International Association for Food Protection WEBINAR

Matrix Additions Part 2: Alternative Approaches for Rapid Pathogen Detection Methods

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Moderator: Jaya Sundaram, WTI, Inc.

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International Association for Food Protection

Presenters

Amanda is the Technical Director of Food Safety at Midwest Laboratories, an ISO 17025 Accredited Laboratory in Omaha, NE. Here she oversees the operational and technical direction of the microbiology and food chemistry laboratories and ensures compliance of analytical and microbiological testing activities with applicable regulatory guidance. Amanda and the microbiology R&D team provide custom projects to clients including method verifications and validations, matrix extension studies, USP suitability/ preparatory testing, and product and process validations. She has her M.S. in Biology from the University of Nebraska at Omaha, with a research focus in molecular genetics and genome technology.

Nisha is a Senior Scientific Affairs Manager at Hygiena. She is responsible for overseeing third-party and internal product validations, certification maintenance/renewals, and global accreditations for multiple divisions within the company. Since joining Qualicon in 2001, Nisha has held a variety of positions within the R&D, Sales and Marketing, Applications and Validations groups. Nisha earned a Bachelor of Science degree in Medical Technology with a minor in Biology from the University of Delaware, and a Master of Science degree in Microbiology from Thomas Jefferson University in Philadelphia. She is also active on the AOAC Modification Task Force and the AOAC Advisory Council.

Introduction

- Food safety is everyone's responsibility
- FSMA has ignited rapid evolution in the food industry
- Professional Development Group (PDG) may provide recommendations, producer must decide whether the specific guidance is appropriate for their circumstances
  - Guidance based on various industry perspectives from four PDG publications
- Focus on US regulated products
  - Must adhere to all international, federal, state, and local laws and regulations related to your products and business

Resources

GENERAL INTEREST PAPER

Microbiological Detection Methods — Assuring the Right Fit

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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

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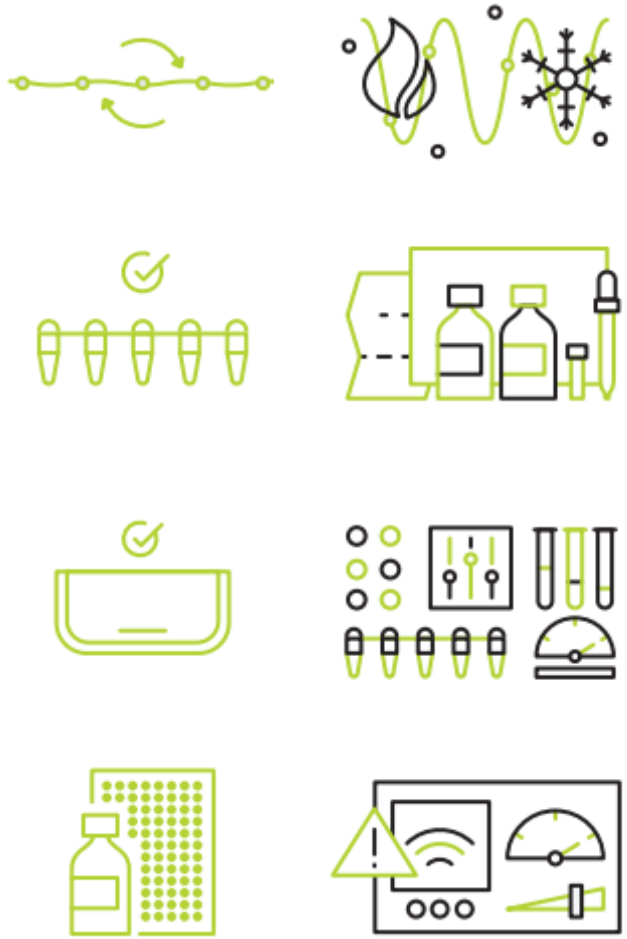
[Selection of Pathogen Strains for Evaluating Rapid Pathogen Test Methods Applied to New Matrices]

Matrix Additions Part 1: Recap

- Verification vs. Validation
- Understand the gap(s) in the scope of validation for rapid pathogen detection methods
- Risk assessment for method performance
- Food matrix grouping based on intrinsic properties
- Selecting enrichment conditions for a matrix evaluation study

Rapid Pathogen Detection Methods

- Certified and/or validated qualitative methods readily available from test kit providers for significant pathogens
- "Fully validated"= AOAC Official Method of Analysis (OMA)
  - Interlaboratory study
- Assay is developed by kit manufacturer
  - Nucleic acid-based: PCR, multiplex PCR, real-time PCR, nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP) and DNA microarray
  - Immunoassays: ELISA and lateral flow



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AOAC OMA vs. AOAC PTM

TABLE 1. Comparison between AOAC and ISO 16140-2 certification requirements for qualitative methods

Study	AOAC appendix J *(1)*		ISO 16140-2:2016 *(9)*
	PTM submission	OMA submission	
Inclusivity/exclusivity	X	X	X
Matrix suitability:[a]			
POD/dPOD	X	X	
LOD_{50}/RLOD			X
Multicomparison			X
Robustness	X		
Lot to lot consistency	X	X	
Interlaboratory/collaborative		X	X

[a]POD, probability of detection, defined as number of positive samples divided by total number of samples in a fractional recovery study; dPOD, difference between probabilities of detection of candidate and reference methods; LOD_{50}, limit of detection, the level of contamination with an expectation of 50% positive test results; RLOD, relative level of detection, the ratio of the LOD of the alternative method and the LOD of the reference method.

TABLE 2. Comparison between AOAC and ISO 16140-2 certification requirements for quantitative methods

Study	AOAC appendix J *(1)*		ISO 16140-2:2016 *(9)*
	Performance tested method	Official methods of analysis	
Inclusivity/exclusivity studies	X	X	X
Matrix suitability	X	X	
Accuracy profile			X
Relative trueness profile			X
Limit of quantification			(X)
Robustness	X		
Lot to lot consistency	X	X	
Interlaboratory/collaborative		X	X

AOAC Matrix Claim

- Scope of matrices included in the validation study & stated in the intended use (applicability statement of the method)

- Broad range of foods claim: 15 matrices from 5 categories
  - ISO 16140-2:2016, Annex A
    Classification of sample types

- Even with a broad range claim, **the specific foods tested need to be evaluated**

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

Raw milk and dairy products	Fresh produce and fruits
Heat-processed milk and dairy products	Processed fruits and vegetables
Raw meat and ready-to-cook meat products (except poultry)	Dried cereals, fruits, nuts, seeds and vegetables
Read-to-eat, ready-to-reheat meat products	Infant formula and infant cereals
Raw poultry and ready-to-cook poultry products	Chocolate, bakery products and confectionary
Read-to-eat, ready-to-reheat meat and poultry products	Multicomponent foods or meal components
Eggs and egg products (derivatives)	Pet food and animal feed
Raw and ready-to-cook fish and seafoods (unprocessed)	Environmental samples (food or feed production
Ready-to-eat, ready-to-reheat fishery products	Primary production samples (PPS)

Why are matrix evaluations needed?

Scope is limited to the matrices included in the method validation study

Example: 10 matrices included in the validation study

Kit producer may have an additional library of validated matrices

Example: 85 validated by kit supplier

<u>Tens of thousands</u> of food products on the market

Thorough validation of ALL matrices at all test portion sizes is not cost-efficient or feasible	Alternative evaluation approaches are necessary

Matrix Evaluation/ Extension Study

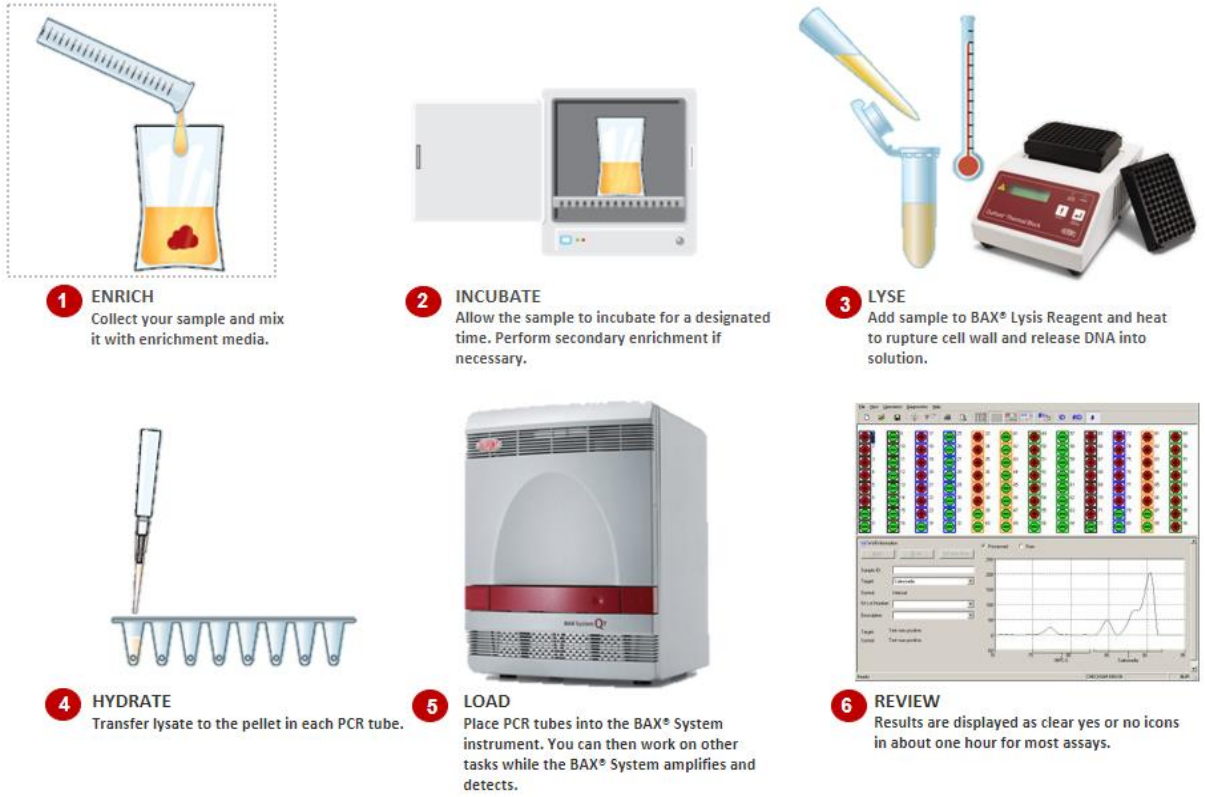
- Process by which test methods are assessed for use with a **matrix of interest**
- Ensure the method is fit-for-purpose for the end user
  - Food manufacturers and/or third-party labs
- To extend the use of a method to a new food or foods not included in the original method validation
- Larger test portion size

Method Overview

1. Enrichment
    - Sample size (test portion)
    - Enrichment media
        - Dilution ratio
        - Enrichment time and temp

2. Sample preparation (Hands-on time)

3. Detection method (Instrument)
    - Result interpretation



<u>*Each part of the method is important for accurate detection of the target analyte</u>

Considerations

- Has the method been validated for that product matrix?
  - If not, is the food category/type that has been validated close enough to your sample type?
    - Have the test portion size, enrichment dilution and incubation time and temp been validated?

Test Portion

The part of the “sample” that is actually tested by the laboratory

Composite test portions

- Lower limit of detection of the method (i.e., 1 CFU per 25 g versus 1 CFU per 375 g)
- **Rigorous evaluation is highly recommended**

"Test 375 g”

- Greater the test portion will increase the sensitivity

… but the method has only been validated at 25 g

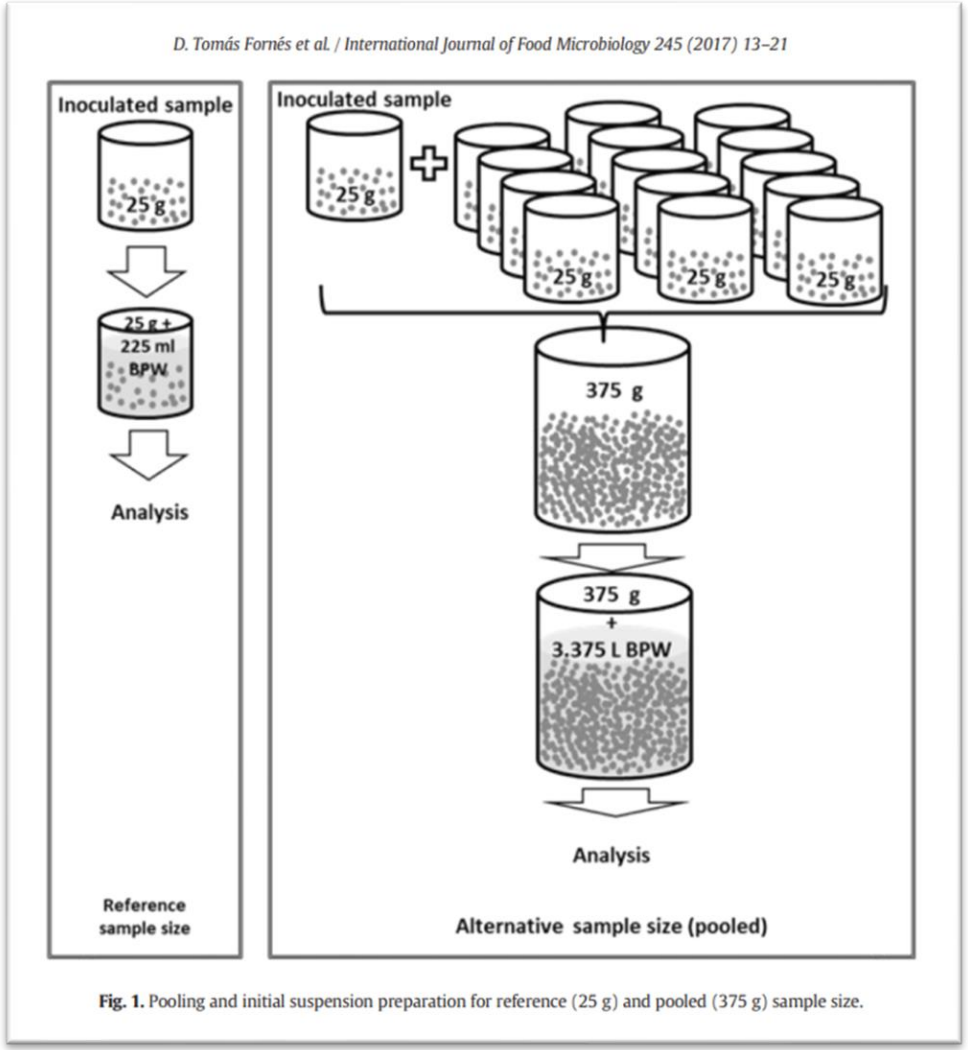


Fig. 1. Pooling and initial suspension preparation for reference (25 g) and pooled (375 g) sample size.

*don’t forget that you need a statistically valid sampling plan!

Sample Enrichment

- Foundation for detection of pathogens

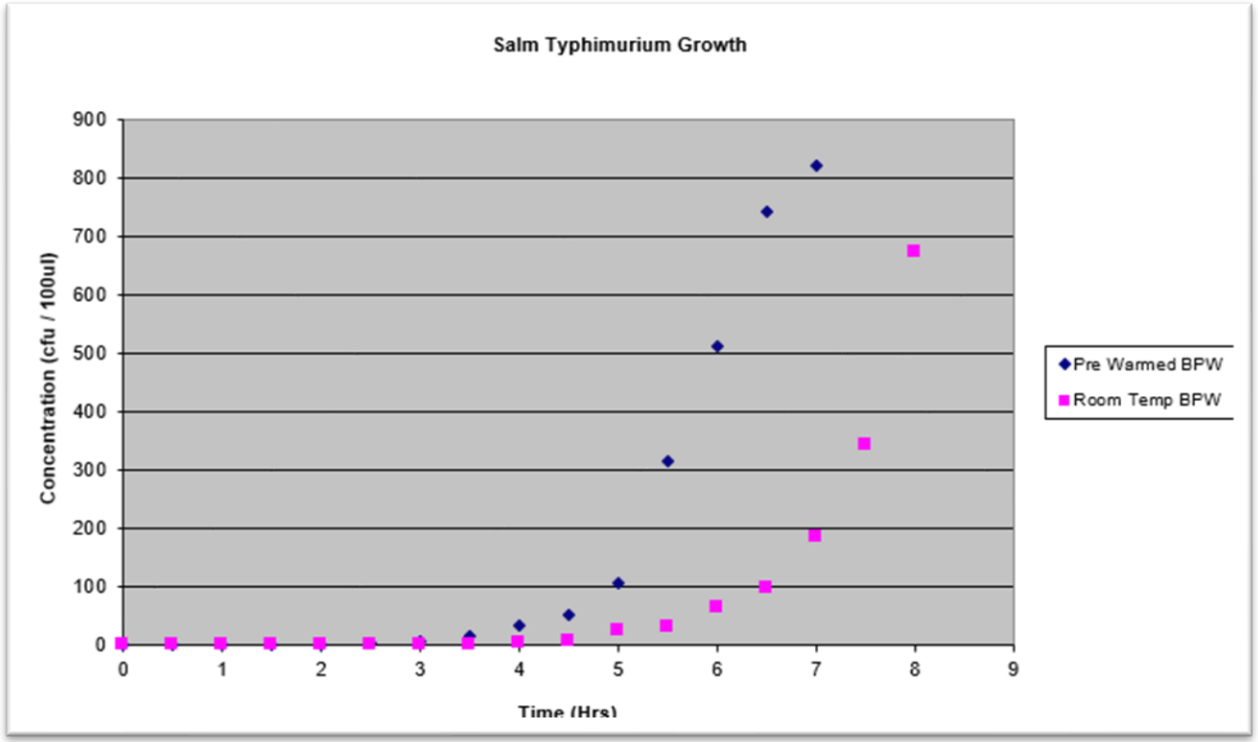
Follow protocol as validated

Media, time, temperature, dilution ratio

Example: **Reduction in enrichment dilution ratio requires validation**

375g refrigerated ground beef in 1.5L of enrichment media

- ✖ Delays in enrichment time/temp = Issues with recovery
- ➡ 45°C for pre-warm temperature



375g Test Portions

- May not be applicable to all matrices
- Example: spices at 25 g using molecular method
  - Enriched in 220 mL skim milk + 230 mL BPW (450 mL media)
  - 375 g sample = scale up 15 times
  - 6.75 Liters of Media = almost 2 gallons
  - Not cost effective or feasible

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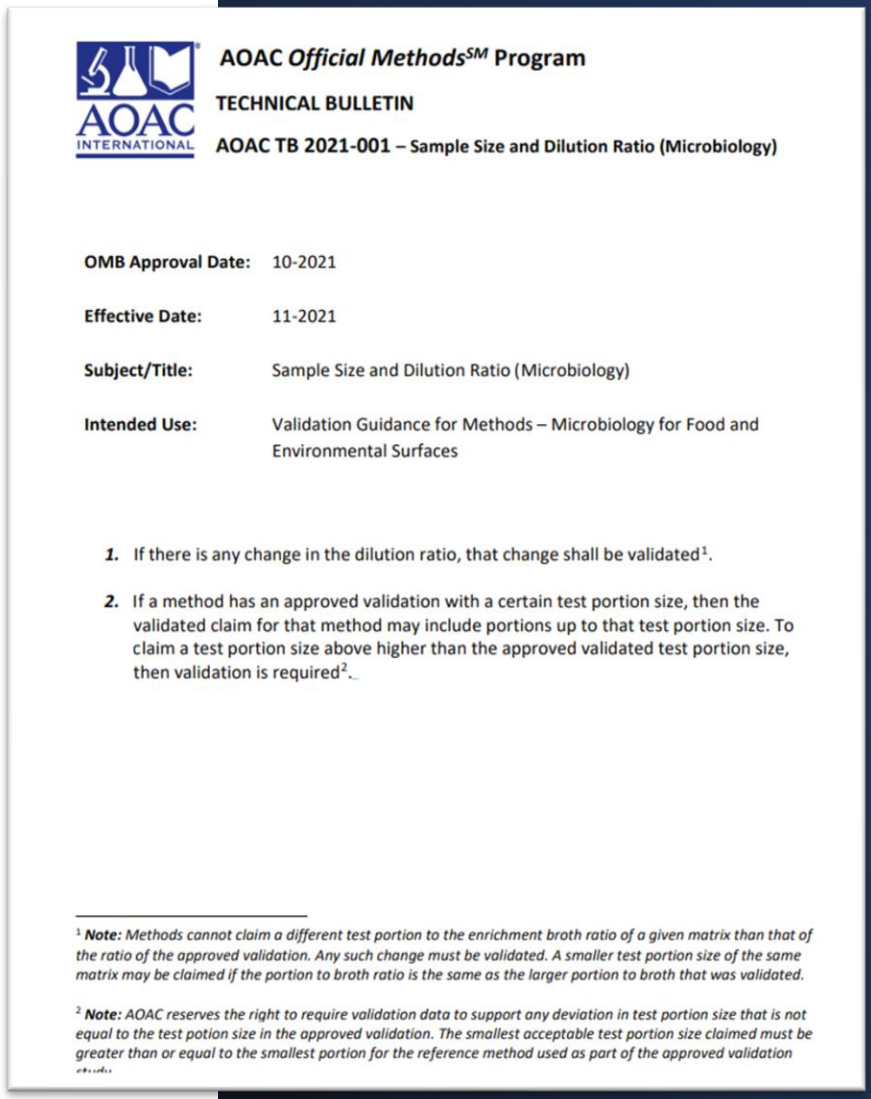
Sample Size and Dilution Ratio

AOAC TB 2023-001

1. If there is **any change in the dilution ratio**, that change shall be validated

2. If a method has an approved validation with a certain test portion size, then the validated claim for that method may include portions up to that test portion size

- **To claim a test portion size above higher than the approved validated test portion size**, then validation is required



AOAC *Official Methods*℠ Program

TECHNICAL BULLETIN

AOAC TB 2021-001 – Sample Size and Dilution Ratio (Microbiology)

OMB Approval Date: 10-2021

Effective Date: 11-2021

Subject/Title: Sample Size and Dilution Ratio (Microbiology)

Intended Use: Validation Guidance for Methods – Microbiology for Food and Environmental Surfaces

1. If there is any change in the dilution ratio, that change shall be validated[1].
2. If a method has an approved validation with a certain test portion size, then the validated claim for that method may include portions up to that test portion size. To claim a test portion size above higher than the approved validated test portion size, then validation is required[2].

[1] ***Note:*** *Methods cannot claim a different test portion to the enrichment broth ratio of a given matrix than that of the ratio of the approved validation. Any such change must be validated. A smaller test portion size of the same matrix may be claimed if the portion to broth ratio is the same as the larger portion to broth that was validated.*

[2] ***Note:*** *AOAC reserves the right to require validation data to support any deviation in test portion size that is not equal to the test potion size in the approved validation. The smallest acceptable test portion size claimed must be greater than or equal to the smallest portion for the reference method used as part of the approved validation*

Potential Inhibitors

- Antimicrobial constituents
  - Example: herbs and spices have antimicrobial or bacteriostatic properties
- Growth inhibitors
  - Example: enzymes and polyphenols
- Molecular inhibitors
  - PCR: collagen, humic acid, calcium ions, and polyphenolic compounds
- Dilution, neutralization or alternative treatment to remove inhibition

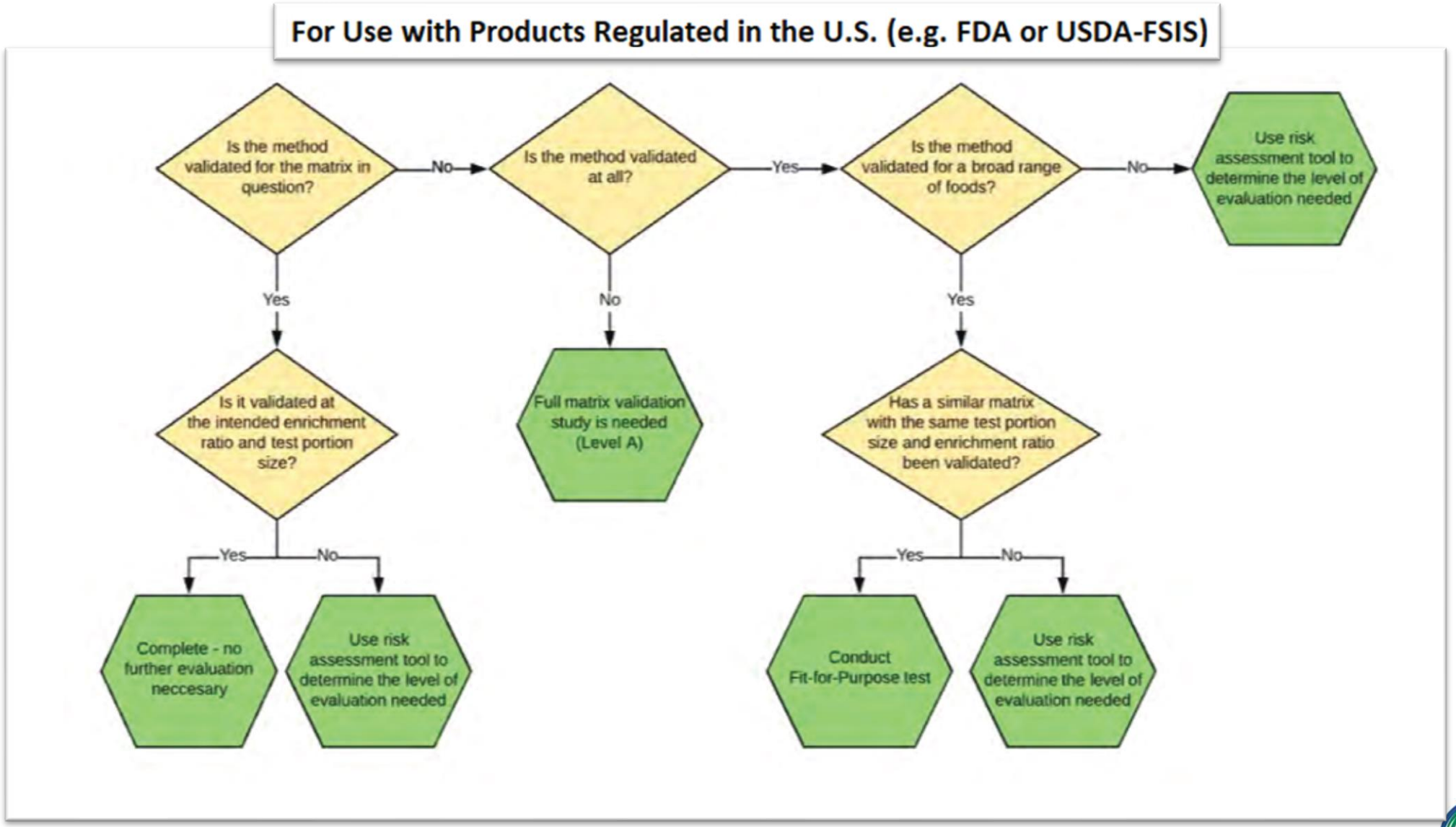
Table B.1 — Example of (food) items and its characteristics

Category	Item	Challenging characteristic
1	1	pH
2	2	Viscosity
3	3	Fat content
4	4	High background microbiota and pH
5	5	Polyphenol

Method Modifications

- For example:
  1. New matrix addition, new media enrichment/time/temperature
  2. New instrumentation
  3. Modification to reagents, manufacturing locations/process
- May or may not affect the established validated performance parameters of the original method
- No “one size fits all” rule or set of rules to govern how modifications will be addressed
  - Some may only necessitate verification
  - Other modifications may require significant validation data to support their use

Fit For Purpose



**Disclaimer: dependent on your geographical location and regulatory body*

Not included: ISO 16140-3

- Different regulatory requirements for different agencies depending on your geographical location

Microbiology of the food chain — Method validation —

Part 3:

Protocol for the verification of reference methods and validated alternative methods in a single laboratory

Microbiologie de la chaîne alimentaire — Validation des méthodes —

Partie 3: Protocole pour la vérification dans un seul laboratoire de méthodes de référence et de méthodes alternatives validées

https://vimeo.com/522329760

Table 3 — Protocols to determine $eLOD_{50}$ and number of replicates needed per inoculation level

Protocol	Inoculation level of the test portion					
	High level $9 \times LOD_{50}$ / test portion	Intermediate level $3 \times LOD_{50}$ / test portion	Low level $1 \times LOD_{50}$ / test portion	3 cfu to 5 cfu /test portion	Blank	Total number of replicates
1	1	4	4	–	1	10
2	–	3	5	–	1	9
3	–	–	–	7	1	8

NOTE The abbreviation of colony forming units is cfu.

Matrix Evaluation/ Extension Study

TABLE 4. Evaluation levels

Evaluation level	Number of spiked test portions	Inoculation level, CFU/test portion	Inoculating cells	Analysis
Full matrix validation	5	2-10	Fresh culture or heat stressed	Presumptive results compared with confirmation results and reference method to demonstrate no statistical difference between the methods
	20	0.2-2		
	5	0		
Moderate matrix evaluation	2	2-10	Fresh culture or heat stressed	Presumptive results compared with confirmation results to demonstrate no deviation in candidate method result compared to culture confirmation
	10	0.2-2		
	2	0		
Minimal matrix evaluation	1-7	20-30	Fresh culture or heat stressed	Candidate detection results for inoculated anduninoculated samples should match input
	0-1	0		

*abbreviated studies may save time and money; they do come with added limitations as a result of the reduced scope of the data obtained

Strain Selection

- Sourced from the same or similar matrix or is commonly isolated from matrix, when possible
  - Strain of interest might now be included in the list of method developers list of strains from the validation
    - For example: outbreak strain not included in inclusivity data- test to see if method detects
- Small in-house and commercial labs may not have the resources
    - Gather, identify and isolate strains or serotypes from naturally contaminated samples
    - Ability to maintain large collections

<u>= Utilize commercially available standardized strains</u>

- Rely on the inclusivity data produced during a method's validation and/or accreditation (see AOAC certificate)

TABLE 2. *Salmonella* serotypes associated with chocolate outbreaks

Salmonella serotype	Alternate strain source	Alternate strain origin	Vehicle	Outbreak date	Reference
Durham	NA[a]	NA	Cocoa powder	1972	(30)
Eastbourne	NCTC 5771	Kauffmann F State Serum Institute, Copenhagen	Chocolate products	1974	(16, 72)
Napoli	NCTC 6853	Italian food handler	Chocolate bars	1982	(31, 34)
Nima	NA	NA	Chocolate coins	1985–1986	(38)
Typhimurium	ATCC 14028	Heart and liver from 4-week-old chickens	Chocolate products	1987	(48)
Oranienburg	ATCC 9239	Outbreak of food poisoning at an Illinois state hospital	Chocolates	2001–2002	(90)
Montevideo	ATCC BAA-710	Human clinical specimen: salmonellosis from tomatoes, 1993	Chocolate tablets	2006	(23)

[a]NA, not applicable.

TABLE 3. Common *Salmonella* serovars available from ATCC on 2 February 2021, with geographical and other source indications

Serovar	Isolates in the ATCC catalog	Geographical association						Other association		
		SE US (86)	Maryland (66)	Louisiana (53)	Seattle (62)	India (50)	Egypt (22)	General	Clinical	Common
Typhimurium	61	X	X		X	X	X	X	X	X
Enteritidis	10	X	X	X	X	X	X	X	X	X
Thompson	4			X						X
Montevideo	2			X		X			X	
Newport	2					X			X	
Pullorum	2					X		X		
Senftenberg	2				X			X	X	X
Braenderup	2			X						
Cerro	2								X	X
Anatum	2									X
Javiana	2									X
Virchow	1							X		
Dublin	1								X	X
Worthington	1									X
London	1									X
Muenchen	1									X
Bredeney	1								X	
Hadar	1									X
Mississippi	1									X

Spiking Procedures for Minimal/ Moderate Matrix Evaluations

Liquid inoculum often used

- Serial dilutions of overnight growth to achieve the targeted inoculation level
- Purchase quantified reference cultures

Best practice is to use appropriately stressed cultures when possible

- Example 1: dry powders + lyophilized cultures
- Example 2: ready-to-eat deli meat + heat stressed culture
- Example 3: frozen vegetables + heat stressed, then frozen culture
- Example 4: perishable items + unstressed culture

Using the Risk-Assessment Tool

- Follow along: link sent in webinar materials
- Tool is accessible through the International Association for Food Protection Applied Laboratory Methods Professional Development Group homepage: https://www.foodprotection.org/upl/downloads/library/matrix-evaluation-level-assessment-tool.xlsx

Example #1: *Salmonella* in Hard-Boiled Eggs

- AOAC-OMA Immunoassay validated for a **broad range of foods**
- Food category: Eggs and egg products (derivatives) ✓

Liquid eggs	*Salmonella enterica ser.* Enteritidis	25g	FDA-BAM Ch. 5
Powdered eggs	*Salmonella enterica ser.* Choleraesuis	25g	FDA-BAM Ch. 5

Validated matrices

VS.

Matrix extension

Method Parameters:

1. Test portion= 25g
2. Enrichment media= BPW
3. Dilution ratio= 1:10
4. Time= 18-24 hours
5. Temp= 35°C

Matrix Evaluation Level Assessment Tool

Home

For Use with Products Regulated in the U.S. (e.g. FDA or USDA-FSIS)

Estimate of need for validation or verification

No.	Question	Action on response	
		Yes	No
0	Is the method validated for this specific matrix, test portion size and enrichment ratio?	**Fit for purpose. Free to use without further evaluation**	Go to 1
1	Is the method validated at all?	Go to 2	Conduct a single laboratory method validation study
2	Is the method validated for a broad range of foods*?	Go to 3	Go to 5
3	Has a similar matrix with the same test portion size and enrichment ratio been validated?	Go to 4	Go to 5
4	Perform Fit-for-Purpose Test.	See "Fit-for-Purpose Test Options" Tab	
5	Conduct risk assessment to determine level of evaluation required.	See "Risk Assessment" Tab	

*Broad range of foods is defined as at least 15 unique matrices across three food categories. Food categories can be found in ISO 16140-2 Annex A.

https://www.foodprotection.org/upl/downloads/library/matrix-evaluation-level-assessment-tool.xlsx

Fit-For-Purpose Study

Home

Recommended Fit-for-Purpose Study Design:

Option	Method	Inoculation level (CFU / test portion)	Number of test portions	Analysis
Fit-for-Purpose Assay	Candidate method	20-30	3	See Fit-for-Purpose Result Analysis Tab
		0	3	
	Reference method	20-30	3	

Additional Fit-for-Purpose Study Designs

Option	Inoculation level (CFU / test portion)	Method	Number of test portions	Expected Result	Notes
FDA 5.1.1 Matrix Verification FDA Emergency Use	20-30	Candidate method	7	7/7 positive	Once 7/7 OR 19/20 spikes are recovered consider matrix verified: no further evaluation necessary. May be performed in parallel with test samples, results of which will be invalidated if spikes are not recovered.
			20	19/20 positive	
USP style Suitability Test	20-30	Candidate method	1	1/1 positive	

https://www.foodprotection.org/upl/downloads/library/matrix-evaluation-level-assessment-tool.xlsx

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Fit-For-Purpose Study Results

Outcome	Method	Detection Results (Correct result / Number tested)		Explanation and response
		Inoculated (expected positives)	Non-inoculated controls (expected negatives)	
1	Candidate method	3/3	3/3	Candidate method is suitable for use with this matrix. Further evaluation is not needed but may be conducted if desired.
	Reference method	3/3	N/A	
2	Candidate method	2/3	3/3	Lack of detection in one of the inoculated samples suggests matrix interference with the assay or pathogen growth issues. Consider dilution, neutralization or alternative treatment to remove inhibition, then repeat Fit-for-Purpose test. If still fails, consider alternative method / platform.
	Reference method	3/3	N/A	
3	Candidate method	3/3	3/3	Suggests matrix inhibition during enrichment with reference method and/or a candidate method that is more sensitive than the reference method. Another possibility is that the inoculum level is much lower than intended. However, candidate method passes Fit-for-Purpose Test and can be used.
	Reference method	<3/3	N/A	
4	Candidate method	<3/3	3/3	Suggests matrix interference with enrichment. Consider dilution or preenrichment medium additive (e.g. Tween) to mitigate matrix interference, then repeat Fit-for-Purpose Assay. If still fails, consider alternative method / platform.
	Reference method	<3/3	N/A	
5	Candidate method	<3/3	<3/3	A "false positive" combined with a "false negative" suggests the samples could have been switched. Investigate. If confirmed, address laboratory procedures and rerun the Fit-for-Purpose Assay.
	Reference method	3/3	N/A	
6	Candidate method	3/3	<3/3	Suggests matrix was contaminated before use, or was cross-contaminated in the laboratory. Investigate including use of confirmatory testing: - If cross-contaminated in the laboratory, address laboratory procedures and rerun Fit-for-Purpose Assay, - If investigation excludes laboratory contamination, suggesting the matrix was contaminated as tested, consider your reporting responsibilities.
	Reference method	3/3	N/A	

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Example #2: *L. monocytogenes* in Vanilla Pudding

- AOAC-OMA LAMP assay validated for queso fresco, **vanilla ice cream**, 4% milk fat cottage cheese, 3% chocolate whole milk
- Food category: Heat-processed milk and dairy products ✓

Validated matrix

VS.

Matrix extension

Method Parameters:

1. Test portion= 25g
2. Enrichment media= UVM Broth
3. Dilution ratio= 1:10
4. Time= 24-28 hours
5. Temp.= 35°C

Matrix Evaluation Level Assessment Tool

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Home

For Use with Products Regulated in the U.S. (e.g. FDA or USDA-FSIS)

Estimate of need for validation or verification

No.	Question	Action on response	
		Yes	No
0	Is the method validated for this specific matrix, test portion size and enrichment ratio?	**Fit for purpose. Free to use without further evaluation**	Go to 1
1	Is the method validated at all?	Go to 2	Conduct a single laboratory method validation study
2	Is the method validated for a broad range of foods*?	Go to 3	Go to 5
3	Has a similar matrix with the same test portion size and enrichment ratio been validated?	Go to 4	Go to 5
4	Perform Fit-for-Purpose Test.	See "Fit-for-Purpose Test Options" Tab	
5	Conduct risk assessment to determine level of evaluation required.	See "Risk Assessment" Tab	

*Broad range of foods is defined as at least 15 unique matrices across three food categories. Food categories can be found in ISO 16140-2 Annex A.

https://www.foodprotection.org/upl/downloads/library/matrix-evaluation-level-assessment-tool.xlsx

for Food Protection

Example #2: *L. monocytogenes* in Vanilla Pudding

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Risk assessment for suggested evaluation level

No.	Question	Yes		No		Your score
		Score	If Yes, Next Step:	Score	If No, Next Step:	
5A	Does the matrix fit into an AOAC or ISO 16140-2:2016 group (and subcategory - if listed) containing a validated representative example?	0	Go to 5B	10	Go to 7A	**0**
5B	Is the similar representative example (from 5A) validated at the intended test portion size, or larger?	0	Go to 5C	0	Go to 5D	**0**
5C	Is the similar representative example (from 5A) validated at the intended enrichment ratio?	0	Go to 6A	0	Go to 5D	**0**
5D	Does the matrix have a high risk association with the target analyte?	10	Go to 6A	4	Go to 6A	**4**
6A	Does the new matrix contain inclusions (e.g. ice cream with almonds vs ice cream)?	0	Go to 6B	0	Go to 7A	
6B	Is the matrix inclusion representative of a matrix already validated by the method?	0	Go to 6C	5	Go to 7A	
6C	Is the new matrix inclusion enrichment procedure the same as the validated representative example (e.g. do almonds have the same enrichment procedure as ice cream?)	0	Go to 7A	2	Go to 7A	
7A	Does the matrix or any inclusions present have known ability to inhibit growth of the organism in enrichment or method detection chemistry?	0	Go to 7B	0	Assessment is complete	0
7B	Has a Fit-for-Purpose Test been performed?	0	Go to 7C	5	Assessment is complete	
7C	Were the results of the Fit-for-Purpose Test acceptable? (e.g. recovery of all inoculated samples)	0	Assessment is complete	Ignore Score Total	Refer to Fit-for-Purpose Result Analysis Tab	
					Total:	**4**

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Example #2: *L. monocytogenes* in Vanilla Pudding

Risk Assessment Score: 4

Return to risk assessment

Test Method Evaluation Levels

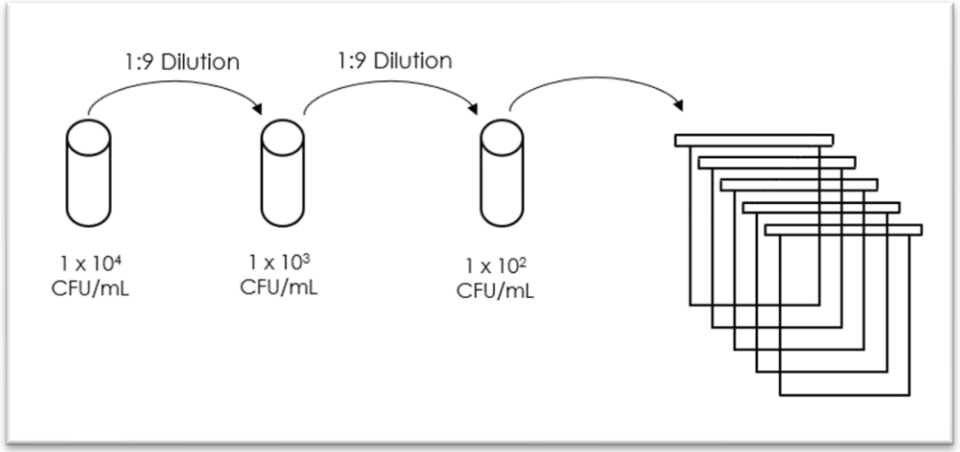
Test method evaluation levels and associated study design schemes determined by risk assessment scoring system.

Level	Inoculation level	Inoculation level, CFU	Number of test portions	Inoculating Cells	Analysis	When to Use:	Notes
Full Matrix Validation	High	20-30	5	Fresh culture or stressed e.g. by heat, drying or freezing	Presumptive results compared to reference method. Equivalent results required as determined by dPOD calculations.	**Risk assessment score of 13+**	If no published validation exists, validation must demonstrate the inclusivity and exclusivity of the method. If a narrow published validation includes inclusivity and exclusivity studies, these need not be repeated. "Low " inoculation level should give 25 - 75 % positivity rate.
	Low	0.2-5	20				
	None	0	5				
Moderate Matrix Evaluation	High	20-30	2	Fresh culture or stressed e.g. by heat, drying or freezing	Presumptive results compared to reference method.	**Risk assessment score of 6-12**	
	Low	0.2-5	10				
	None	0	2				
Minimal Matrix Evaluation	High	20-30	1 - 7	Fresh culture or stressed e.g. by heat, drying or freezing	100 % correct response	**Risk assessment score of 2-5**	
	None	0	0-1				

All matrices should be processed at intended-use test portion size and enrichment protocol

"Minimal Matrix Evaluation"

- Recommended for a risk assessment score of **2 to 5**
- Screen for obvious detection issues
  1-7 spiked test portions and
  0-1 uninoculated samples
- Test portion spiked with < 30 CFU of the target analyte
  - 7/7 spikes show recovery of the organism



- **Matrix spikes yield positive results = verified**
- Uninoculated sample(s) do not have cross-reaction (false positive)

Example #3: *L. monocytogenes* in Strawberry Ice Cream

Validated matrix

VS.

Matrix extension

Inclusion= strawberry pieces

Risk assessment for suggested evaluation level

No.	Question	Yes		No		Your score
		Score	If Yes, Next Step:	Score	If No, Next Step:	
5A	Does the matrix fit into an AOAC or ISO 16140-2:2016 group (and subcategory - if listed) containing a validated representative example?	0	Go to 5B	10	Go to 7A	**0**
5B	Is the similar representative example (from 5A) validated at the intended test portion size, or larger?	0	Go to 5C	0	Go to 5D	**0**
5C	Is the similar representative example (from 5A) validated at the intended enrichment ratio?	0	Go to 6A	0	Go to 5D	**0**
5D	Does the matrix have a high risk association with the target analyte?	10	Go to 6A	4	Go to 6A	
6A	Does the new matrix contain inclusions (e.g. ice cream with almonds vs ice cream)?	0	Go to 6B	0	Go to 7A	**0**
6B	Is the matrix inclusion representative of a matrix already validated by the method?	0	Go to 6C	5	Go to 7A	**5**
6C	Is the new matrix inclusion enrichment procedure the same as the validated representative example (e.g. do almonds have the same enrichment procedure as ice cream?)	0	Go to 7A	2	Go to 7A	
7A	Does the matrix or any inclusions present have known ability to inhibit growth of the organism in enrichment or method detection chemistry?	0	Go to 7B	0	Assessment is complete	0
7B	Has a Fit-for-Purpose Test been performed?	0	Go to 7C	5	Assessment is complete	**5**
7C	Were the results of the Fit-for-Purpose Test acceptable? (e.g. recovery of all inoculated samples)	0	Assessment is complete	Ignore Score Total	Refer to Fit-for-Purpose Result Analysis Tab	
					Total:	**10**

Example #3: *L. monocytogenes* in Strawberry Ice Cream

Risk Assessment Score: 10

Return to risk assessment

Test Method Evaluation Levels

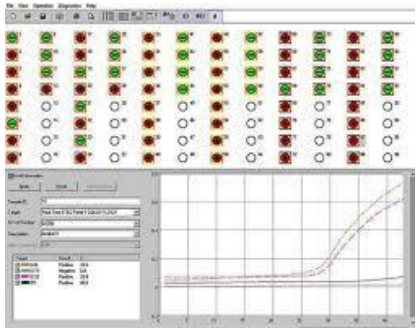
Test method evaluation levels and associated study design schemes determined by risk assessment scoring system.

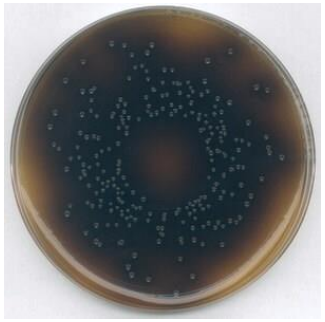
Level	Inoculation level	Inoculation level, CFU	Number of test portions	Inoculating Cells	Analysis	When to Use:	Notes
Full Matrix Validation	High	20-30	5	Fresh culture or stressed e.g. by heat, drying or freezing	Presumptive results compared to reference method. Equivalent results required as determined by dPOD calculations.	**Risk assessment score of 13+**	If no published validation exists, validation must demonstrate the inclusivity and exclusivity of the method. If a narrow published validation includes inclusivity and exclusivity studies, these need not be repeated. "Low " inoculation level should give 25 - 75 % positivity rate.
	Low	0.2-5	20				
	None	0	5				
Moderate Matrix Evaluation	High	20-30	2	Fresh culture or stressed e.g. by heat, drying or freezing	Presumptive results compared to reference method.	**Risk assessment score of 6-12**	
	Low	0.2-5	10				
	None	0	2				
Minimal Matrix Evaluation	High	20-30	1 - 7	Fresh culture or stressed e.g. by heat, drying or freezing	100 % correct response	**Risk assessment score of 2-5**	
	None	0	0-1				

All matrices should be processed at intended-use test portion size and enrichment protocol

“Moderate Matrix Evaluation”

- Recommended for a risk assessment score of **6 to 12**
  - 2 high-level 2-10 CFU/test portion
  - 10 low-level (fractional) 0.2-2 CFU/test portion
  - 2 uninoculated test portions
- Paired results align with cultural confirmation
- No false positive or false negative results





Example #4: *E. coli* O157:H7 in Flour

- Real-Time PCR Method AOAC PTM validated for: Raw beef products, raw milk, spinach and lettuce

RISKS:

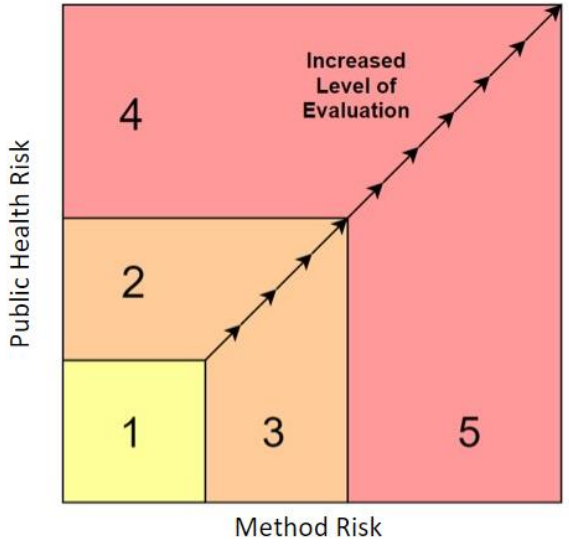
➡ Food Category not validated

Fresh produce and fruits

VS.

Dried cereals, fruits, nuts, seeds and vegetables

➡ Matrix associated with O157 outbreaks



Alternative Approaches for Qualitative Microbiological Method Matrix Additions - International Association for F

Example #4: *E. coli* O157:H7 in Flour

Risk assessment for suggested evaluation level

No.	Question	Yes		No		Your score
		Score	If Yes, Next Step:	Score	If No, Next Step:	
5A	Does the matrix fit into an AOAC or ISO 16140-2:2016 group (and subcategory - if listed) containing a validated representative example?	0	Go to 5B	10	Go to 7A	10
5B	Is the similar representative example (from 5A) validated at the intended test portion size, or larger?	0	Go to 5C	0	Go to 5D	
5C	Is the similar representative example (from 5A) validated at the intended enrichment ratio?	0	Go to 6A	0	Go to 5D	
5D	Does the matrix have a high risk association with the target analyte?	10	Go to 6A	4	Go to 6A	
6A	Does the new matrix contain inclusions (e.g. ice cream with almonds vs ice cream)?	0	Go to 6B	0	Go to 7A	
6B	Is the matrix inclusion representative of a matrix already validated by the method?	0	Go to 6C	5	Go to 7A	
6C	Is the new matrix inclusion enrichment procedure the same as the validated representative example (e.g. do almonds have the same enrichment procedure as ice cream?)	0	Go to 7A	2	Go to 7A	
7A	Does the matrix or any inclusions present have known ability to inhibit growth of the organism in enrichment or method detection chemistry?	0	Go to 7B	0	Assessment is complete	0
7B	Has a Fit-for-Purpose Test been performed?	0	Go to 7C	5	Assessment is complete	5
7C	Were the results of the Fit-for-Purpose Test acceptable? (e.g. recovery of all inoculated samples)	0	Assessment is complete	Ignore Score Total	Refer to Fit-for-Purpose Result Analysis Tab	
				Total:		**15**

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Example #4: *E. coli* O157:H7 in Flour

Risk Assessment Score: 15

Return to risk assessment

Test Method Evaluation Levels

Test method evaluation levels and associated study design schemes determined by risk assessment scoring system.

Level	Inoculation level	Inoculation level, CFU	Number of test portions	Inoculating Cells	Analysis	When to Use:	Notes
Full Matrix Validation	High	20-30	5	Fresh culture or stressed e.g. by heat, drying or freezing	Presumptive results compared to reference method. Equivalent results required as determined by dPOD calculations.	**Risk assessment score of 13+**	If no published validation exists, validation must demonstrate the inclusivity and exclusivity of the method. If a narrow published validation includes inclusivity and exclusivity studies, these need not be repeated. "Low " inoculation level should give 25 - 75 % positivity rate.
	Low	0.2-5	20				
	None	0	5				
Moderate Matrix Evaluation	High	20-30	2	Fresh culture or stressed e.g. by heat, drying or freezing	Presumptive results compared to reference method.	**Risk assessment score of 6-12**	
	Low	0.2-5	10				
	None	0	2				
Minimal Matrix Evaluation	High	20-30	1 - 7	Fresh culture or stressed e.g. by heat, drying or freezing	100 % correct response	**Risk assessment score of 2-5**	
	None	0	0-1				

All matrices should be processed at intended-use test portion size and enrichment protocol

"Full Matrix Validation"

- Recommended for a risk assessment score of **13+** using the Matrix Evaluation Level Assessment Tool
- **Will be covered in Part 3**
- Define the appropriate method protocol for the new matrix
- Parameters based on **AOAC Appendix J- 4.1.3 Matrix Study**

Evaluation level	Number of spiked test portions	Inoculation level, CFU/test portion	Inoculating cells	Analysis
Full matrix validation	5	2-10	Fresh culture or heat stressed	Presumptive results compared with confirmation results and reference method to demonstrate no statistical difference between the methods
	20	0.2-2		
	5	0		

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Questions

Submit questions into the chat

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December 13, 2023, 11:00 AM Building a Culture – The Tools and Tips You Need to Succeed

December 14, 2023, 9:00 AM Impact of Water Use and Reuse in Food Production and Processing on Food Safety at the Consumer Phase: Focus on the Fresh Fruit and Vegetable Products Sector

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