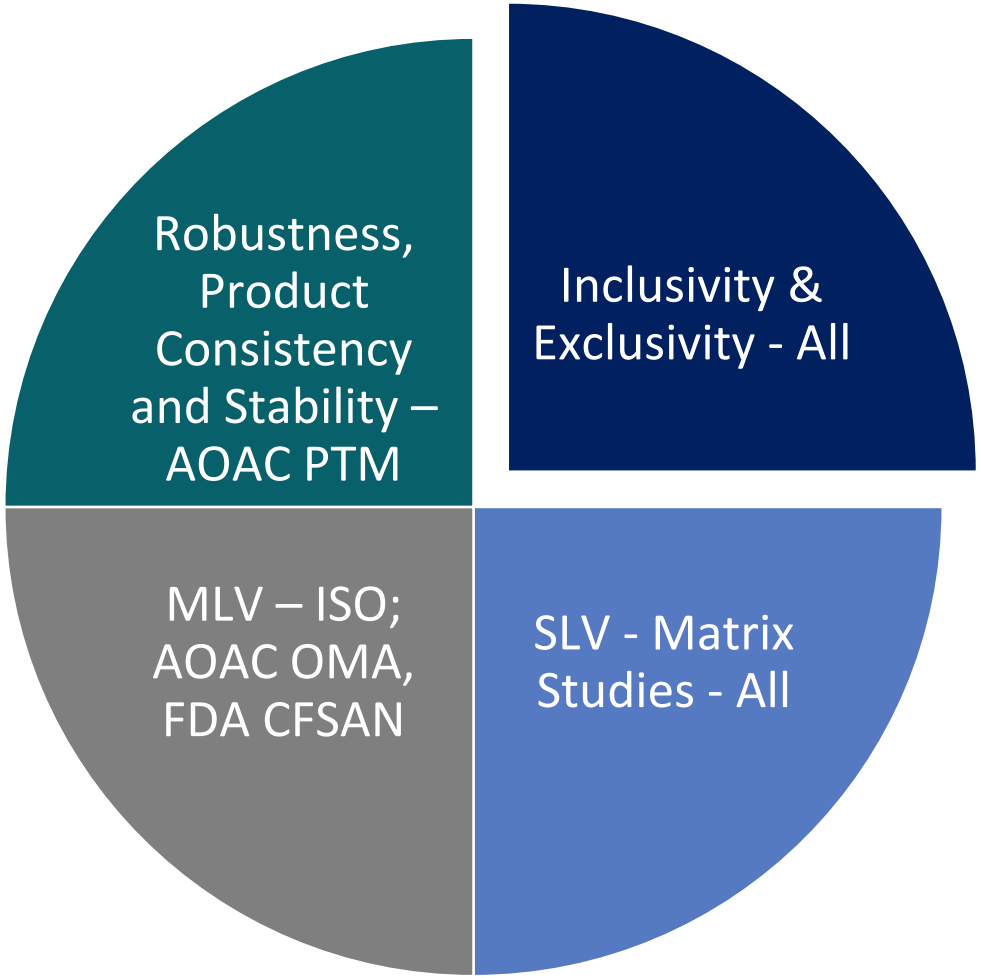
Verification
Demonstrates that the laboratory can effectively perform the method

Validation Guidance

- **Global Validation Guidance:**
  - ISO 16140 series (-1, -2, -4, -5, -6, -7) *Microbiology of the food chain – Method validation*
  - AOAC INTERNATIONAL Appendix J – *Guidelines for the Validation of Microbiological Methods for Food and Environmental Surfaces*
- **North American Validation Guidance**
  - US FDA CFSAN – *Guidelines for the Validation of Analytical Methos for the Detection of Microbial Pathogens in Food and Feeds, Ed 3.0*
  - USDA FSIS – *FSIS Guidance for Test Kit Manufacturers, Laboratories: Evaluating the Performance of Pathogen Test Methods*
  - Health Canada – *The Compendium of Analytical Methods* (Parts 1, 2A, 2B, 3, 4, 6, 7 and 9)
- **Conformity Assessment Organizations:**

Validation Guidance

- **Inclusivity and Exclusivity**
  - 50 target (100 for Salmonella) tested at 10x LOD of method
  - 30 non-target tested at high concentration
- **Matrix Study - SLV**
  - Qualitative - 3 levels of contamination (5 x control, 20 x low and 5 to 20 x high)
  - Quantitative – 3 to 4 levels of contamination (5 x low, medium and high; control if artificially contaminated)
    - Additional matrix study tests required by ISO (sensitivity, relative trueness)
  - Bulk inoculation
  - Stressing/equilibration of inoculum and matrix



Verification Guidance

- **Global Verification Guidance**
  - ISO 16140-3:2021 *Microbiology of the food chain – Method validation – Part 3: Protocol for the verification of validated reference and validated alternative methods in a single laboratory*
- **North American Verification Guidance**
  - US FDA CFSAN - Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds (3rd Ed. October, 2019)
  - Health Canada - Part 5: Guidelines to Verify Standard Food Microbiological Methods for Implementation in Routine Testing (April, 2015)

Verification Guidance

ISO 16140-3	Method must be fully validated (collaboratively studied)
	Implementation and food item verification
	Multiple options - Qualitative: 8 to 10 replicates depending on protocol used Quantitative: Factorial study design, Comparison to traditional plating methods
US FDA CFSAN	Method must be collaboratively studied
	Six inoculated (< 30 CFU/test portion) and non-inoculated replicates.
	If FP/FN, full SLV should be performed (20 replicates)
Health Canada	Qualitative - Detection limit study: Artificial contamination of 5 levels (+ control) with 3 replicates tested at each Recovery study: Each protocol must be tested with 3 to 5 replicates
	Quantitative – Reproducibility data available: 10 replicates measured in duplicate No reproducibility data: Factorial study design with 10 -20 replicates measured in duplicate

Responsibilities for Validation and Verification

- **Validation**
  - Primarily technology providers and expert laboratories
  - If method modified or extended, end user would perform validation
- **Verification**
  - All end users: Third party-contract laboratories, manufacturers, reference laboratories

Ensuring the Reliability of a Test Method

Sampling

Contamination will often be:

a. Heterogeneous
b. Very low numbers

Sampling is critical

Test

Test should be adequate for the hazard

a. Use of indicator microorganism test
b. Use of a pathogen detection test

Defined through risk-based analysis

Method

Method should be capable of:

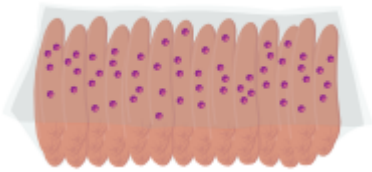
a. Promote conditions to enable microbial recovery and detection
   - Enrichment conditions
   - Technology for detection

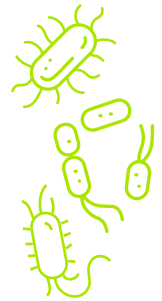
Method should be fit for purpose

Testing data is used to make decisions thus it is critical to clearly define sampling procedures and method selection to ensure reliable results

Is the Sampling Plan Robust Enough?

Contamination level 10%

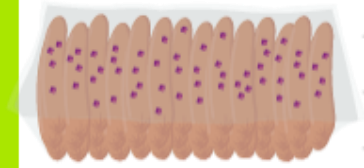


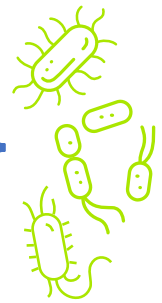


Units in a lot	Pathogen contamination (%)	Contaminated units	Samples analyzed	Probability of detecting a contaminated lot
50,000	0.001 (0.1%)	50	x 2900	95%
	0.01 (1%)	500	x 300	
	0.05 (5%)	2500	x 60	
	0.1 (10%)	5000	x 28	

x1

Contamination level 0.01%





Units in a lot	Pathogen contamination (%)	Contaminated units	Samples analyzed	Probability of detecting a contaminated lot
10,000	0.0001 (0.01%)	1	x1	0%
50,000	0.0001 (0.01%)	5		
100,000	0.0001 (0.01%)	10		
1,000,000	0.0001 (0.01%)	100		

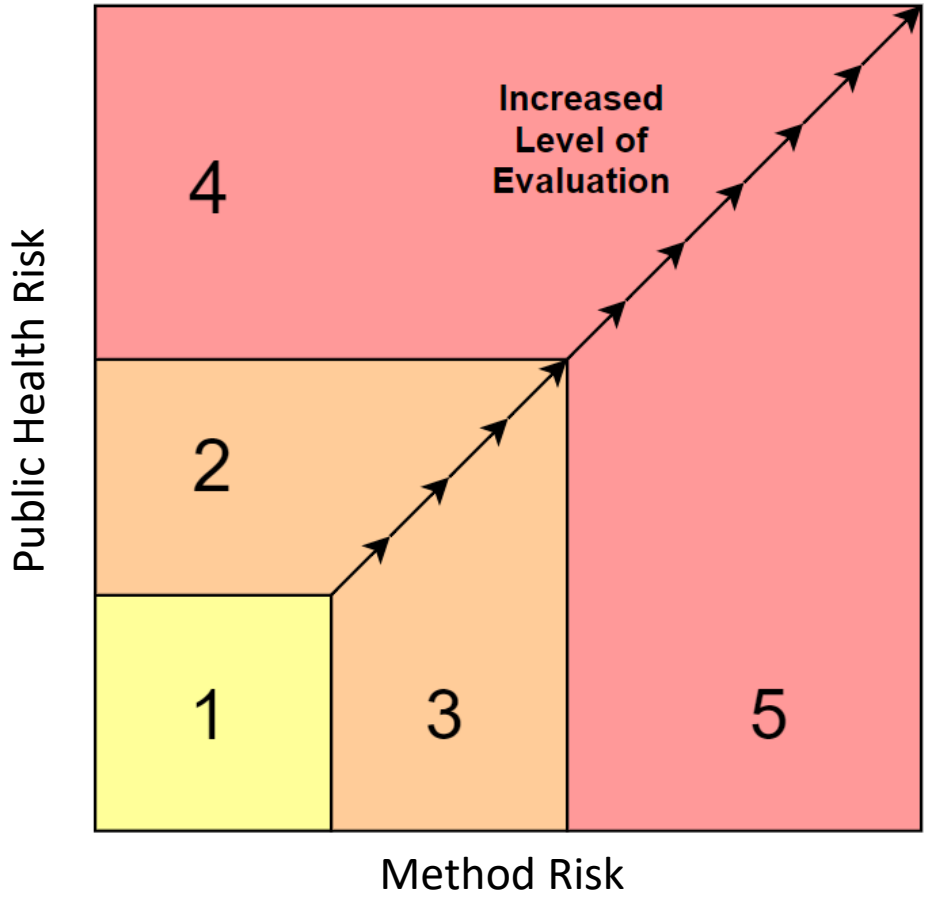
Image courtesy: Neogen|3M Food Safety

Validation Challenges for Food Manufacturers

- **Is a method validated?**
  - Diversity and complexity of matrices
  - Closeness of product to validation claims of method
- **Use of pathogenic organisms in a production facility area and risk of contamination**
- **Adequate laboratory space, equipment and technical skill needed to perform validation and verification**

Method Risk Assessment

1. **Low Risk for Public Health and Method Performance**
   a) No outbreaks or recalls associated with matrix
   b) No pathogen risk reported with the matrix
   c) Matrix already validated for method
2. **Moderate Risk for Public Health**
   a) No outbreaks and recalls associated with matrix
   b) Pathogen risk has been reported
3. **Moderate Risk for Method Performance**
   a) Matrix validation data for similar products
4. **High Risk for Public Health**
   a) Outbreaks and recalls associated with matrix
   b) Inherent pathogen risk with product
5. **High Risk of Method Performance**
   a) No matrix validation data



Alternative Approaches for Qualitative Microbiological Method Matrix Additions - International Association for Food Protection

Alternative Matrix Evaluation Approaches

Method validation schemes use food matrix categorization to simplify the work needed to demonstrate that methods are effective and fit-for-purpose across similar foods.

Grouping of food types based on intrinsic factors is a common way to address the number of studies and/or complexity of the studies used for matrix addition.

Planning for a Matrix Extension

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If a matrix has not been evaluated there are two critical risks to method performance:

1. Enrichment

Would the method allow propagation of the target organism to detectable levels in the new matrix?

- Simplification from a two-stage to a single-stage enrichment
- Composite test portions (25 g vs 375 g)
- Use of proprietary media

2. Technology detection

Would the new matrix interfere with the assay's chemistry or technology?

- DNA amplification inhibitors
- Sample pH interference
- Reporting system inhibitor compounds (fluorescence)
- Analytical limit of detection

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Evaluation of a Matrix's Intrinsic Factors

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Table 1. Chemical and physical food attributes (intrinsic factors) considered in grouping matrices

pH	Surface structure
Water activity	Salt
Natural occurring inhibitors – cocoa polyphenols, enzymes	Sugar
% Fat	Added humectants – Polysaccharides, Dietary Fiber, Hydrocolloid, Pectin
% Protein	Emulsifiers
% Fiber	Fermentation products and byproducts
% Carbohydrate	Microbial inhibitors and preservatives used in formulation
Added organic acids	Type of processing – roasted, high pressure processing, irradiated
Microbial load – active cultures, raw agricultural product, meat	Physical form – dried, intermediate moisture food, high moisture

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Food Matrix Grouping Approach: Uncategorized Foods

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The Interagency Food Safety Analytics Collaboration

- Food categorization by food type then by food processing
  - pasteurized fluid dairy products,
  - unpasteurized fluid dairy products,
  - pasteurized solid, and
  - semisolid dairy products

However, these schemes only group select products, leaving many uncategorized for industry to assess. For example

- cheese powder concentrates
- proprietary spice blends

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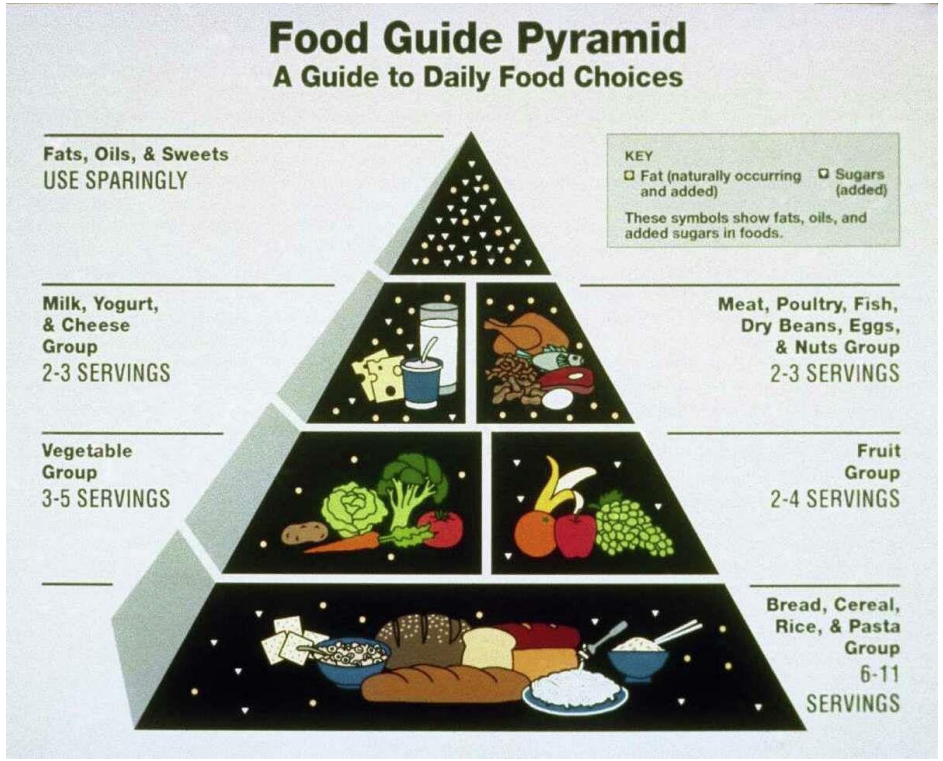
Other Types of Groupings



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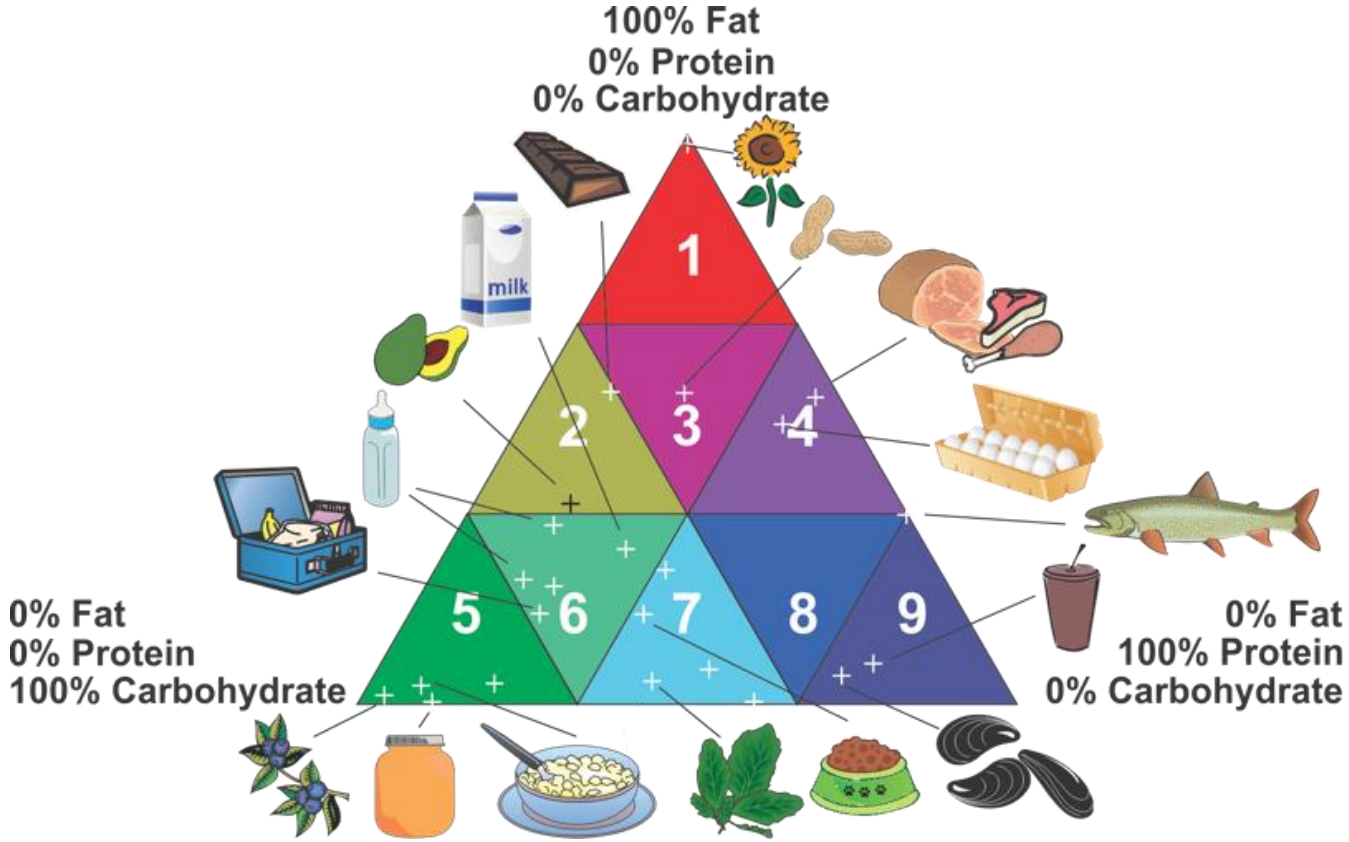

Food Pyramid (nist.gov)

Commonality in Enrichment Procedures

- Within a test method, there is often a significant core in enrichment conditions for the claimed validated matrices.
- A common core in enrichment conditions increases the confidence that a method can recover a pathogen of concern, even in an unevaluated matrix
- **In a matrix extension, choosing an enrichment condition used by that method for a matrix from a similar validated category is a good starting point**
- However, if modification occurs, 'full validation' may be required
  - Modified enrichment media
  - Additional dilutions
  - Unique intrinsic properties of the matrix

Core Method Conditions and Validated Matrices

(Example for *Salmonella*)

	Enrichment core conditions						Categories								
Reference	Test portion size	Enrichment stage	Ratio[a]	Broth identity	Time (h)	Temp (°C)	Dairy products	Meat and poultry	Egg products	Seafood	Fruits and vegetables	Miscellaneous foods	Animal feed	Spices	Environmental samples[b]
AOAC OMA 2011.03 *(8)*	Follow BAM or MLG	Primary	Follow FDA BAM or USDA MLG				X	X	X	X	X	X	X	X	X
		Secondary		SX2	22–26	42 ± 1									
AOAC OMA 2013.01 *(9)*	25 g, 375 g, 30 mL, sponge, swab	Single	0.1, 0.25	BPW + proprietary supplement	22–24	42 ± 1	X	X	X	X	X	X	X	X	s, p, t
AOAC OMA 2014.01 *(11)*	25 g, 100 g, 325 g, 375 g, sponge	Primary	0.1	BPW	18–24	41.5 ± 1		X	X	X	X		X		s
		Secondary		RV[c]	24[c]	41.5 ± 1[c]									
AOAC OMA 2016.01 *(12)*	100–375 g, 325 g, 30 mL, sponge, swab	Single	0.1	ISO BPW	24	37 ± 1		X	X			X	X		X
			0.25	ISO BPW (prewarmed)	18–24	41.5 ± 1[c]		X							s, c, t
AOAC OMA 2017.06 *(14)*	25 g, 375 g, 30 mL, sponge, swab	Single	0.1, 0.25, 0.5	BPW (prewarmed)	21 ± 1	37 ± 1	X	X	X		X		X		s, t, p, c
					10 ± 2[d]	36 ± 1[d]									
FDA BAM[e] Chap. 5 *(34)*	25 g, sponge, frog legs, pig ears	Primary	0.1	Lactose	24 ± 2	35	X	X	X	X	X	X	X		X
		Secondary		RV and TT	24 ± 2	42 ± 0.2 35 ± 2									
USDA MLG[f] Chap. 4.10 *(29)*	25 g, 100 g, 325 g, 30 mL, sponge	Primary	0.1, 0.2, 0.5	BPW	22–24	35 ± 2		X	X	X		X			X
		Secondary		RV and TT	22–24	42 ± 0.5									
ISO 6579-1:2017 *(24)*	25 g	Primary	0.1	BPW (prewarmed)	18 ± 2	34–38	X	X	X	X	X	X	X		X
		Secondary		RVS and MKTTn	24	41.5 37									

Alternative Approaches for Qualitative Microbiological Method Matrix Additions - International Association for Food Protection

Core Method Conditions and Validated Matrices

(Example for *Listeria spp*)

		Enrichment core condition					Categories								
Reference	Test portion size	Ratio[a]	Stage	Broth identity	Time (h)	Temp (°C)	Dairy products	Meat and poultry	Egg products	Seafood	Fruits and vegetables	Miscellaneous foods	Animal feed	Spices	Environmental samples[b]
AOAC OMA 996.14 *(5)*	25 g, sponge, swab	0.1	Primary	Modified Fraser with LiCl	26–30	30 ± 1	X	X		X	X				s, c, r
			Secondary	BLEB	22–26	30 ± 1									
AOAC OMA 999.06 *(6)*	25 g	0.1	Single	BLEB	48–50[c]	30 ± 1	X	X			X				
AOAC OMA 2004.06 *(7)*	25 g	0.1	Primary	Demi-Fraser	24–26	30 ± 1	X	X		X	X				
			Secondary	Fraser without FAC	24–26	30 ± 1									
AOAC OMA 2013.10 *(10)*	25–125 g, sponge, swab	0.1	Primary	LPT	26–30	30 ± 1	X	X		X	X	X		X	s, c, p, t
			Secondary	LPT	22–26	30 ± 1									
AOAC OMA 2016.07 *(13)*	25–125 g, sponge, swab	0.1, 0.2, in 10, 100, or 225 mL	Single	Demi-Fraser	28–30	37 ± 1	X	X		X	X				s, c, p
FDA BAM Chap. 10 *(35)*	25 g, sponge, swab	0.1	Single	BLEB + pyruvate	24–48	30	X	X	X	X	X	X	X		X
USDA MLG Chap. 8.11 *(30)*	25 g, 125 g, sponge(s), filter	0.1	Primary	UVM	20–24	30 ± 2		X	X	X					X
			Secondary	MOPS-BLEB	18–24	35 ± 2									
ISO 11290-1: 2017 *(25)*	25 g	0.1	Primary	Demi-Fraser	24–26	30	X	X		X	X				X
			Secondary	Fraser	22–26	37									

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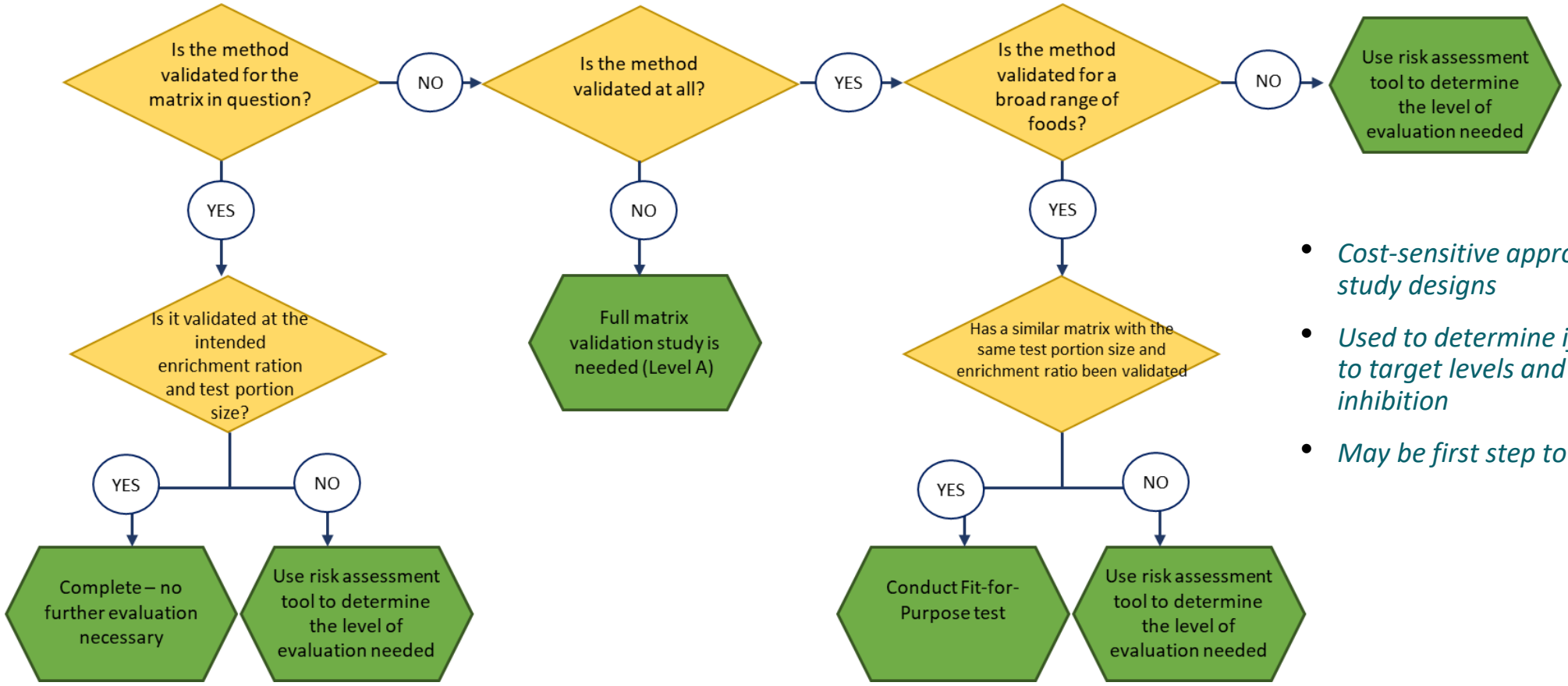
Fit for Purpose Decision Tree



- *Cost-sensitive approach with many possible study designs*
- *Used to determine if matrix allows propagation to target levels and demonstrates no matrix inhibition*
- *May be first step toward full validation study*

If you would like to learn more

GENERAL INTEREST PAPER

Microbiological Detection Methods — Assuring the Right Fit

Patrick M. Bird,[1] Megan S. Brown,[2]* Joy E. Dell'Aringa,[3] LeAnne A. Hahn,[4] J. David Legan,[2] Ryan D. Maus,[4] Stephanie Pollard[5] and Laurie S. Post[4]

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SUMMARY

The food safety industry is in the midst of rapid evolution. Leaders and scientists alike are approaching new regulatory requirements set forth by the Food Safety Modernization Act to ensure analytical methods, designed to detect hazards, are fit-for-purpose for their specific commodities. Simultaneously, the food industry is innovating at a tremendous rate. Unique ingredients and formulations are being developed, novel processing methods are being deployed, and new products are entering the market. The food safety community is scrutinizing analytical approaches to ensure that new and existing methods are appropriate for the bevy of products being tested. In addition, the industry is working to understand and agree upon the most prudent scientifically and economically sound approaches to method validation and verification. In this introductory article, the International Association for Food Protection Applied Laboratory Methods Professional Development Group discusses the needs and considerations for assessing fit-for-purpose approaches in the food analytical laboratory.

OVERVIEW

The first major change in U.S. food safety legislation since the Food Drug and Cosmetics Act of 1938 occurred in 2011, when the Food Safety Modernization Act (FSMA) was passed. This law emphasizes prevention of entry of foodborne contaminants into the market *(3)* and builds on approaches already implemented in industry, such as the Hazard Analysis Critical Control Point (HACCP) principles, to identify risks, apply control measures with defined critical limits, and verify effectiveness in mitigating those risks *(3)*. FSMA calls these control measures "Preventive Controls" and requires that "the owner, operator, or agent in charge of a facility" must verify that their food safety preventive controls "are effectively and significantly preventing the occurrence of identified hazards." This demand for verification is driving a large increase in laboratory testing, especially as food businesses expand environmental monitoring and increase the analysis of raw materials and finished products for pathogens, spoilage organisms, allergens and other adulterants. To facilitate this increase in testing, manufacturers are relying more and more on commercial or private laboratories to help them meet this demand by producing accurate results that are both efficient and cost effective.

In addition to testing that is driven by regulatory changes, globalization of the food supply, shorter product development timelines, and reformulation of existing products *(4)* to meet consumer trends create huge numbers of new food products that must be tested. In the U.S. alone, 21,435 new packaged food and beverage products for consumers were introduced in 2016, almost double the 11,853 introduced in 1998 *(11)*. These new products may be the result of incremental changes, such as the advent of Greek yogurt, which grew from nothing in 2005 to 44% of the yogurt market by 2014 *(10)*, or they may result from more radical innovations, such as the addition of probiotic cultures to various foods, including juices, chips, chocolate bars, pet food, and others. Products are also becoming more "exotic", as in the case of insect-based foods *(8)* such as energy bars made from cricket flour. All such foods may come in multiple flavors, varieties (e.g., nonfat, sugar free), and forms (e.g., freeze-dried bites), resulting in a complexity of forms and formulations that may interfere with pathogen detection methods.

The USDA Trends in Food Recalls *(12)* reported a doubling in recalls between 2004 and 2013 and suggested a number of possible reasons, including:

- increased regulatory oversight
- increased product and environmental sampling
- improvements in technology and detection
- better product and ingredient traceability
- increased audits and inspections, and
- new food types available in the market.

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[Microbiological Detection Methods – Assuring the Right Fit]

GENERAL INTEREST PAPER

Alternative Approaches for Qualitative Microbiological Method Matrix Additions

Megan S. Brown,[1]* Patrick M. Bird,[2] Sharon Brunelle,[3] W. Evan Chaney,[4] Charles A. Kennett,[5] J. David Legan,[1] Ryan D. Maus,[6] Laurie Post[6] and Stephanie Pollard[7]

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SUMMARY

Most commonly used pathogen detection methods have undergone a rigorous validation through third-party certification bodies such as AOAC INTERNATIONAL, Association Française de Normalisation, MicroVal, and others. These validations focus on sensitivity, robustness, and inclusivity and exclusivity of the assay target(s) for the matrices submitted to the certification body. This creates a list of officially validated matrices that falls far short of what is seen routinely during end-user testing. Thorough validation of all matrices at all test portion sizes is neither cost efficient, practical, nor arguably necessary. Here, we provide guidance on alternate evaluation approaches using a food-similarity grouping and a risk-based questionnaire to help end-users determine an appropriate level of evaluation of their method of choice. In reducing the burden of evaluation for many matrices, these alternative approaches may allow more matrices to be evaluated, thus strengthening confidence in method application and ultimately leading to a safer food supply.

OVERVIEW

The Food Safety Modernization Act, passed in 2011, emphasizes prevention of entry of foodborne contaminants into the market *(33)*. This act focuses on the establishment of verified "preventive controls" to reduce or eliminate identified hazards in the food production environment. This has led not only to a dramatic increase in laboratory testing of raw ingredients, finished food products, and environmental samples but also to questions on what "verified" means. Most foodborne pathogen test methods are validated for specific applications by a third-party certification body such as AOAC INTERNATIONAL (AOAC), Association Française de Normalisation, MicroVal, NordVal International, or Health Canada. However, third-party validation studies often include only a small number of matrices or a different test portion size than is commonly tested in the field (e.g., 25 versus 375 g, respectively). Because test methods cannot be validated for every possible matrix at every test portion size, there is a substantial gap in data between third-party certified matrices and end-user fit-for-purpose analytical testing needs. In this article, we aim to provide suggestions for practical, risk-based approaches to address that gap in qualitative microbiological methods by focusing on matrix grouping and levels of test method evaluation. In support of this aim, we have created a Matrix Evaluation Level Assessment Tool (available at https://www.foodprotection.org/upl/downloads/library/matrix-evaluation-level-assessment-tool.xlsx) that guides the user through a set of questions to help determine the degree of test method evaluation needed for a new matrix.

Need for alternative method evaluation approaches

Rapid methods for the qualitative microbiological testing of foods are used extensively throughout the food industry for detection of low concentrations of pathogens. Typically, method validation studies are conducted through recognized third-party certification bodies by the rapid method developer or test kit manufacturer with a limited group of food matrices and associated method parameters such as test portion size, nutrient media, and enrichment conditions. Because the scope of the validation is limited to the matrices included in the method validation study, the responsibility for ensuring that methods are fit-for-purpose is left to end-users such as food manufacturers and third-party laboratories. This responsibility often means conducting matrix addition studies to extend the method scope to a new matrix or a new test portion size. Here, we use the term "evaluation" to encompass the process by which test methods are assessed for use with a matrix of interest. This is an attempt to distinguish this process from definitions of verification or validation used by regulatory and accreditation bodies.

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[Alternative Approaches for Qualitative Microbiological Methods Matrix Additions]

GENERAL INTEREST PAPER

Evaluating Microbiological Method Equivalence – A Decision Guide

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SUMMARY

Using an appropriate method is a key step in generating reliable results; and, when those results are to be used to make safety-critical decisions, method selection becomes even more important. For microbiological testing, there are national and international standard methods and various other widely accepted methods. Performance of such methods has usually been validated through some kind of collaborative process or independent review. An independent review may have resulted in some kind of certification. Method validation, with or without independent certification, demonstrates that a method has performance equivalent to an established reference method. Many circumstances can arise that cause a laboratory to change methods. In such an event, how is a laboratory to determine that two methods are equivalent to one another if neither of them is a reference method? In this paper we outline a thought process to guide this decision. The process involves comparing existing validation and/or certification data to determine whether two or more methods have been compared against the same reference method for the matrices of interest using a rigorous experimental and statistical approach. If they have, the methods may be considered equivalent, and a laboratory simply needs to verify its ability to perform them. If they have not, then a formal validation may be needed.

OVERVIEW

In previous articles by the International Association for Food Protection Interest Group on Verification and Validation, the increasing need for the most prudent, scientifically and economically sound approaches to method validation and verification was discussed *(5)*. Suggestions for practical, risk-based approaches to address this need focused on matrix grouping and levels of test method evaluation in a second publication *(6)*. Following on this theme, this current article discusses another approach that would alleviate verification and validation testing pressure and reduce the burden of evaluation, particularly when one is faced with choosing between two or more validated methods. The most direct comparison is when the methods are validated for the same target analyte in the same validated matrix. When can we consider these methods equivalent to one another without a direct comparison between them? How does the reference method affect this consideration? What if the validations do not include exactly the same matrices? What other factors would play into method selection? These questions and more are the subject of frequent decisions in microbiology laboratories around the world.

Before addressing these questions, it is helpful to have a basic understanding of the process of method validation. The starting point for validation of a new method is the existing reference method against which the new method is compared. There are minor differences in the definition of reference among sources *(1, 8, 15)*, but all are from recognized sources such as the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM), the U.S. Department of Agriculture (USDA) *Microbiology Laboratory Guidebook* (MLG), the Health Canada *Compendium of Analytical Methods*, standards from the International Organization for Standardization (ISO), and national standards from countries throughout the world. Traditionally all are cultural methods.

The developer of a new qualitative method evaluates several performance parameters including inclusivity, exclusivity, robustness, and stability and the ability to detect the target in a range of matrices following guides to validation such as AOAC Appendix J *(1)* or ISO 16140-2 *(9)*. There are

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[Evaluating Microbiological Method Equivalence – A Decision Guide]

GENERAL INTEREST PAPER

Selection of Pathogen Strains for Evaluating Rapid Pathogen Test Methods Applied to New Matrices

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SUMMARY

Before first use of a validated method, laboratories verify their ability to apply the method as designed. In routine laboratory operations, new matrices will appear occasionally, with insufficient data ensuring method performance for the matrix. Approaches have been documented to the "fitness for purpose" testing then required, but the question of how to select the pathogen strain or strains for this activity has received scant attention. This article reviews factors that may influence strain selection for method evaluation, including processing environment, geographical origin or proximity, seasonality, environmental factors, intrinsic characteristics of matrices, public health data, and the logistics, cost, and complexities involved in managing large challenge-strain collections. We conclude that food safety is served best when laboratories conduct method application studies for new matrices with one or more appropriately stressed members of a small, conveniently managed panel of challenge strains. However, if stakeholders have clear knowledge of a strong link between the matrix and a particular strain of concern, that would be a reason to favor acquisition and use of that strain. The worst approach is to not conduct application studies because of perceived limitations in accessing one or more highly specific strains.

OVERVIEW

Analytical methods for detecting microbial pathogens must be validated. Method validation is defined in International Standards Organisation (ISO) 16140-2 *(43)* as "the establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specific intended use are fulfilled." Validation is a rigorous experimental process that examines inclusivity, exclusivity, sensitivity, and robustness.

Inclusivity testing determines a method's ability to detect strains or isolates of the target pathogen and should cover the genetic, serological, and biochemical diversity of the target. Certification bodies such as the Association of Official Analytical Collaboration (AOAC) International, Association Française de Normalisation, and others typically require 50 strains of the target pathogen for inclusivity testing. However, in the case of *Salmonella*, there are more than 2,500 recognized serotypes; therefore, the inclusivity requirement increases to at least 100 serotypes *(3)*. At the time of writing, AOAC International is asking for these representative serotypes to include three strains from each of the *Salmonella enterica* subspecies and *Salmonella bongori (36)*. Selection of suitable strains for method validation is critical to understanding method limitations *(8, 10)*.

Once the method is formally validated and accredited, its performance in an individual laboratory should be verified before use. Method verification is defined in ISO 16140-3 *(44)* as "the demonstration that a validated method performs, in the user's hands, according to the method specification determined in the validation study and is fit for its intended purpose." Verification within a single laboratory may include only a single strain *(44, 83)*.

The use of stressed microorganisms during validation of microbiological methods is intended to mimic the sublethal stress that may occur as a result of product manufacturing or environmental management procedures and thus the ability of the method to recover and detect low numbers of these viable organisms. ISO 16140-2 *(43)* prescribes stresses related to processing conditions, including heat (50°C for 15 min), cold or freezing, pH, and low water activity (a_w), along with resource competition from a high intrinsic background microflora. Guidelines for AOAC International certification *(3)* have similar requirements. Parameters for imposing stress on the challenge strains may

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[Selection of Pathogen Strains for Evaluating Rapid Pathogen Test Methods Applied to New Matrices]

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Questions

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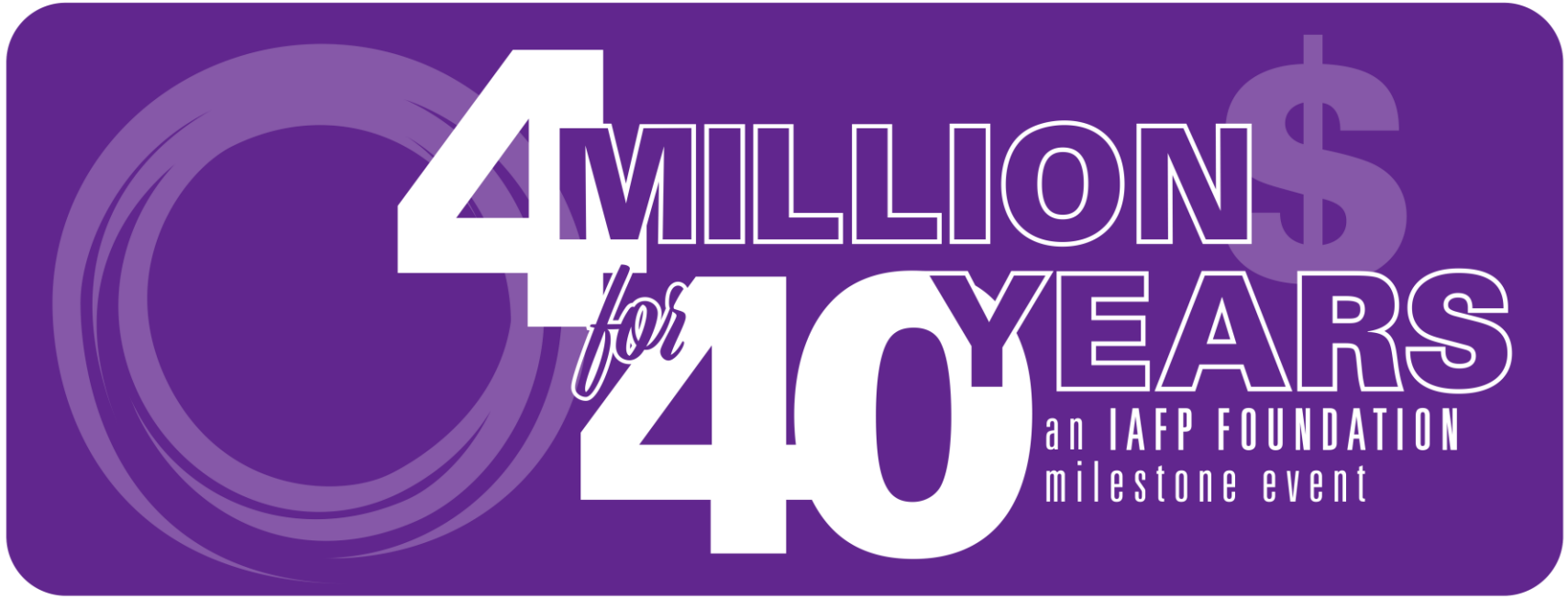

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