FDA U.S. FOOD & DRUG ADMINISTRATION

Memorandum

To: Foods Program Governance Board
From: FDA Foods Program Regulatory Science Steering Committee (RSSC)
Date: October 17, 2019
Subject: Guidelines for the Validation of Microbiological Methods for the FDA Foods Program, 3rd Edition

The FDA Foods Program Regulatory Science Steering Committee (RSSC), made up of representatives from the Center for Food Safety and Applied Nutrition (CFSAN), the Center for Veterinary Medicine (CVM), the Office of Regulatory Affairs (ORA), the National Center for Toxicological Research (NCTR), and the Office of the Chief Scientist of the FDA, is charged with the task of prioritizing, coordinating and integrating human food- and animal food-related science and research activities across the operating units of FDA's Foods Program.

As a regulatory agency tasked with ensuring the safety of the nation's food supply, it is imperative that the laboratory methods needed to support regulatory compliance, investigations and enforcement actions meet the highest analytical performance standards appropriate for their intended purposes. Development of standardized validation requirements for all regulatory methods used in our laboratories to detect chemical and radiological contaminants, as well as microbial pathogens, is a critical step in ensuring that we continue to meet the highest standards possible.

The attached document, now formally adopted by the RSSC, updates and renews the requirements that must be fulfilled in the evaluation of microbiological methods to be used in our testing laboratories and supersedes the prior guidelines. These updated guidelines are posted on FDA's Foods Program Methods website. Please share these microbiological methods validation guidelines with anyone who may be conducting or supervising microbiological methods validation projects or otherwise needs to be aware of these updated requirements.

As one of the hierarchical committees under the RSSC, the Microbiological Methods Validation Subcommittee (MMVS) is charged with providing guidance and oversight to all validation studies and is principally responsible for the content of these Guidelines, with input from the Microbiology Research Coordination Group (MRCG) and associated Technical Advisory Groups. Additional questions and comments about the Guidelines may be directed to the MMVS or MRCG.

Thank you,

Selen A. Stromgren -S

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Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds

Edition 3.0

U.S. Food and Drug Administration Foods Program

October 2019

ACKNOWLEDGMENT

The first edition of these guidelines was published in 2019 at the request of the US FDA Foods Program. In cooperation with members of the Foods Program Regulatory Science Steering Committee, direct input, review, and consent were provided by the following FDA research and regulatory offices:

Center for Food Safety and Applied Nutrition

Office of Regulatory Science
Office of Food Safety
Office of Applied Research and Safety Assessment

Center for Veterinary Medicine
Office of Research
Office of New Animal Drug Evaluation

Office of Regulatory Affairs
Office of Regulatory Science

APPROVAL PAGE

This document is approved by the FDA Foods and Veterinary Medicine (FVM) Regulatory Science Steering Committee (RSSC). The **FVM RSSC Project Manager is responsible for updating the document as change requirements are met, and disseminating updates to the RSSC and other stakeholders, as required.**

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Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds
Edition 3.0

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1.0 INTRODUCTION

1.1 Purpose

The Foods Program within the U.S. Food & Drug Administration is responsible for ensuring the safety of the nation's food and feed supply. FDA accomplishes this through education; inspection; data collection; standards setting; prompt investigation of outbreaks; and, enforcement actions when appropriate. The effectiveness of the FVM Enterprise is highly dependent on the quality and performance of the laboratory methods used within the FDA. To ensure that all laboratory methods meet the highest analytical standards possible for their intended purpose, the FDA Regulatory Science Steering Committee (RSSC) has established these criteria by which all FVM microbiological methods shall be evaluated and validated.

1.2 Scope

These criteria apply to all FDA laboratories that develop and participate in the validation of analytical food and feed methods for Agency-wide implementation in a regulatory capacity. This includes all research laboratories, and ORA labs where analytical methods may be developed or expanded for potential regulatory use. At the time of final approval by the RSSC, this document will supersede all other intra-agency documents pertaining to food- and feed-related method validation criteria for microbial analytes. In addition, this guidance is a forward-looking document; the requirements described here will only apply to ***newly***-developed methods and those for which significant modifications have been made to an existing method. Once a method has been validated, it can be implemented by other laboratories following the method verification process.

1.3 Administrative Authority and Responsibilities

All criteria established in this document for analytical method validation have been adopted and approved by the RSSC. As stated in the Methods Development, Validation and Implementation Program SOP (APPENDIX 3), The Methods Validation Subcommittee (MVS) will have oversight responsibility for all validation studies (See Section 2.2.1).

1.4 The Method Validation Subcommittee

Under the authority of the SRSC, a Microbiology Methods Validation Subcommittee (MMVS) will oversee all microbiology method validation concerns. The MMVS is governed by the organizational structure, roles and responsibilities as detailed in its charter (See APPENDIX 2). Briefly, the MMVS will oversee and coordinate – in collaboration with the originating laboratory – all collaborative laboratory validation studies (planning and implementation) for microbiological methods developed within the FDA FVM Enterprise to support regulatory analytical needs. This includes the evaluation of Single Laboratory Validation (SLV) results and the evaluation of any subsequent collaborative validation study plan. Unless otherwise stated, most correspondence between the method developer(s) and the MMVS will be by email using the following address: Microbiology.MVS@fda.hhs.gov.

1.5 General Responsibility of the Originating Laboratory

It is the responsibility of the originating (developing) laboratory to ensure proper adherence to all criteria described in the document. The originating laboratory must work in close consultation with the MMVS and/or its designated Technical Advisory Group (TAG) throughout the collaborative laboratory validation process. It is always a good idea to have the MMVS review validation protocols early in the validation process. It will be the responsibility of the originating laboratory to include their respective QA/QC manager in all aspects of the validation process and to ensure proper adherence to all criteria described in this document.

1.6 Method Validation Definition

Method validation is a process by which a laboratory confirms by examination, and provides objective evidence, that the particular requirements for specific uses are fulfilled. It serves to demonstrate that the method can detect and identify an analyte or analytes:

- In one or more matrices to be analyzed.
- In one or more instruments or platforms.
- With a demonstrated sensitivity, specificity, accuracy, trueness, reproducibility, ruggedness and precision to ensure that results are meaningful and appropriate to make a decision.
- Reliably for its intended purpose. Intended purpose categories include, but may not be limited to emergency/contingency operations; rapid screening and high throughput testing; and confirmatory analyses.
- After the method developer has conducted experiments to determine or verify a number of specific performance characteristics that serve to define and/or quantify method performance.

1.7 Applicability

This document establishes evaluation criteria for methods to detect, identify, and quantify all microbial analytes that may now be, or have the potential to be associated with foods and feeds *i.e.* any microbiological organism of interest (target organism) or the genetic material *i.e.* DNA, RNA, toxins, antigens, or any other product of these organisms. If not specifically identified, all information contained in the accompanying tables should be extrapolated to the microbial analyte of interest. Such applicable areas of methods development and evaluation include, but are not limited to, the following:

- Qualitative assays *i.e.* detection assays
- Quantifiable assays *i.e.* real-time PCR
- Analyte-specific
    - Bacteriological, *e.g.*
        - *Salmonella* spp.
        - Pathogenic *Escherichia coli*
        - *Listeria monocytogenes*

        - *Shigella* spp.
        - *Vibrio* spp.
        - *Campylobacter* spp.
    - Microbial toxins (excluding marine biotoxins. See Chemistry method validation guidelines)
    - Viral pathogens, *e.g.*
        - Hepatitis A virus
        - Norovirus
        - Enterovirus
    - Parasitic protozoan pathogens, *e.g.*
        - *Cryptosporidium*
        - *Cyclospora cayetanensis*
    - Indicator organisms
- Bioengineered analytes, *e.g.*
    - Genetically-modified foods (GMOs)
- Applications
    - Pre- and selective enrichment
    - Microbial analyte recovery and concentration
    - Screening, high-throughput, confirmation
- Procedures
    - Phenotypic, *e.g.*
        - Biochemical characterization for identification
        - Antibiotic resistance traits for identification
        - Antigenic characterization for identification
    - Genetic, *e.g.*
        - Nucleic acid isolation/concentration/purification
        - Polymerase Chain Reaction
            - Conventional
            - Real-time
            - Reverse transcription
        - Sequencing, *e.g.*
            - Whole genome
            - Selective sequencing
            - Single nucleotide polymorphism (SNP) analysis
        - Strain-typing applications
- Immunological
    - Antibody capture
    - ELISA
    - Flow cytometry

1.8 Requirements

Method validation shall be required for:

- Submission of a new or alternate method.
- Major modifications to an existing, validated method (See Section 5.0).

2.0 CRITERIA AND GUIDANCE FOR THE VALIDATION OF FDA-DEVELOPED METHODS

This section provides validation criteria and guidance for all FVM-developed or any existing validated method(s) that has been significantly modified (*See* Section 5.0).

2.1 Validation Definitions

2.1.1 The Reference Method

The reference method is defined as that method by which the performance of an alternate method is measured or evaluated. Validation studies must include comparison to a recognized reference method to demonstrate equivalence or increased performance, the significance of which must be determined statistically. For bacterial analytes, reference methods are generally culture-based and result in a pure isolate. The FDA Bacteriological Analytical Manual (BAM), the USDA Microbiology Laboratory Guidebook (MLG) and ISO culture methods contain recognized reference culture methods. **FDA BAM reference methods take precedence over all other reference methods unless otherwise determined by the MMVS.** It is recognized that this requirement may either not be practical or possible in all instances. In such cases, consultation between the originating laboratory and the MMVS will be necessary to define the most appropriate reference method. ***All*** new methods ***must*** be validated against an agreed-upon reference method if existing.

2.1.2 The Alternate Method

The alternate method refers to the newly developed or modified method that is to be evaluated against the performance of a recognized reference method by a defined validation process.

2.1.3 The Originating Laboratory

The originating laboratory refers to the laboratory that developed the method and has completed the SLV requirements.

NOTE: An “originating laboratory” can be more than a single laboratory when 2 or more laboratories combine their resources to develop and validate a method. In such cases, none of the laboratories so combined may act as a Collaborating Laboratory.

2.1.4 The Collaborating Laboratory

The collaborating laboratory refers to the laboratory (or laboratories) other than the originating laboratory involved in Multi-Laboratory method validation studies. A collaborating laboratory may have more than one collaborator analyzing collaborative study samples, so long as individual collaborators work independently of one another.

2.2 The Method Validation Process

Within the Foods Program, method validation exercises confirm by examination (and the provision of objective evidence) that the particular requirements for a method have been fulfilled. All methods used by the FDA in support of its regulatory and compliance roles must be validated according to the guidelines established by the FVM Enterprise. Three levels of scrutiny are defined below and serve to demonstrate that the method can detect, identify and, where applicable, quantify an analyte or analytes to a defined standard of performance. The hierarchy of criteria within the validation process also provides general characteristics on the method's utility and insights for its intended use.

2.2.1 Method Validation

2.2.1.1 Single-laboratory Validation (SLV)

The originating lab has done a more comprehensive initial study with defined inclusivity/exclusivity levels as shown in Tables 1. If available, a comparison has been done to an existing reference method. Results of the SLV has been evaluated and approved by the MMVS. This is the first step in the validation process for methods designed for routine regulatory applications. All FDA SLV protocols should be reviewed by MMVS before research is initiated.

Intended Use: Methods validated to this level of scrutiny can be used immediately for emergencies Slightly higher false-positive rates may be acceptable as all samples analyzed will require confirmatory testing.

2.2.1.2 Independent Laboratory Validation (ILV)

The purpose of an ILV is to determine if a method can be successfully performed by a laboratory other than the originating laboratory. An ILV study may be required for methods extensions and method modifications that do not require a multi-laboratory validation (MLV) study. Generally speaking, an ILV is required under 2 circumstances: 1) For entirely new methods or modified methods that have not been fully validated through a MLV study; or, 2) For methods extensions of fully validated methods (through MLV study), where the sample preparation procedure has been changed for a particular matrix or set of matrices. It is not required for methods extensions where the method remains unchanged and where the scope of the method is being extended to include additional matrices. Determination of the need for an ILV is at the discretion of the MMVS. **(See Table 1)**

Intended Use: Methods validated to this level of scrutiny can be used immediately for emergencies only and not for regulatory purposes unless the purpose of the ILV is for the extension of a fully validated method. Slightly higher false-positive rates may be acceptable as all samples analyzed will require confirmatory testing.

2.2.1.3 Multi-laboratory Validation (MLV)

A MLV study is an inter-laboratory study in which collaborators in multiple laboratories use a defined method of analysis to analyze identical portions of homogeneous materials to assess the performance characteristics obtained for that method of analysis (W. Horwitz, IUPAC, 1987). It is designed to measure **Reproducibility**, so that it can be determined if the method can be successfully performed by laboratories other than the originating laboratory. For methods having more than one sample preparation or enrichment scheme, it is necessary to test one matrix per sample preparation or enrichment scheme.

The criteria defined for this level of scrutiny (to be performed by the originating and collaborating labs) are closely aligned with other recognized and established validation criteria for collaborative studies *e.g.* AOAC, ISO. This includes criteria for inclusivity/exclusivity, analyte contamination levels, competitor strains, aging, and a comparison to an existing, recognized reference method when available.

Intended Use: All methods validated to this level of scrutiny are acceptable for use in all regulatory circumstances *e.g.* confirmatory analyses; regulatory sampling, outbreak investigations, and surveillance and compliance support.

2.3 Validation Criteria

Tables 1, 2, 3 and 4 contain the general criteria that must be met in order to successfully achieve a defined level of validation for a new or modified method. Table 1 describes general guidelines for qualitative methods to detect conventional microbial foodborne pathogens. Table 2 applies to detection methods for microbial analytes that face unique isolation and/or enrichment challenges. Table 3 describes general guidelines for identification or confirmatory methods. Table 4 describes general guidelines for quantifiable methods. Table 5 gives general parameters for emergency validation studies. The criteria contained within these tables also distinguish between qualitative and quantitative methods; and, those requirements to be carried out by the originating and collaborating laboratories respectively.

2.3.1 Validation Criteria for Qualitative Methods to Detect Conventional Microbial Food-borne Pathogens

2.3.1.1 Definition

A method that identifies analyte(s) based on chemical, biological, or physical properties; method of analysis whose response is either the presence or absence of the analyte detected either directly or indirectly in a certain amount of sample. Most qualitative methods are or can be made at least “semi-quantitative” to provide rough estimates of the amount of analyte present.

2.3.1.2 Criteria

Table 1 pertains to bacterial pathogens (and other pathogenic microorganisms) that meet the following general characteristics:

- Not limited by strain availability; ability to fully comply with inclusivity and exclusivity requirements.
- Are capable of cultural enrichment in a timely manner.
- Can be enumerated.

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

Criteria	Single Laboratory Validation Study	Independent Laboratory Validation Study	Multi-Laboratory Validation Study
Participating Laboratory	**Originating Laboratory**	**Collaborator**	**Collaborators**
# of Target Organism (Inclusivity)[b]	50 (unless 50 aren't available)[c,d]	[e]NA	NA
# of Non-Target Organism (Exclusivity) [b]	30 strains[f]	NA	NA
# of Collaborators Providing Usable Data	NA	1	10
# of Foods	1 or more[g]	1 or more[g]	1 or more[g]
# of Analyte Levels/Food Matrix	3 levels: Minimum of two inoculated levels (one fractional[h] and one 1 log higher) and one uninoculated level	3 levels: Minimum of two inoculated levels (one fractional and one 1 log higher) and one uninoculated level	3 levels: Minimum of two inoculated levels (one fractional and one 1 log higher) and one uninoculated level
Replicates per Food at Each Level Tested	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)[i]	8 per level
Aging of Inoculated Samples Prior to Testing	Yes[j]	Yes[j]	Yes[j]
Addition of Competitor Strain[k]	In 1 food at +1 log>analyte at fractional positive analyte level	In 1 food at +1 log>analyte at fractional positive analyte level	In 1 food at +1 log>analyte at fractional positive analyte level
BAM Reference Method Comparison Requirement[l]	Yes, if available	Yes, if available	Yes, if available

[a] Analysts should consult with MMVS to determine appropriate statistics before initiating study.

[b] Using pure cultures without a food matrix. See Appendix 6 for suggested strains.

[c] The target concentration for testing is 10 to 100 times the LOD_{50} of the candidate method. Inclusivity testing is only necessary for new methods and where deemed necessary by MMVS. Inclusivity testing unnecessary for methods extensions to new matrices.

[d] 100 serotypes for *Salmonella* testing.

[e] Not Applicable

[f] At growth limit, i.e., 10^9 CFU/ml for target organisms. Exclusivity testing is only necessary for new methods and where deemed necessary by MMVS. Exclusivity testing unnecessary for methods extensions to new matrices. Exclusivity non-target organisms are grown in a non-selective rich medium.

[g] For FDA regulatory use, methods are only valid for foods that have been tested; the MMVS may require that a new method be validated for 3 foods within a food category (See APPENDIX 5). See Section 5 for further guidance on matrix extension criteria.

[h] Must be adjusted to achieve fractional positive results (one or both methods *i.e.* the reference and alternate methods must yield 50%±25% of tests positive) at this level; the high-level inoculum should be approximately1 log greater than that used to achieve fractional results. All 5 replicates at the high inoculum should yield positive results.

[i] Independent lab test portions are blind coded.

[j] Period of aging depends on food being tested. Perishable foods should be aged under refrigeration for 48 – 72 h. Frozen and shelf stable foods should be aged for a minimum of 2 weeks at -20°C or at room temperature, respectively. MMVS can change aging requirements if circumstances warrant.

[k] An appropriate competitor is one that gives similar reactions in enrichment and detection systems. Natural background microflora can fulfill this requirement if it present in the matrix at a level 1 log greater than the target analyte. Perform aerobic plate counts on all foods tested to determine levels of background microflora. This requirement may be waived or modified by consent of the FDA MMVS should the pathogen be unstable in the matrix.

[l] Independent Laboratory and Multi-Laboratory Validation Studies should use the BAM reference method if available. Other reference methods can be used per MMVS approval.

2.3.1.3 Detection of Microbial Analytes That Present Unique Isolation and/or Enrichment Challenges

Tables 2 provides validation criteria for microbial pathogens characterized as difficult to isolate, limited resources for extensive inclusivity and exclusivity studies, and either **non-culturable** for enrichment purposes or, enrichment cannot be accomplished in a timely manner.

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

Criteria	Single Laboratory Validation Study	Independent Laboratory Validation Study	Multi-Laboratory Validation Study
Participating Laboratory	**Originating Laboratory**	**Collaborator**	**Collaborators**
# of Target Organism (Inclusivity)[b]	[c]TBD	[d]NA	NA
# of Non-Target Organism (Exclusivity)[e]	TBD	NA	NA
# of Collaborators Providing Usable Data[f]	NA	1	Minimum of 5[g]
# of Foods	1 or more[g]	1 or more[g]	1 or more[g]
# of Analyte Levels/Food Matrix	3 levels: Minimum of two inoculated levels (one fractional[h] and one1 log higher[i]) and one uninoculated level	3 levels: Minimum of two inoculated levels (one fractional and one1 log higher) and one uninoculated level	3 levels: Minimum of two inoculated levels (one fractional and one 1 log higher) and one uninoculated level
Replicates per Food at Each Level Tested	≥3[j]	≥3[j]	≤8[j]
Aging of Inoculated Samples Prior to Testing [k]	Yes	Yes	Yes
Addition of Competitor Strain[l]	In 1 food at +1 log>analyte at fractional positive analyte level	In 1 food at +1 log>analyte at fractional positive analyte level	In 1 food at +1 log>analyte at fractional positive analyte level
BAM Reference Method Comparison Requirement[m]	Yes, if available	Yes, if available	Yes, if available

***Analysts should consult with MMVS to determine appropriate statistics before initiating study.**

[a] Such examples include but are not limited to RNA food-borne viruses, and protozoan parasites. See APPENDIX 3 Sections V and VI.

[b] Using pure cultures without a food matrix. See Appendix 6 for suggested strains. The target concentration for testing is 10 to 100 times the LOD_{50} of the candidate method. Inclusivity testing only necessary for new methods and where deemed necessary by MMVS. Inclusivity testing unnecessary for methods extensions to new matrices.

[c] TBD to be determined in consultations with the originating laboratory, the MMVS, and subject matter experts.

[d] Not Applicable.

[e] Exclusivity testing only necessary for new methods and where deemed necessary by MMVS. Exclusivity testing unnecessary for methods extensions to new matrices. Exclusivity non-target organisms are grown in a non-selective rich medium.

[f] Labs providing data are required to run study on same PCR platform.

[g] Where circumstance and resources permit.

[h] Must be adjusted to achieve fractional positive results (one or both methods *i.e.* the reference and alternate methods must yield 50%±25% of tests positive) at this level, advisable to include when possible one additional level at +1 log.

[i] All test samples inoculated at this level should yield 100% positive results.

[j] Number of required replicates is at the discretion of MMVS. Consult with the MMVS.

[k] Period of aging depends on food being tested. Perishable foods should be aged under refrigeration for 48 – 72 h. Frozen and shelf stable foods should be aged for a minimum of 2 weeks at -20°C or at room temperature, respectively. This requirement may be waived by consent of the FDA MMVS should the pathogen be unstable in the particular matrix

[l] An appropriate competitor is one that gives similar reactions in enrichment and detection systems. Natural background microflora can fulfill this requirement as long as it present in the matrix at a level 1 log greater than the target analyte. Perform aerobic plate counts on all foods tested to determine levels of background microflora. This requirement may be waived or modified by consent of the FDA MMVS should the pathogen be unstable in the particular matrix.

[m] Independent Laboratory and Multi-Laboratory Validation Studies should use the BAM reference method available. Other reference methods can be used per MMVS approval.

2.3.2 Validation Criteria for Identification Methods

2.3.2.1 Definition

A method used to confirm the identity of a microbial analyte *e.g.* serotyping.

2.3.2.2 Criteria

Table 3- General Guidelines for the Validation of Identification Methods for Microorganisms

Criteria	Single Laboratory Validation Study	Independent Laboratory Validation Study	Multi-Laboratory Validation Study
Participating Laboratory	**Originating Laboratory**	**Collaborator**	**Collaborators**
# of Target Organism (Inclusivity)[a]	variable[b,d]	N/A	16[d]
# of Non-target Organism (Exclusivity) [a]	variable[c,d]	N/A	8[d]
# of Collaborators Providing Usable Data	NA	1	10
Reference Method Comparison Requirement	No[e]	No	No

[a] At 10^3 CFU/mL for target organisms and non-target organisms grown in a non-selective rich medium. 10^3 CFU/reaction for molecular methods *e.g.* PCR. See Appendix 6 for suggested strains.

[b] Family level (non-*Salmonella*), 200 target strains; Genus level (non-*Salmonella*), 150 target strains; Species level (non-*Salmonella*), 100 target strains; (Sub)type level (non-*Salmonella*), 25 target strains; *Salmonella* genus or species level, 150 serotypes covering subspecies and major serogroups; *Salmonella* serotype level, at least 25 strains per serotype claimed.

[c] Family level (non-*Salmonella*), at least 100 non-target strains; Genus level (non-*Salmonella*), at least 100 non-target strains; Species level (non-*Salmonella*), at least 100 different non-target strains (50 strains from non-target genus & 50 strains from non-target species within the target genus); (Sub)type level (non-*Salmonella*), 100 different non-target strains (25 strains from non-target genus, 25 strains from non-target (sub)type within the target species, & add up to the total of 100 different non-target strains); *Salmonella* genus and species level, at least 100 different non-target strains; *Salmonella* serotype level, 25 strains from non-target genus and 75 strains from non-target serotypes within the target subspecies.

[d] Should be evaluated together in one single study; inclusive and exclusive samples should be intermingled and blinded

[e] Strains used in ID studies will be fully characterized (e.g., biochemically, serologically and/or genetically in sufficient detail for its identity to be known) before use.

2.3.3 Validation Criteria for Quantifiable Methods to Detect Conventional Microbial Food-borne Pathogens

2.3.3.1 Definition

A method that provides an estimate of the amount of analyte present in the test sample, expressed as a numerical value in appropriate units, with trueness and precision which are fit for the intended purpose.

2.3.3.2 Criteria for Quantitative Methods

Table 4- General Guidelines for the Validation of Quantitative Detection Methods for Microbial Analytes

Criteria	Single Laboratory Validation Study	Independent Laboratory Validation Study	Multi-Laboratory Validation Study
Participating Laboratory	**Originating Laboratory**	**Independent Laboratory**	**Collaborating Laboratories [a]**
# of Target Organism (Inclusivity)[b]	50 (unless 50 are not available)	NA[b]	NA≠
# of Non-target Organism (Exclusivity)[b]	30 strains	NA≠	NA≠
# of Collaborators Providing Usable Data	NA	1	10
# of Foods	1 or more[c]	1 or more[c]	1 or more[c]
# of Analyte Levels/Food Matrix	4 levels: Low medium and high inoculum levels[d] and one uninoculated level	4 levels: Low medium and high inoculum levels[d] and one uninoculated level	4 levels: Low medium and high inoculum levels[d] and one uninoculated level
Replicates per Food at Each Level Tested	5 replicates per level for a total of 20 replicates per method	5 replicates per level for a total of 20 replicates per method	Two test portions per level for a total of 8 test portions
Aging of Inoculated Samples Prior to Testing[e]	Yes	Yes	Yes
Addition of Competitor Strain[f]	In 1 food at +1 log>analyte at highest analyte level	In 1 food at +1 log>analyte at highest analyte level	In 1 food at +1 log>analyte at highest analyte level
BAM Reference Method Comparison Requirement [g]	Yes, if available	Yes, if available	Yes, if available
Confirmation of Test Portions	Varies[h]	Varies	Varies

[a]. If the required number of laboratories are not available, two or more analysts from the same laboratory can submit data generated independently. Minimum of 3 to 5 laboratories are required.

[b] Using pure cultures without a food matrix. See Appendix 6 for suggested strains.

[c] Where circumstance and resources permit.

[d] The low level should be at or near the limit of detection; medium and high levels should be chosen to span the analytical range of the alternate method.

[e] Period of aging depends on food being tested. Perishable foods should be aged under refrigeration for 48 – 72 h. Frozen and shelf stable foods should be aged for a minimum of 2 weeks at -20°C or at room temperature, respectively.

[f] An appropriate competitor is one that gives similar reactions in enrichment and detection systems. Natural background microflora can fulfill this requirement as long as it present in the matrix at a level 1 log greater than the target analyte. Levels of background microflora assessed through the use of an aerobic plate count assay to determine background microflora

[g] Independent Laboratory and Multi-Laboratory Validation Studies should use the BAM reference method. Other reference methods can be used per MMVS approval.

[h] Representative isolates must be confirmed is a specific claim is made. If the claim is an *E. coli* count, then representative colonies should be confirmed. If the claim is for a total viable count, then colonies do not have to be confirmed.

2.3.3.3 Validation Criteria for Emergency Usage

A method used when there is no validated method for a pathogen/matrix pair during an outbreak/public health emergency. Emergency methods may not be used for routine sample analysis: i.e. survey.

This level has the lowest level of validation. All the work will have been done by one or more labs. Sensitivity and specificity (inclusivity and exclusivity) has been tested, but only included a limited number of strains. The MMVS, Agency Subject Matter Experts (SMEs) and the originating laboratory may identify additional criteria for evaluation. Once the crisis has past and it has been determined that there is a need for further validation, procedures outlined in this document must be followed.

Intended Use: Emergency needs. These are methods developed or modified for the detection of an analyte, or a matrix not previously recognized or identified as a threat to food safety or public health. Performance of the method at this level will determine, in part, whether further validation is useful or warranted.

NOTE: *Under emergency situations where the rapid development and deployment of a method is needed to immediately address an outbreak event, Emergency Use criteria should be followed as closely as the situation will allow.*

Table 5. General guidelines for validation of qualitative emergency methods for conventional microbial foodborne pathogens.

Criteria	Conventional Pathogens	Pathogens that present unique isolation/enrichment challenges
Participating Laboratory	**Originating Laboratory**	**Originating Laboratory**
# of target organism (inclusivity)[a]	TBD[b]	TBD
# of non-target organism (exclusivity)[a]	TBD	TBD
# of laboratories providing usable data	1	1[c]
# of foods	1 or more[c]	1 or more[c]
# of analyte levels/food matrix	TBD	TBD
Replicates per food at each level tested	TBD	TBD
Aging of inoculated samples prior to testing	No	No
Addition of competitor strain	No	No
BAM Reference Method Comparison Requirement[d]	TBD	TBD

[a]Using pure cultures without a food matrix.
[b]To Be Determined in consultation with the originating laboratory, the MMVS, and subject matter experts.
[c]Where circumstances and resources permit.
[d]Studies should be performed using the most effective reference method available if any reference method is available.

2.4 Method Validation Operational Aspects

2.4.1 General Considerations

- All correspondence *e.g.* proposals, validation reports etc., with the MMVS will be initiated via email using the following address: Microbiology.MVS@fda.hhs.gov.
- As defined in the SRSC Document titled "Method Development, Validation and Implementation SOP (See APPENDIX 3), all method validation plans must be submitted to and approved by the MMVS prior to initiating any methods validation work beyond the single lab validation stage. See APPENDIX 4 and consult with the MMVS for proposal formatting.
- The number of laboratories submitting usable data in all the above tables represents the minimum number allowable for a successful validation study. It is suggested that 4 additional labs be considered for participation, since a variety of unforeseen circumstances can cause data sets to be rejected.
- The following elements must be addressed in all proposals for method validation studies (in non-emergency use situations).

    - Intended use or applicability statement for the method being validated.
    - Applicability of paired vs. unpaired sampling/testing.
    - Statistical methods must be employed to verify equivalent or statistically-significant improvement of performance between the new method and the reference method (or in some cases, the originally validated method) to include but not limited to sample means and the degree of accuracy. The MVS biostatistician will provide guidance on applicable statistical tools that will be employed on a case-by-case basis (See 2.4.2 Assessment for additional details).
    - Use of an appropriate reference method as determined in consultation with the MMVS. The reference method shall never be modified; comparison with a modified reference method renders the validation study invalid.
    - Where possible, the use of an accredited independent source for sample preparation and distribution.
    - Strain selection for inclusivity and exclusivity testing – This facet of the validation study it to assess the reliability and specificity of the alternate method.

    - Individual laboratories within the FVM research enterprise maintain their own inventories of microbial analyte collections. These collections, strains and serovars derived from food surveillance programs, food-borne outbreak investigations, and clinical specimens, are available to all Agency scientists. Access is governed by "U.S. Food and Drug Administration Foods Program Internal Strain Sharing Standard Operating Procedure" (http://inside.fda.gov:9003/downloads/oc/officeoffoods/scienceandresearchteam/ucm353743.pdf).
        - The choice of inclusivity strains should reflect the genetic, serological, and/or biochemical diversity of the organisms involved, as well as other factors such as virulence, frequency of occurrence and availability. Inclusivity testing is performed on purified cultures.

- The choice of exclusivity strains should closely reflect related, potentially cross-reactive organisms. Other factors such as virulence, frequency of occurrence and availability should be considered. Exclusivity testing is performed on purified cultures.
- Species/strains specified for use in inclusivity and exclusivity panels must be traceable to the source. The source and origin of each species/strain should be documented. See Appendix 6 for suggested inclusive and exclusive microbial analytes. This is not an exhaustive list and should serve only as a reference resource and a guide to aid the developer.
- It is understood that it is not always possible to meet the inclusivity/exclusivity requirements listed herein. For example, only limited numbers of strains may be available for emerging pathogens, certain viruses or parasites. Under such circumstances, the MMVS or its designee will work in concert with the originating laboratory to test their methods with the maximum number of available strains when the developer is unable to comply with the requirements of this document.

- Suitability and availability of naturally-contaminated samples in the proposed validation study.
- Inoculum preparation, spiking methodology, and uniformity of contamination (when artificially-contaminated samples will be used).
- Sample preparation, naturally-occurring microflora, and the requirement for aerobic plate counts (APC) to verify background microflora. APCs should always be performed on test portions/test samples.
- Need for inclusion of competitive microflora. For food matrices that exhibit low naturally-occurring microflora background (as determined by APC), validation studies will adhere to AOAC-established parameter *i.e.*1 log greater than microbial analyte being tested. Selection of competitive microflora to be used will be done in consultation with the MMVS.
- Selection of spiking levels (when artificially-contaminated samples will be used).
- Matrix aging to assess method robustness.
- Microbial analyte stress, cell injury, and matrix-derived inhibition of analyte enrichment/growth.
- Selection of appropriate foods. - Assays to detect various pathogens will be validated individually based upon the historical outbreak record and epidemiological link between matrix, pathogen, and illness. Some examples are provided in Appendix 5. Extension of a method to include additional food matrices will require additional validation studies. See Section 5.0.
- Formation of composited samples. In some instances, it may be necessary to validate composited samples. For example, in the case of *Salmonella* an analytical unit is 25 g and a composite sample is 375 g. A composite test portion is formed by adding fourteen uninoculated 25 g test portions to one inoculated 25 g test portion for a total of 375 g. The composite is compared to a 25 g inoculated test portion that is analyzed with the reference method. Reference and composite method sample sizes vary from method to method, but the validation comparison is

always reference method (inoculated x grams) to composite (inoculated x g + uninoculated y g).

- Inocula designed to yield fractional positive results. Samples for both the reference method and the test method must achieve 50%±25% positive results (See APPENDIX 1: Glossary of Terms, for a complete description of fractional recovery).
-

2.4.2 Assessment of Validation Results

- Acceptable false negative and false positive rates will be established in consultation with the MMVS. Factors that will influence this decision may include, but not be limited to the replicate number and intended use (emergency, screening, confirmatory).
- False positive and false negative rates, for a MLV study, will be evaluated in total (across all labs/data sets).
- Method equivalence determinations and employing appropriate statistical measurements. Statistical algorithms must be employed to test for significance differences (superiority or equivalence) and for data disqualification *(see below),* the preferred method of statistical analysis is Relative Limit of Detection (RLOD). Selection of a statistical approach will be dictated by the type and scope of the study and will be determined through consultations between the originating lab and the MMVS during the planning phase of any validation study.
- Data sets derived from a validation exercise can be disqualified. Examples include but may not be limited to:

    - Negative controls (un-inoculated controls) yield a positive outcome-an indicator of lab/operator error.
    - Deviation from the prescribed method.
    - Quality control deficiencies e.g. homogeneity and stability. Statistically-supported outliers (Quantifiable methods).
    - Failure to achieve fractional results within specified ranges (across all labs/data sets).

3.0 CRITERIA AND GUIDANCE FOR THE VALIDATION OF FDA-DEVELOPED NUCLEIC ACID SEQUENCE-BASED ASSAYS

Information regarding the development, validation and implementation of nucleic acid sequence-based identification methods can be found in *"Guidelines for the Validation of Analytical Methods for Nucleic Acid Sequence-Based Analysis of Food, Feed, Cosmetics and Veterinary Products".* These criteria apply to all CFSAN, CVM, and ORA laboratories that develop and participate in the validation of targeted nucleic acid sequence-based analytical methods for food, feed, cosmetics, and veterinary products for Agency-wide implementation in a regulatory capacity.

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4.0 CRITERIA AND GUIDANCE FOR THE VALIDATION AND VERIFICATION OF COMMERCIALLY- AVAILABLE MICROBIOLOGICAL DIAGNOSTIC KITS AND PLATFORMS

4.1 Definitions

4.1.1 Validation of an Alternative Method

Demonstration that adequate confidence is provided when the results obtained by the alternative method *i.e.* the commercially-available kit, are comparable to or exceed those obtained using the reference method using the statistical criteria contained in the approved validation protocol.

4.1.2 Verification

Method verification is a process by which a laboratory confirms by examination, and provides objective evidence, that the particular requirements for specific uses are fulfilled. It serves to demonstrate that the method can detect and identify an analyte or analytes:

- The confirmation by examination and the provision of objective evidence that specified requirements have been fulfilled.
- To assess the performance of a method in the user's laboratory against the specifications of the method established during the validation.
- To assess the method performance on items included in the scope of the method and tested routinely by the user laboratory.
- To demonstrate that the method functions (without any adaptation) in the user's laboratory on matrices not included in the original method validation.

4.2 Criteria

4.2.1 Commercially-available Microbiological Diagnostic Kits Whose Performance Parameters Have been Fully Validated Against the BAM Reference Method, Unless Unavailable, in a Multi-Laboratory Validation Study Monitored and Evaluated by a Third Party Methods Validation Body e.g. AOAC-RI (OMA Methods), AFNOR, MicroVal, etc.

Each lab must perform an in-house verification for the *"first use"* of an alternate method in this category. For subsequent use(s) of the method, lab controls will be used per lot to re-verify the method.

4.2.1.1 Verification Requirements *(per lab)*

- Six replicates of the inoculated matrix and six replicates of the un-inoculated matrix are tested and confirmed by both the alternative and the reference method. If no false positive or false negative results are obtained, then the alternate method is verified to function in the user's laboratory on any matrix included in the scope of the method, and the new matrix (if any) which was used for in-house verification.

- Each commodity to be tested should be spiked with a level close to the detection limit, usually <30 cfu of analyte per 25 g food sample or any other specified sample size (e.g.,<30 cfu/375 g composite) to determine if there is any interference from the matrix.
- If unacceptable false positive or false negative results are observed (as defined for the intended use of the method), then the study must be expanded to a full SLV (Table1) to define the operating characteristics of the method with the new matrix. Consult Section V: Food Matrix Extension for more detailed information.

  NOTE: The matrix chosen for verification should be one routinely tested by the user laboratory. The verification criteria described above apply only for foods which were part of this in-house "first use" verification and/or the collaborative study by an independent accrediting body. The use of such kits for food matrices that were not included in the original collaborative study must be preceded by a food matrix extension study. If a matrix extension was performed under the auspices of a third-party methods validation organization or by any other entity, the study report should be carefully examined to determine that there are not significant differences between the method validated in the original study and the method validated in the extension. (See Section 5: Food Matrix Extension)

4.2.2 Commercially-available Microbiological Diagnostic Kits Whose Performance Parameters are Supported by Data Obtained Through an Independent Laboratory Validation Protocol Without a Collaborative Study and Evaluated by a Third-Party Methods Validation Organization e.g. AOAC-RI (Performance Tested Methods).

All methods fitting into this description ***must*** be validated according to the criteria defined for Agency-developed (FDA) microbiology methods (*See* Section 2; e.g. must be validated through Multi-Laboratory validation study).

5.0 METHOD MODIFICATION AND METHOD EXTENSION CRITERIA FOR EXISTING VALIDATED MICROBIOLOGY METHODS

Modifications to an existing validated method may be made for any number of reasons and may or may not affect the established validated performance parameters of the original method. There is no *"one size fits all"* rule or set of rules to govern how a modification will be addressed.

Some modifications (*e.g.* ease-of-use capabilities, availability/substitution of reagents or instrumentation, sample handling/sample processing adaptations, etc.) may only necessitate verification against the original method according to criteria detailed in Section 4.2.1.1 (Verification), whereas other modifications may require significant Validation data to support their use as found in Table 1. It is recommended that

statistical analyses be performed on the verified performance specifications to support implementation of the modification. If applicable,these include, but are not limited to:

- The *t* test for significance of difference between the two sample means to determine degree of accuracy. The *t* Stat value must be less than or equal to the *t* critical value.
- The F test for significance of difference between the two sample variances to determine degree of precision. The F value must be less than or equal to the F critical value.

More extensive modifications that may influence method sensitivity, specificity, precision and accuracy (quantifiable methods), *e.g.* changes in sample preparation procedures, time/temperature requirements for non-selective and selective enrichment media; or, altering chemistry parameters for molecular methods for example may require either limited (SLV or ILV Study) or a MLV Study as described in Table 1.

Any decision on how such modifications are viewed and the approach to be taken will reside with the MMVS.

Specific criteria for matrix and platform extension to existing methods are described in greater detail in Sections 5.1 and 5.2

5.1 Matrix Extension and Verification

FDA ORA microbiology labs analyze a huge variety of food matrices. Even so, methods used in FDA field laboratories for regulatory purposes must be evaluated for *each* food.

Very often however, validation studies can neither address all the varied matrices nor fully anticipate what matrix or matrices will be involved in emergency situations or outbreak investigations – two scenarios where samples must be analyzed immediately.

Though it is generally assumed that the more closely related a new food matrix is to a previously-validated matrix for the detection of a defined analyte, the greater the probability that the method will perform similarly with the new matrix, the method must nonetheless be verified for all new matrices. This is to ensure that the new matrix will neither produce high false positive (matrix is free from cross reactive substances) nor high false negative rates (matrix is free of inhibitory substances).

As described below, either a verification process or additional validation studies will be required before any given validated method can be used to test a food (or foods) not included in the original method validation. Close consultation between method developers, laboratory managers, QMS managers and the MVS will aid in determining which approach is more applicable for any given situation.

NOTE: Criteria described in sections 5.1.1 and 5.1.2 only apply to situations in which no additional modifications to the method have been made. In those cases where food matrix extension is accompanied by additional modifications to the method, an SLV or Independent Laboratory Validation as described in Table 1 may be required. This decision will be at the discretion of the MMVS.

5.1.1 Matrix Verification Guidance for New Foods From the Same Category Used for the Original or Subsequent Validation Studies

In instances where a method will be used to test a food (or foods) from the same category of food (See APPENDIX 5) included in the original validation study, ORA laboratories will analyze the matrix in question concurrently with a matrix spike. The matrix spike will consist of a 25-gram to a maximum of 375-g of product spiked with an inoculum of 30 cells or less of the target analyte. Good Quality Control Procedures must be followed to ensure the spike is representative of the analytical testing. Situations where a spike control should be greater than 25 g (max 375 g) include: follow up to a reconditioning process (where the product was known to be contaminated with the target analyte), items that contain potentially inhibitory compounds, and/or historical knowledge of inhibitory issues with a product that indicates a 375-g matrix spike is advisable. Twenty-five g matrix spikes will be sufficient in most cases. Negative spike results invalidate the analysis and the sample must be analyzed using the conventional culture procedure.

ORA labs may continue to perform individual sample matrix spikes for matrices that have not been fully validated for the method. Matrix spike results will be entered into Field Accomplishment Computerized Tracking System (FACTS) and data will be evaluated and classified according specific food, and matrix spike results. When a specific food has yielded at least seven positive and no negative results using matrix spikes; or, a >95% confidence level (19 of 20 positives), the method will be considered verified for that food product. The method can then be used for that food without further positive spike controls.

The ORA Office of Regulatory Science will maintain and update lists detailing the expansion of food matrices for methods used by ORA laboratories; these lists will be posted on the ORA Office of Regulatory Science website.

5.1.2 Matrix Extension Guidance for New Foods From a Different Category Than That Used for the Original Method Validation Study

In instances where a method will be used to test a food (or foods) for which it has not previously been validated ***and*** the food (or foods) is not within the same category of food (See APPENDIX 5) included in the original validation study, then a SLV and possibly an ILV will be required as described in Table 1.

Note: For bacteria/culturable, a category consists of 3 food types; for non-culturable organisms, a category consists of 2 food types.

5.2 Platform Extension

Platform extension refers to the proposed use of a new, similarly functioning instrument into approved method that ***differs*** from the one used in the original validation study. Such platform differences may include (but not be limited to) being of similar function and capacity but from a different manufacturer; from the same manufacturer but with significantly different performance parameters (i.e. capacity, capabilities); or, represent the next generation for that type of instrumentation to include newer technology and/or reagent reformulations.

The use of specialized instrumentation (and in many cases their accompanying proprietary reagents) dictate the performance standards of validated analytical methods. Therefore, it cannot be assumed that the impact on the method's performance from any interchangeability of instrumentation will be negligible. Performance comparability must be assessed.

In general, platform extension validation must be done by comparing the proposed new platform to the previously validated platform. The scope of the validation study may vary from case to case and will be dependent on such factors as reformulation of buffers, primers, probes, alternative proprietary chemistries, threshold of detection sensitivity, etc. Each case will be judged independently through examination of publicly accessible data, input from SMEs, the method developer, and the MMVS.

In planning platform extension validation, the method developer and the MMVS, must determine what aspect of the technology will be compared in order to determine how the study should proceed. In some instances, a platform extension study may require only a simple verification process. Other instances, however, may necessitate an SLV or Independent Validation Study as described in Table 1.
For molecular methods, frozen extracts from the original validation study may be used for platform extensions with the consent of the MMVS. Make sure that all controls are included.

6.0 Further Considerations

These guidelines may not address all methods validation issues. In cases where they do not, the reader should refer to internationally recognized Microbiological Validation Guidelines such as AOAC International's Appendix J (http://www.eoma.aoac.org/app_j.pdf) or the International Organization for Standardization's 16140:2016 (and subsequent editions). The validation protocols found in these internationally recognized guidelines do not supersede the requirements of FDA's Microbiological Guidelines, but can provide methods developers and laboratorians with useful information. Contact MMVS for more information (**Microbiology.MVS@fda.hhs.gov**).

APPENDIX 1
Glossary of Terms

Action level: Level of concern for an analyte that must be reliably detected, identified or quantified in a sample.

Accuracy: A measure of the degree of conformity of a value generated by a specific procedure to the assumed or accepted true value, and includes precision and bias.

Alternate method: The newly developed or modified method that is to be evaluated against the performance of a recognized reference method by a defined validation process.

Analytical batch: An analytical batch consists of samples which are analyzed together with the same method sequence and same lots of reagents and with the manipulations common to each sample within the same time period or in continuous sequential time periods. A set of measurements or test results taken under conditions that do not vary within a 24 hour time period.

Analyte: Component measured by the method of analysis. In the case of microbiological methods, it is the microorganism or associated by-products (e.g., enzymes or toxins).

Applicability: The analytical purpose for which a method has been validated.

Bias: The difference between the expectation of the test results and an accepted reference value.

> **NOTE**: *Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to the bias. A larger systematic error difference from the accepted reference value is reflected by a larger bias value.*

Calibration: The set of operations which establish, under specific conditions, the relationship between values of quantities by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards.

Certified Reference Material (CRM): Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes metrological traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence (slightly modified from VIM04)

> **NOTE:** *The term "Standard Reference Material" (SRM) is the name of a certified reference material (CRM), which is the trademark name of a certified*

reference material that has been certified and is distributed by the National Institute of Standards and Technology (NIST).

Detection limit: A detection limit is the lowest amount of analyte in a sample which can be detected but, not necessarily quantified, as an exact value. It is often called the limit of detection (LOD), which is the lowest concentration level that can be determined as statistically different from a blank at a specified level of confidence. It is determined from the analysis of sample blanks and samples at levels near the expected LOD (see ISO 11843, CLSI EP17).

Exclusivity: Specificity; the ability of the method to distinguish the target from similar but genetically distinct non-target. It is the lack of interference in the alternative method from a relevant range of non-target strains, which are potentially cross-reactive.

Food category: A group of specific related foods. Appendix 5 lists nine recommended food categories: meat products, poultry, fish and seafood products, fruit- and vegetable-based products, dairy products, chocolate/bakery products, animal feeds, pasta, and miscellaneous.

Food matrix: Components that comprise the food sample.

Food product: Any substance usually composed primarily of carbohydrates, fats, water and/or proteins that can be eaten or drunk by an animal or human for nutrition or pleasure. See APPENDIX 5 for examples of representative food products.

Food type: An item that is processed, partially processed or unprocessed for consumption. APPENDIX 5 lists various types such as raw, heat processed, frozen, fermented, cured, smoked, dry, low moisture, etc.

Fractional recovery: Validation criterion that is satisfied when a common set of samples (e.g., inoculation level), yields a partial number of positive determinations and a partial number of negative determinations within a replicate set of samples. The proportion of positive samples should approximate 50% (±25%) of the total number of replicates in the set. A set of replicate analyses are those replicates analyzed by on method (either reference or alternate). In the context of the entire data set, values outside the prescribed fractional range (50%±25%) may be considered. For example, for studies where a larger number of test portions were analyzed, (i.e., 60), a larger fractional range may be acceptable. Other parameters may be considered on an individual basis.

Inclusivity: Sensitivity; the ability of the method to detect a wide range of targets by a defined relatedness e.g. taxonomic, immunological, genetic composition.

Incurred samples: Naturally-contaminated test samples.

Laboratory: An entity that performs tests and/or calibrations. When a laboratory is part of an organization that carries out activities additional to sample preparation, testing and calibration, the term laboratory refers only to those parts of that organization that are involved in the sample preparation, testing and calibration process. A laboratory's activities may be carried out at a permanent, temporary, or remote location.

Limit of Quantification (LOQ): Lowest amount or concentration of analyte that can be quantitatively determined with an acceptable level of uncertainty, also referred to as the limit of determination.

Linearity: Defines the ability of the method to obtain test results proportional to the concentration.

Matrix blank: A quality control sample of a specified amount of matrix that does not contain the analyte of interest.

Matrix spike: An aliquot of a sample prepared by adding a known quantity of target analytes to a specified amount of matrix and subjected to the entire analytical procedure to establish if the method or procedure is appropriate for the analysis of a specific analyte in a particular matrix.

Method blank: Quality control sample that does not contain the analytes of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples.

Method Detection Limit (MDL; also known as LOD): Lowest amount or concentration of analyte that a specific method can statistically differentiate from analyte-free sample matrix. This is dependent on sensitivity, instrumental noise, blank variability, sample matrix variability, and dilution factor.

Minimum Detectable Concentration (MDC): An estimate of the minimum true concentration of analyte that must be present in a sample to ensure a specified high probability (usually >95%) that the measured response will exceed the detection threshold (i.e., critical value), leading one to conclude correctly that the analyte is present.

Minimum Quantifiable Concentration (MQC): The smallest concentration of analyte whose presence in a laboratory sample ensures the relative standard deviation of the measurement does not exceed a specified value, usually 10 percent.

Multi-Laboratory validation (MLV) study: An MLV study is an inter-laboratory study in which each laboratory uses the defined method of analysis to analyze identical portions of homogeneous materials to assess the performance characteristics obtained for that method of analysis. It is designed to measure inter-laboratory reproducibility, so that it can be determined if the method can be successfully performed by laboratories other than the originating laboratory. For methods having more than one sample preparation or enrichment scheme, it is necessary to test one matrix per sample preparation or enrichment scheme.

Precision: Degree of agreement of measurements under specified conditions. The precision is described by statistical methods such as a standard deviation or confidence limit. See also Random Error. Repeatability expresses the precision under the same operating conditions over a short period of time. Intermediate precision expresses within-laboratory variations, such as different days, different analysts, and different equipment. Reproducibility expresses the precision between laboratories.

Qualitative method: A method that identifies analyte(s) based on chemical, biological, or physical properties; method of analysis whose response is either the presence or absence of the analyte detected either directly or indirectly in a certain amount of sample. Most qualitative methods are or can be made at least "semi-quantitative" to provide rough estimates of the amount of analyte present.

Quantifiable method: A method that provides an estimate of the amount of analyte present in the test sample, expressed as a numerical value in appropriate units, with trueness and precision which are fit for the purpose.

Random error: The irreproducibility in making replicate measurements resulting from random changes in experimental conditions that affects the precision of a result. The distribution of random errors usually follows a Gaussian bell-shaped curve. See also Precision.

Range: The interval of concentration over which the method provides suitable precision and accuracy.

Recovery: Proportion of incurred or added analyte which is extracted and measured from the analytical portion of the test sample.

Reference material: A material or substance, one or more of whose property values are sufficiently homogenous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

Reference standard: A standard, generally having the highest metrological quality available at a given location in a given organization, from which measurements are made or derived. Note: Generally, this refers to recognized national or international traceable standards provided by a reference standard producing body such as the National Institute of Standards and Technology (NIST).

Relative Limit of Detection: The limit of detection of the alternate method divided by the limit of detection of the reference method.

Repeatability: The closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement.

Ruggedness or robustness: The ability of a method to resist changes in test results when subjected to minor deviations in experimental conditions of the procedure. Ruggedness testing examines the behavior of an analytical process when subtle small changes in the environment and/or operating conditions are made, akin to those likely to arise in different test environments.

Screening method: A method intended to detect the presence of an analyte in a sample at or above some specified concentration (target level).

Sensitivity: The lowest concentration that can be distinguished from background noise or the smallest amount of a substance or organism that can accurately be measured by a method or test system is the analytical sensitivity. However, sensitivity is commonly defined as the slope of the calibration curve at a level near the LOQ.

> **Source :** The origin of a test sample. A sample matrix may have variability due to its source. For example, a water sample may have variable characteristics, and therefore, may show method results variability, depending on whether the sample source is drinking water, ground water, surface water, or waste water. Different food sources are defined as different commercial brands. Different water sources could be from different areas of a reservoir. Different plant or soil sources could be samples from the different areas of a plot or field. Different sediment sources could be samples from different areas of a water body.
>
> **NOTE:** The number of sources for a food method validation study may be determined by the number and selection of matrices analyzed in the method validation study. For example, if a variety of food matrices with differing physical and chemical properties are selected, the number of sources for each food sample matrix may be one or more. For a method validation study analyzing one food matrix, 3-5 sources of the food matrix are recommended.

Specificity: It is the ability of the method to distinguish the target from similar but genetically distinct non-target organisms. Methods with low specificity have high false positive rates.

Standard Reference Material (SRM): A certified reference material issued by NIST in the United States. An SRM is certified by NIST for specific chemical or physical properties and is issued with a certificate that reports the results of the characterization and indicates the intended use of the material (www.nist.gov/SRM).

Strain: A group of microorganisms of the same species having distinctive hereditary characteristics not typical of the entire species; a subset of a bacterial species differing from other bacteria of the same species by minor but identifiable differences

Systematic error: A form of measurement error, where error is constant across trials. This may also be referred to as Bias.

Target level: The level at which an analyte can be reliably identified or quantified in a sample.

Trueness: The degree of agreement of the expected value from a measurement with the true value or accepted reference value. This is related to systematic error (bias).

Uncertainty: The parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand. (VIM, 1993)

Validation, method: The confirmation by examination and the provision of objective evidence that the particular requirements for the specific use of a method are fulfilled.

Validation of an alternative method: Demonstration that adequate confidence is provided when the results obtained by the alternative method are comparable to those obtained using the reference method using the statistical criteria contained in the approved validation protocol.

Verification: The confirmation by examination and provision of the objective evidence that specified requirements for the performance of a method have been fulfilled by an individual laboratory. Also, the means used to demonstrate that the method functions (without any adaptation) in the user's laboratory on matrices not included in the original method validation.

APPENDIX 2
RSSC Method Validation Subcommittee Charter

Available at:
https://www.fda.gov/media/93508/download

APPENDIX 3
Method Development, Validation and Implementation SOP

Available at:
Methods Development, Validation, and Implementation Program (MDVIP) Standard Operating Procedures

APPENDIX 4
FVM Microbiology Method Validation Study Application

Available at:

Please contact MMVS for application at Microbiology.MVS@fda.hhs.gov.

APPENDIX 5

Examples of Food Types and Associated Microbiological Contaminants

Appendix 5.1-Food Categories Relevant to Foodborne Pathogenic Microorganisms

(*AOAC Classification of Food Categories, Feldsine et al., (2002) JAOACI 85(5) 1197 – 1198)*

Food type	*Yersinia*	*Clostridium perfringens*	*Listeria mono.*	*E. coli O157*	*Staph. aureus*	*Campy*	*Salmo-nella*	*B. cereus*	*C. cayetan-ensis*	*C. parvum / hominis*	HAV/ Noro-virus
Meats											
raw	x		x	x		X	x	x			
heat processed			x	x	x		x				
frozen			x	x			x				
fermented			x	x			x				
cured		x	x		x		x				
other		dishes / gravy	pate								
Poultry											
raw	x					X	x				
heat processed							x				
frozen							x				
other		dishes / gravy									
Seafood											
raw	x		x	x		X	x				x
heat processed							x				x
frozen			x	x			x				x
shellfish	x			x		X	x				x
smoked		x	x		x		x				
other							x				
Fruits & Vegetables											
unpasteurized juice				x			x			X	
raw	x		x	x		X	x		X	X	x
heat processed		x									
frozen			x				x		X	X	x
dry								x			
juice/concentrate				x			x				
low moist							x				
nut meats			x	x			x				
others											
Dairy											
raw	x		x	x	x	X	x	x			
heat processed			x					x			
frozen			x	x	x		x	x	w/ berries		w/berries
Fermented?			x	x	x		x				
dry					x		x	x			
ice cream			x				x		w/ berries		w/berries
cheese			x	x			x				

Chocolate / bakery											
low moist							x				
dry powder							x				
milk chocolate							x				
other					pastry			custard			
Animal feed											
low moist							x				
pet food							x				
Pasta											
uncooked							x				
Miscellaneous											
Dressings			x	x			x				
spices		x					x				
mayonnaise			x	x		X	x				
flour			x			X	x				
egg / derivatives				x			x				
cereal/rice								x			

Appendix 5.2 - AOAC Food Categories Relevant to Non-pathogenic Microorganisms

Product	Yeast & Mold	Lactics	Total Viable	Coliform	*E. coli*
Meat					
raw	x	x	x	X	x
heat processed		x	x	X	
frozen	x		x	X	x
Fermented	x	x	x		
cured		x	x		
Poultry					
raw	x	x	x	X	x
heat processed		x	x	X	
frozen	x		x	X	x
other			x		
Seafood					
raw	x	x	x	X	x
heat processed		x	x	X	
frozen	x		x	X	x
smoked	x	x	x	X	
Fruits & Vegetables					
raw	x	x	x	X	x
heat processed			x	X	
frozen	x		x	X	
dry	x		x	X	
fermented	x		x		
cured/salted	x		x		
juice/concentrate	x	x	x		
low moist	x		x		
Dairy					
raw	x	x	x	X	x
heat processed			x	X	
frozen	x		x	X	x
Fermented	x				x
dry			x	X	
Choc/bakery					
low moist / IMF	x		x	X	
dry			x	X	
milk chocolate	x		x	X	
Animal feed					
low moist	x		x	X	
dry pet	x		x	X	x
Pasta					
uncooked	x		x	X	
Miscellaneous					
dressings	x	x	x	X	x
spices			x		x
mayonnaise	x	x	x		x
egg / derivatives			x	X	

cereal / rice			x	X	

Representative Food Products in Categories

Meats:
Ground beef, ground pork, meat by-products, glandular products, frog legs, rabbit carcasses, lamb, sausage, frankfurters, lunch meat, beef jerky, meat substitutes

Poultry:
Ground chicken, ground turkey, cooked chicken, raw chicken parts

Seafood:
Raw shrimp, fish sticks, surimi, raw fish filet, raw oysters, raw mussels, raw clams, cooked crawfish, smoked fish, pasteurized crabmeat

Fruits & Vegetables:
Fresh / frozen fruits or dried fruits, orange juice, apple juice, apple cider, tomato juice, melon cubes, berries
Pecans, walnuts, peanut butter, coconut, almonds
Lettuce, spinach, kale, collard greens, cabbage, bean sprouts, seed sprouts, spent water from sprouts, peas, mushroom, and green beans

Dairy:
Yogurt, cottage cheese, hard and soft cheeses, raw or pasteurized liquid milk (skim, 2% fat, whole, buttermilk), infant formula, coffee creamer, ice cream, nonfat dry milk / dry whole milk, dried buttermilk, dried cheese spray

Chocolate / bakery:
Frosting and topping mixes, candy and candy coating, milk chocolate

Animal feed:
Dry pet food, meat and bone meal, chicken and feather meal

Uncooked Pasta:
Uncooked noodles, macaroni, spaghetti

Miscellaneous:
Shell eggs, liquid whole eggs, oral or tube feedings containing egg, dried whole egg or dried egg yolk, dried egg whites
Oregano, pepper, paprika, black pepper, white pepper, celery seed or flakes, chili powder, cumin, parsley flakes, rosemary, sesame seed, thyme, vegetable flakes, onion flakes, onion powder, garlic flakes, allspice
Wheat flour, casein, cake mixes, whey, nonfat dry milk/dry whole milk, corn meal, dried whole egg or dried egg yolk, dried egg whites, soy flour, dried yeast, cereals, dried buttermilk, dry cheese spray

APPENDIX 6
Strains and Serovars for Inclusivity and Exclusivity Panels
(*abridged)*

- This appendix is meant to serve as a guide or starting point for the method developer as they construct exclusive and inclusive panels for method validation and is not intended to be exhaustive.
- Inclusivity/exclusivity panels should be comprised of fully characterized (biochemically, genetically, antigenically as appropriate) strains as to its exact identity. Strains are not restricted to national culture collections, such as ATCC, but they must be fully traceable to the original source.
- Access to microbial analyte strain and serovar and collections within the FVM research enterprise is governed by "U.S. Food and Drug Administration Foods Program Internal Strain Sharing Standard Operating Procedure", which is available by contacting **Microbiology.MVS@fda.hhs.gov.**

I. *E. coli O157:H7*

	Serotype	Genotype		
		***stx*1**	***stx*2**	***uid*A-O157:H7/H-**
EHEC	O157:H7	+	+	+
	O157:H7	+	-	+
	O157:H7	-	+	+
	O157:H7	-	-	+
	O157:H-	+	+	+
	O157:H-	-	+	+
STEC	O68:H-	+	+	-
	O48:			
	O45:H2			
	O137:H41			
	O111:H-			
	O22:H8			
	O15:H27			
	O4:H-			
	O26:H11	+	-	-
	O26:H-			
	O45:H2			
	O85:H-			
	O103:H2			
	O103:H6			
	O111:H11			
	O125:H-			
	O126:H27			
	O146:H21			
	*E coli, stx*1 insert			
	O14:H19	-	+	-
	O28:H35			
	O48:H21			
	O55:H7			
	O104:H21			
	O121:H19			
	O165:H25			
	*E. coli, stx*2 insert			
Non-toxigenic *E. coli*	Non-O157:H7	-	-	-
	O55:H7			
	O157:H16			
	O157:H45			

Exclusivity

	Serotype	Genotype		
		*stx*1	*stx*2	*uid*A-O157:H7/H-
Shigella dysenteriae		+	-	-
Hafnia alvei		-	-	-
1.9 Morganella morganii		-	-	-
Citrobacter fruendii		-	-	-
Lectercia adecarboxylata		-	-	-
Hafnia alvei		-	-	-
Shigella sonnei		-	-	-
1.10 Shigella boydii		-	-	-
Shigella flexneri		-	-	-
Citrobacter fruendii		-	-	-
Salmonella Grp. 30		-	-	-
Salmonella Lansing Grp.P		-	-	-
Klebsiella pneumoniae		-	-	-
Listeria monocytogenes		-	-	-
Listeria innocua		-	-	-
1.11 Listeria ivanovii		-	-	-
Listeria seeligeri		-	-	-
Listeria welshimeri		-	-	-
Vibrio cholerae	O1 Inaba	-	-	-
Vibrio parahaemolyticus	O4	-	-	-
Vibrio vulnificus		-	-	-
Staphylococcus aureus		-	-	-
Rhodococcus equi		-	-	-
Lactobacillus sp.		-	-	-
Lactobacillus sp.		-	-	-
Salmonella Typhimurium		-	-	-

Streptococcus pyogenes	-	-	-
Algaligenes faecalis	-	-	-
Salmonella choleraesuis	-	-	-
Yersinia entercolitica	-	-	-
Yersinia entercolitica	-	-	-
1.12	**Enterobacter cloacae**	-	-	-

II. *Salmonella* *(inclusivity)*

Note: (Derived from the Defense Science Office (DSO) of the Defense Advance Research Projects Agency (DARPA) Systems and Assays for Food Examination (SAFE) Program.

IIa. *Salmonella*: Subspecies Set

SAFE Designation	Original Designation	Serotype	Subsp.
1	02-0061	Newport	I
2	02-0062	Enteritidis	I
3	02-0105	Heidelberg	I
4	02-0115	Typhimurium	I
5	2433	Typhi	I
6	CNM-1029/02	4,5,12:b:-	I
7	CNM-3578/03	Hadar	I
8	CNM-3663/03	Virchow	I
9	CNM-3685/03	Brandenburg	I
10	00-0163	II 58:l,z13,z28:z6	II
11	00-0324	II 47:d:z39	II
12	01-0227	II 48:d:z6	II
13	01-0249	II 50:b:z6	II
14	CNM-169	II 53:lz28:z39	II
15	CNM-176	II 39:lz28:enx	II
16	CNM-4290/02	II 13,22:z29:enx	II
17	CNM-466/03	II 4,12:b:-	II
18	CNM-5936/02	II 18:z4,z23:-	II
19	01-0089	IIIa 41:z4,z23:-	IIIa
20	01-0204	IIIa 40:z4,z23:-	IIIa
21	01-0324	IIIa 48:g,z51:-	IIIa
22	02-0111	IIIa 21:g,z51:-	IIIa
23	CNM-247	IIIa 51:gz51:-	IIIa
24	CNM-259	IIIa 62:g,z51:-	IIIa
25	CNM-3527/02	IIIa 48:z4,z23,z32:-	IIIa
26	CNM-7302/02	IIIa 48:z4,z23:-	IIIa
27	01-0170	IIIb 60:r:e,n,x,z15	IIIb
28	01-0221	IIIb 48:i:z	IIIb
29	01-0248	IIIb 61:k:1,5,(7)	IIIb
30	02-0188	IIIb 61:l,v:1,5,7	IIIb
31	CNM-3511/02	IIIb 48: z10: e,n,x,z15	IIIb
32	CNM-4190/02	IIIb 38:z10:z53	IIIb
33	CNM-750/02	IIIb 60:r:z	IIIb
34	CNM-834/02	IIIb 50:i:z	IIIb
35	01-0133	IV 50:g,z51:-	IV
36	01-0147	IV 48:g,z51:-	IV
37	01-0149	IV 44:z4,z23:-	IV
38	01-0276	IV 45:g,z51:-	IV
39	01-0551	IV 16:z4,z32:-	IV
40	CNM-1904/03	IV 11:z4,z23:-	IV
41	CNM-4708/03	IV 6,7:z36:-	IV

42	ST-16	IV 16:z4,z32:-	IV
43	ST-21	IV 40:g,z51:-	VII
44	ST-22	IV 40:z4,z24:-	VII
45	94-0708	V 48:i:-	*S. bongori*
46	95-0123	V 40:z35:-	*S. bongori*
47	96-0233	V 44:z39:-	*S. bongori*
48	CNM-256	V 60:z41:-	*S. bongori*
49	CNM-262	V 66:z41:-	*S. bongori*
50	95-0321	V 48:z35:-	*S. bongori*
51	1121	VI 6,14,25:z10:1,(2),7	VI
52	1415	VI 11:b:1,7	VI
53	1937	VI 6,7:z41:1,7	VI
54	2229	VI 11:a:1,5	VI
55	811	VI 6,14,25:a:e,n,x	VI

IIb. *Salmonella*: Outbreak Cluster Set

SAFE Designation	Original Designation	Serotype
56	AM04695	Typhimurium / DT104b
57	K0507	Typhimurium
58	H8289	Typhimurium
59	H8290	Typhimurium
60	H8292	Typhimurium
61	H8293	Typhimurium
62	H8294	Typhimurium
63	2009K0191	Typhimurium
64	2009K0208	Typhimurium
65	2009K0224	Typhimurium
66	2009K0226	Typhimurium
67	2009K0230	Typhimurium
68	2009K0234	Typhimurium
69	2009K0350	Typhimurium
70	AM03380	Typhimurium / DT104
71	AM01797	Typhimurium / DT104
72	AM03759	Typhimurium / DT104
73	CDC_07-0708	I 4,[5],12:i:-
74	CDC_08-0061	I 4,[5],12:i:-
75	CDC_08-0134	I 4,[5],12:i:-
76	CDC_07-835	I 4,[5],12:i:-
77	CDC_07-934	I 4,[5],12:i:-
78	CDC_07-922	I 4,[5],12:i:-
79	CDC_07ST000857	Enteritidis
80	CDC_08-0253	Enteritidis
81	CDC_08-0254	Enteritidis

IIc. *Salmonella*: Food Set

SAFE Designation	Original Designation	Serotype
82	2105 H	Saphra
83	1465 H	Rubislaw
84	2069 H	Michigan
85	2308 H	Urbana
86	885 H	Vietnam
87	3030 H	Tornow
88	768 H	Gera
89	1941 H	Fresno
90	3029 H	Brisbane
91	4000 H	Agona
92	1501 H	Muenchen
93	1097 H	Senftenberg
94	1250 H	Muenster
95	1 H	Montevideo
96	1070 H	Johannesburg
97	2080 H	Javiana
98	3170 H	Inverness
99	1061 H	Cubana
100	1158 H	Cerro
101	1988 H	Alachua

III. *Listeria spp.*

Organism	Isolate #	Isolate Information	Serology
		Food Isolates	
L. monocytogenes	15b42	cucumber	4
	3365	mackerel	4b6
	3312	cheese	1a1
	15b27	radish	1
	2388	coleslaw	1
	2478	raw milk	1
	3313	shrimp	1a1
	3326	roast beef	1a1
	3358	milk product	1a2
	3363	cook snow crab	1a2
	3756	beef & gravy Rh-	1
	15b72	apple juice	1
	15b85	cream ch. & veg	1
	15c14	avocado pulp	1
	15c22	fontina cheese	1
	15a90	turkey ham	3b
	2450	veg. mix	1
	2475	cold cut sand.	1
	2492	ice cream	1
	3291	popsicle	1a1
	3318	lobster	1a2
	3321	raw shrimp	4b6
	3332	Mexican-style cheese	4b6
	3359	surimi scallops	1a1
	3362	pollock fish	1a1
	3558	cheese	4b
	3644	red bean ice bar	4b6
	3662	cheese	4b6
	15b70	cheddar cheese	4
L. monocytogenes	2369	**Patient Isolates**	1
	2370		1
	15b55		1
	15b65		1
	3555		4
	3664		1a1
	3666		4b6
	3668		4b6
	15a82		4
	15b56		4
	15b58		4
	15b81		1
	15b82		4
L. monocytogenes	3315	**Environmental Isolates (swab)**	1a1
	3286		1a2
	3308		1a2

	3360		1a1
L. monocytogenes	KC 1710	Other Isolates	4a7,9
	ATCC 19114		4a
	V-7		1a1
	ATCC 15313		1
	Scott A		4b6
	ATCC 19116		4c
	ATCC 19115		

Organism	Isolate #	Organism	Isolate #
L. innocua	3107	*L. welshimeri*	2230
	3124		2231
	3516		3425
	3654		3441
	3758		3659
	6273		15b05
	3181		15b06
	3270		15b16
	3390		15b46
	3392		15b48
	3552		15b50
	3757	*Hafnia alvei*	6410
	15a93	*E. coli*	6365
	15a94	*Morganella morganii*	13b67
	15a95	*Shigella dysenteriae*	13c94
	15b30	*Citrobacter freundii*	13d26
	15b31	*E. coli*	13d64
	15b51	*Leclercia adecarboxylata*	13d65
	15a92	*Hafnia alvei*	13d66
	ATCC 33090	*Shigella sonnei*	13g01
L. ivanovii	2244	*Shigella boydii*	13g18
	3106	*Shigella flexneri*	13g19
	3417	*Citrobacter freundii*	6251
	6274	*Salmonella Grp. 30*	6269
L. ivanovii	15a96	*Salmonella lansing Grp. P*	6270
	15a97	*Klebsiella pneumonia*	6271
	15a98	*Vibrio cholerae*	6277
	15b24	*Vibrio parahaemolyticus*	6278
	ATCC 19119	*Vibrio vulnificus*	6279
L. seeligeri	2232	*Staphylococcus aureus*	ATCC 25923
	2233	*Rhodococcus equi*	6281
	2243	*Lactobacillus spp.*	6282
	2302	*Lactobacillus spp.*	6286
	3110	*Salmonella typhimurium*	6290
	3126	*Streptococcus pyogenes*	ATCC 19615
	3389	*Alcaligenes faecalis*	ATCC 8750
	3423	*Salmonella choleraesuis*	ATCC 6539
	3439	*Yersinia entercolitica*	1269

L. seeligeri (continued)	3451	*Yersinia entercolitica*	1270
	3517	*E. coli*	13a80
	3531	*Enterobacter cloacae*	18g53
	3656		
	6275		
	15b07		
	15b08		
	15b09		
	15b26		
	15b28		
	15b49		

IV. *Shigella*

Inclusive Panel

Genus	Species (Group)	Serotype
Escherichia	*Escherichia coli*, Enteroinvasive	
Shigella	Provisional	Unknown
Shigella	*bodyii* (C)	1
		2
		3
		4
		5
		6
		7
		8
		9
		10
		11
		12
		13
		14
		15
		16
		17
		18
Shigella	*dysenteriae* (A)	1
		2
		3
		4
		5
		6
		7
		8
		9
		10
		11
		12
		13
		14
		15
Shigella	*flexneri* (B)	1
		1a
		1b
		2
		2a
		2b
		3
		3a
		3c
		4
		4a
		5
		5a
		5b
		6
Shigella	*flexneri*, provisional (B)	Unknown
Shigella	*sonnei* (D)	

IV. *Shigella* *(continued)*

Bacteria strain	Strain no.	Source*
Acinetobacter baumannii	19606	ATCC
Aeromonas caviae	15468	ATCC
Aeromonas hydrophila	7966	ATCC
Bacillus licheniformis	12759	ATCC
Bacillus sphaericus	4525	ATCC
Bacillus stearothermophilus	12016	ATCC
Bacillus subtilis	6633	ATCC
Bordetella bronchiseptica	10580	ATCC
Burkholderia cepacia	25608	ATCC
Citrobacter freundii	255	PRLSW
Citrobacter freundii	food isolate	PRLSW
Citrobacter freundii	68	MNDAL
Citroabcter younger	food isolate	PRLSW
Clostrodium sporogenes	11437	ATCC
Edwardsiella tarda	254	PRLSW
Enterobacter aerogenes	13048	ATCC
Enterobacter aerogenes	11	VADCLS
Enterobacter cancerogenus	food isolate	PRLSW
Enterobacter cloacae	260	PRLSW
Enterobacter cloacae	71	MNDAL
Enterococcus durans	6056	ATCC
Enterococcus faecalis	7080	ATCC
Erysipelothrix rhusiopathiae	19414	ATCC
Enterotoxgenic *E. coli*	H10407	CFSAN
Enterotoxgenic *E. coli*	C600/pEWD299	CFSAN
Enterotoxgenic *E. coli*	65	MNDAL
Escherichai coli O157:H7	43890	ATCC
Escherichai coli O157:H7	43888	ATCC
Escherichai coli O157:H7	43895	ATCC
Escherichai coli O157:H7	68-98	CDC
Escherichai coli O157:H7	24-98	CDC
Escherichai coli O157:H7	20-98	CDC
Escherichai coli O157:H7	16-98	CDC
Escherichai coli O157:H7	63	MNDAL
Escherichai coli O157:H7	4	VADCLS
Escherichai coli O157:H44	26	VADCLS
Escherichia coli O111:NM	04.SB.00067	OCPHL
Escherichia coli O143:H4	05.SB.00141	OCPHL
Escherichia coli	8739	ATCC
Escherichia coli	25922	ATCC
Escherichia coli (hemo +)	food isolate	PRLSW
Escherichia coli (hemo +)	28	VADCLS
Escherchia coli (sorbitol –)	food isolate	PRLSW
Escherchia coli (sorbitol –)	food isolate	PRLSW
Escherchia coli	64	MNDAL
Escherchia coli	74	MNDAL
Escherichi coli	8	VADCLS
Klebsiella pnenumoniae	13883	ATCC
Klebsiella pnenumoniae	75	MNDAL
Klebsiella oxytoca	66	MNDAL
Leclercia adecarboxylata	23216	ATCC

Leclercia adecarboxylata	73	MNDAL
Listeria innocua	33090	ATCC
Listeria ivanovii	19119	ATCC
Listeria monocytogenes	19115	ATCC
Listeria monocytogenes	H2446	CDC
Listeria monocytogenes	H8393	CDC
Listeria monocytogenes	H8494	CDC
Listeria monocytogenes	H8395	CDC
Listeria seeligeri	35967	ATCC
Morganella morganii	257	PRLSW
Paenibacillus polymyxa	7070	ATCC
Pantoea agglomerans	food isolate	PRLSW
Pasteurella aerogenes	27883	ATCC
Plesiomonas shigelloides	51903	ATCC
Proteus mirabilis	7002	ATCC
Proteus mirabilis	food isolate	PRLSW
Proteus kauseri	13315	ATCC
Proteus vulgaris	69	MNDAL
Providencia alcalifaciens	51902	ATCC
Providencia rettgeri	76	MNDAL
Providencia stuartii	257	PRLSW
Pseudomonas aeruginosa	27853	ATCC
Pseudomonas aeruginosa	9027	ATCC
Pseudomonas aeruginosa	67	MNDAL
Pseudomonas mendocina	food isolate	PRLSW
Rhodococcus equi	6939	ATCC
Salmonella Gaminara	8324	ATCC
Salmonella diarizonae	12325	ATCC
Salmonella Abortusequi	9842	ATCC
Salmonella diarizonae	29934	ATCC
Salmonella diarizonae	252	PRLSW
Salmonella Mbandaka	253	PRLSW
Salmonella Tennessee	249	PRLSW
Salmonella Lexington	248	PRLSW
Salmonella Havana	241	PRLSW
Salmonella Baildon	61-99	CDC
Salmonella spp.	78-99	CDC
Salmonella spp.	87-03	CDC
Salmonella spp.	98-03	CDC
Salmonella Braenderup	H 9812	CDC
Salmonella Enteritidis	59	MNDAL
Salmonella Heidelberg	60	MNDAL
Salmonella Kentucky	61	MNDAL
Salmonella Newport	62	MNDAL
Salmonella Typhimurium	30	VADCLS
Serratia liquefaciens	27592	ATCC
Serratia liquefaciens	70	MNDAL
Sphingomonas paucimobilis	72	MNDAL
Staphylococcus aureus	6538	ATCC
Staphylococcus aureus	25923	ATCC
Staphylococus epidermidis	14990	ATCC
Staphylococcus xylosus	29971	ATCC
Streptococcus equi subsp. equi	9528	ATCC
Streptococcus gallolyticus	9809	ATCC
Streptococcus pyogenes	19615	ATCC

Vibrio cholerae	14035	ATCC
Vibrio cholerae	14033	ATCC
Vibrio parahaemolyticus	17802	ATCC
Vibrio vulnificus	27562	ATCC
Yersinia enterocolitica	51871	ATCC
Yersinia enterocolitica	27729	ATCC
Yersinia kristensenii	33639	ATCC

ATCC: American Type Culture Collection
OCPHL: Orange County Public Health Laboratory, CA
CDC: Centers for Disease Control and Prevention
PRLSW: Pacific Regional Laboratory – Southwest, FDA
CFSAN: Center for Food Safety and Applied Nutrition, FDA
VADCLS: Virginia Division of Consolidated Laboratory Services
MNDAL: Minnesota Department of Agriculture Laboratory

V. *Food-borne RNA Viruses*

These panels were developed and adopted by the FDA BAM Council, 2009-2015

Inclusivity requirements

Target	Single Laboratory Validation Study	Independent Laboratory Validation Study		Multi-laboratory Validation Study
Norovirus	1 Strain Genogroup I 1 Strain Genogroup II	2 Strains - Genogroup I 5 Strains - Genogroup II		10 Strains – Genogroup I 20 Strains – Genogroup II
Hepatitis A	HM175/18f (subgenotype 1B) ATCC #VR-1402	5 Strains[a]		20 Strains[b]
Enterovirus	Poliovirus 1 (attenuated) ATCC #VR-1562	5 Strains[c]		30 Strains[d]

Hepatitis A virus Panels

Independent Laboratory Validaton Study ([a]should include the following strains):

HM175/18f (subgenotype 1B)	ATCC #VR-1402
HAS-15 (subgenotype 1A)	ATCC #VR-2281

Multilaboratory Valdation Study

([b]should include the following strains):

HM175/18f (subgenotype 1B)	ATCC #VR-1402
HAS-15 (subgenotype 1A);	ATCC #VR-2281
LSH/S	ATCC #VR-2266
PA219 (subgenotype IIIA)	ATCC #VR-1357

Enterovirus Panels

Independent Laboratory Validation Study Level ([c]should include the following strains):

Coxsackievirus A3	ATCC #VR-1007
Echovirus 1	ATCC #VR-1038

Multi-laboratory Validation Study ([d]should include the following strains):

Coxsackievirus A3	ATCC #VR-1007
Echovirus 1	ATCC #VR-1038
Echovirus 21	ATCC #VR-51

V. *Food-borne RNA Viruses:* *(continued)*

Exclusivity Panel

Target	Single Laboratory Validation Study	Independent Laboratory Validation Study		Multi-laboratory Validation Study
Norovirus	10 strains[a]	20 strains[b]		40 strains[b]
Hepatitis A	10 strains[c]	20 strains[d]		40 strains[d]
Enterovirus	10 strains[e]	20 strains[f]		40 strains[f]

Norovirus Panels

Single Laboratory Validation Study *([a]must include)*:

Panel A

HM175/18f (subgenotype 1B)	ATCC #VR-1402 (or equivalent)
Feline calicivirus	ATCC #VR-2057
Murine calicivirus	

Independent and Multi-laboratory Validation *([b]must include):*

Panel A representatives ***plus:***

Panel B

HAV; (subgenotype 1A)	ATCC #VR-2281 (or equivalent)
Coxsackievirus A3	ATCC #VR-1007 (or equivalent)
Echovirus 1	ATCC #VR-1038 (or equivalent)
Rotavirus;	ATCC #VR-2018 (or equivalent)
Astrovirus	
San Miguel Sea lion virus	(if available)
Escherichia coli (1)	
Salmonella spp.(1)	
Shigella spp.(1)	
Vibrio spp. (1)	
Listeria spp. (1)	

Hepatitis A virus Panels

Single Laboratory Validation *([c]must include):*

Panel C

norovirus genogroup I	
norovirus genogroup II	
Coxsackievirus A3	ATCC #VR-1007 (or equivalent)

independent and Multi-laboratory validations *([d]must include)*:

Panel C representatives ***plus***

Panel D

Echovirus 1	ATCC #VR-1038 (or equivalent)
Rotavirus	ATCC #VR-2018 (or equivalent)
Feline calicivirus	ATCC #VR-2057
Astrovirus	
Escherichia coli (1)	
Salmonella spp.(1)	
Shigella spp.(1)	
Vibrio spp. (1)	
Listeria spp. (1)	

Enterovirus Panels:

Single Laboratory validation *([e]must include):*

Panel E

norovirus genogroup I	
norovirus genogroup II	
HM175/18f (subgenotype 1B)	ATCC #VR-1402 (or equivalent)

Independent and Multi-laboratory validations *([f]must include)*:

Panel E representatives ***plus***

Panel F

HAV (subgenotype 1A)	ATCC #VR-2281 (or equivalent)
Rotavirus	ATCC #VR-2018 (or equivalent)
Feline calicivirus	ATCC #VR-2057
Escherichia coli (1)	
Salmonella sp.(1)	
Shigella spp.(1)	
Vibrio spp. (1)	
Listeria spp. (1)	

VI. Protozoan Parasites

A. *Cyclospora cayetanensis*

a. Inclusive Panel

As many geographic and outbreak isolates as are available

b. Exclusive Panel

***Cyclospora* spp.**

C. cercopitheci
C. colobi
C. papionis

***Eimeria* spp.**

E. acervulina
E. bovis
E. burnetti
E. maxima
E. mitis
E. mivati
E. necatrix
E. nieschulzi
E. praecox
E. tenella

Additional Microorganisms

Cryptospordium spp
Apicomplexa
Bacterial isolates

B. *Cryptosporidium* spp.

Inclusive Panel

C. hominis
C. parvum (multiple strains available*)*

Exclusive Panel

C. baileyi
C. canis
C. cuniculus
C. felis
C. meleagridi
C. muris
C. serpentis
Cyclospora ssp.
Apicomplexa
Bacterial isolates