

Guidelines for the Validation of Microbiological Methods for the FDA Foods Program

Edition 4.0

**U.S. Food and Drug Administration Foods Program
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APPROVAL PAGE

The FDA Foods Program Regulatory Science Steering Committee (RSSC) approved this document. The Microbiology Method Validation Subcommittee (MMVS) 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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1.0 INTRODUCTION

1.1 Purpose

The Foods Program (FP) within the U.S. Food and Drug Administration (FDA) is responsible for ensuring the safety of the nation's human and animal food supply. The FDA accomplishes this through education, inspection, data collection, standards setting, prompt investigation of outbreaks, and enforcement actions when appropriate. The effectiveness of the FP 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 FP Regulatory Science Steering Committee (RSSC) has established these criteria by which all FP 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 human and animal food microbiological methods for Agency-wide implementation in a regulatory capacity. This includes all research and field laboratories 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 human and animal food-related method validation criteria for microbial analytes, including environmental samples collected from food production and adjacent areas. 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.

1.4 The Microbiology Methods Validation Subcommittee

Under the authority of the RSSC, the Microbiology Methods Validation Subcommittee (MMVS) will oversee all matters related to microbiology method validation. The MMVS is governed by the organizational structure, roles, and responsibilities detailed in the FDA FP Method Validation Subcommittee (MVS) Charter ([Appendix 2](#)). Briefly, the MMVS will oversee and coordinate with the originating laboratory on all planning and implementation of laboratory validation studies for microbiological methods developed within the FDA FP Enterprise to support regulatory analytical needs. This includes the evaluation of single-laboratory validation (SLV) results and the evaluation of any subsequent multi-laboratory validation (MLV) study proposals and results. Unless otherwise stated, all correspondence between the method developer(s) and the MMVS will be initiated via email to the current MMVS Chair (listed on the internal [FDA FP Regulatory Science Method Development, Validation, and Implementation Program \(MDVIP\)](#) SharePoint Site). The MMVS Chair can be contacted externally at Microbiology.MVS@fda.hhs.gov.

1.5 General Responsibility of the Originating Laboratory

It is the responsibility of the originating (method-developing) laboratory to ensure proper adherence to all criteria described in this 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. MMVS approval is not required for SLV study proposals, but the originating laboratory is encouraged 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 or specialist 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 requirements for intended 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.
- Using one or more instruments or platforms.
- With a demonstrated sensitivity, specificity, trueness, precision, reproducibility, and ruggedness to ensure that results are meaningful and appropriate to make a decision regarding the status of a regulatory sample.
- 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 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 human and animal foods, and their production environment. This includes any microbiological organism of interest (target organism) or 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 method development and evaluation include, **but are not limited to**, the following examples:

- Qualitative detection methods – e.g., real-time polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP)
- Quantitative methods – e.g., quantitative real-time PCR (qPCR), digital PCR (dPCR), most probable number (MPN), enumeration by direct plating)
- Analyte-specific methods
 - Bacterial pathogens
 - *Salmonella* spp.
 - Pathogenic *Escherichia coli*

- *Listeria monocytogenes*
 - *Shigella* spp.
 - *Vibrio* spp.
 - *Campylobacter* spp.
 - *Cronobacter* spp.
 - Other foodborne bacterial pathogens
- o Microbial toxins (excluding marine biotoxins. See the chemistry method validation guidelines, [Appendix 2](#))
- o Viral pathogens
 - Hepatitis A virus
 - Norovirus
 - Other foodborne/waterborne viruses
- o Parasitic protozoan pathogens
 - *Cryptosporidium*
 - *Cyclospora cayetanensis*
- o Indicator organisms
 - *E. coli* and coliform bacteria
- Applications
 - o Pre-enrichment and selective enrichment
 - o Microbial analyte recovery and concentration
 - o Screening, high-throughput, confirmation
- Procedures
 - o Phenotypic
 - Biochemical characterization for identification
 - Antimicrobial resistance traits
 - Antigenic characterization for identification
 - o Genotypic (also see the guidelines for the validation of nucleic acid sequence-based methods, [Appendix 2](#))
 - Nucleic acid isolation/concentration/purification
 - Polymerase chain reaction (PCR)
 - Conventional (PCR)
 - Real-time (qPCR)
 - Reverse transcription (RT-PCR, RT-qPCR)
 - Digital (dPCR)
 - Loop-mediated isothermal amplification (LAMP)
 - Sequencing
 - Whole genome
 - Targeted amplicon sequencing
 - Single nucleotide polymorphism (SNP) analysis
 - Strain-typing applications
- Immunological

- o Antibody capture
- o ELISA
- o Flow cytometry

1.8 Requirements

Method validation shall be required for:

- Submission of a new (alternative) method.
- Major modifications to an existing validated method (See [Section 5.0](#)).

2.0 CRITERIA AND GUIDANCE FOR VALIDATION OF FDA-DEVELOPED METHODS

This section provides validation criteria and guidance for all FP-developed or existing validated methods that have been significantly modified (See [Section 5.0](#)).

2.1 Validation Definitions

2.1.1 Reference Method

The reference method is defined as the method by which the performance of an alternative method is measured or evaluated. Validation studies must include comparison to a recognized reference method (if existing) to demonstrate equivalence or increased performance, the significance of which must be determined statistically. The FDA Bacteriological Analytical Manual (BAM), the USDA Microbiology Laboratory Guidebook (MLG) and the International Organization for Standardization (ISO) methods contain recognized reference 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 Alternative Method

The alternative 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 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 Collaborating Laboratory

The collaborating laboratory refers to the laboratory (or laboratories) other than the originating laboratory involved in independent laboratory validation (ILV) and MLV 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 FP, method validation studies confirm by examination (and the provision of objective evidence) that the 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 FP Enterprise. Three levels of validation 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)

An SLV is carried out by the originating FDA laboratory to determine if the alternative method performs as well or better than the reference method, if available (Tables 1-4). An SLV report of the results is submitted to the MMVS for evaluation and approval (with input by the appropriate TAG). This is the first step in the validation process for methods designed for routine regulatory applications.

Intended Use: Methods validated to this level can be implemented immediately if the MMVS determines an MLV is not required (e.g., matrix extensions or minor modifications of methods that have been previously MLV validated).

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 method extensions and method modifications that do not require an MLV (see section 5.0). An ILV may also be performed if only one regulatory laboratory (not the originating laboratory) will be using the method. Determination of the need for an ILV is at the discretion of the MMVS (See Tables 1-4). An ILV report of the results is submitted to the MMVS for evaluation and approval (with input by the appropriate TAG).

Intended Use: Methods validated to this level can be implemented immediately by the regulatory laboratory that performed the ILV if the MMVS determines an MLV is not required.

2.2.1.3 Multi-laboratory Validation (MLV)

An MLV study is an inter-laboratory study in which collaborators in multiple FDA regulatory laboratories (and state laboratories if needed) analyze test portions originating from the same sample batch to assess the performance characteristics of the alternative method compared to the reference method, if available. Test portions are prepared by one organizing laboratory prior to distribution to the testing laboratories. An MLV is primarily designed to measure reproducibility and determine if the alternative 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 validation (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 International, ISO). This includes criteria for analyte contamination levels, competitor strains, aging, and a comparison to an existing, recognized reference method when available (Tables 1-4).

Both the MLV proposal and MLV final report are submitted to the MMVS for evaluation and approval using the **Application for MLV Proposal and Final Report Review** form ([Appendix 2](#)). The MLV proposal must be approved by the MMVS prior to initiation of the MLV study.

Intended Use: Methods validated to this level can be implemented immediately.

2.3 Validation Criteria

Tables 1, 2, 3, 4, and 5 contain the general criteria that must be met to successfully achieve a defined level of validation for a new or modified method. The criteria contained within these tables also distinguish between qualitative and quantitative methods and the requirements to be carried out by the originating and collaborating laboratories respectively.

- [Table 1](#) describes general guidelines for qualitative methods to detect conventional microbial analytes.
- [Table 2](#) applies to qualitative detection methods for microbial analytes with unique isolation and/or enrichment challenges.
- [Table 3](#) describes general guidelines for identification/characterization for microbial analytes.
- [Table 4](#) describes general guidelines for quantitative methods for microbial analytes.
- [Table 5](#) gives general parameters for emergency validation studies for microbial analytes.

2.3.1 Validation Criteria for Qualitative Methods to Detect Conventional Microbial Analytes

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 microbial analytes 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.

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

[Table 2](#) provides validation criteria for microbial analytes 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.

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Table 1: General guidelines for 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}	NA ^e	NA
# of Non-Target Organism (Exclusivity) ^b	30 strains ^f	NA	NA
# of Collaborators Providing Usable Data ^g	NA	1	10
# of Foods ^{h,i}	3 or more	1 or more	1 or more
# of Analyte Levels/Food Matrix	3 levels: Minimum of two inoculated levels (one fractional ^j and one 1 log higher) and one uninoculated level	3 levels: Minimum of two inoculated levels (one fractional ^j and one 1 log higher) and one uninoculated level	3 levels: Minimum of two inoculated levels (one fractional ^j 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) ^k	8 per level ^k
Aging of Inoculated Samples Prior to Testing ^l	Yes	Yes	Yes
Addition of Competitor Strain ^m	In 1 food at +1 log>target analyte at fractional positive analyte level	In 1 food at +1 log>target analyte at fractional positive analyte level	In 1 food at +1 log>target analyte at fractional positive analyte level
BAM Reference Method Comparison Requirement ⁿ	Yes, if available	Yes, if available	Yes, if available

^aRecommend method developers consult with the MMVS biostatistician to determine appropriate statistics.

^bUsing purified analytes tested individually without a food matrix. See [Appendix 4](#) for suggested strains. Inclusivity/exclusivity testing is only necessary for new methods and when deemed necessary by the MMVS. Inclusivity/exclusivity testing is unnecessary for method extensions to new matrices, and minor modifications to existing reference methods.

^cThe target concentration for testing is 10 to 100 times the LOD50 of the alternative method. Inclusivity organisms are grown in a rich, non-selective medium.

^d100 serotypes for *Salmonella* testing.

^eNot Applicable

^fAt growth limit (e.g., 10⁹ CFU/ml) for non-target organisms. Exclusivity non-target organisms are grown in a rich, non-selective medium.

^gLabs providing data are required to run study on the same instrument platform.

^hIncludes environmental samples ([Appendix 3](#)). For FDA regulatory use, methods are only valid for matrices that have been tested; the MMVS may require that a new method be validated for 3 foods within a food category (See [Appendix 3](#)). See [Section 5.0](#) for further guidance on matrix extension criteria.

ⁱFor the number of different types of multi-ingredient foods (e.g., salsa) to be tested, consultation with the MMVS is required prior to initiation of the study. Certain types of complex environmental samples may also fall under this category and require MMVS consultation.

^jMust be adjusted to achieve fractional positive results (i.e., the reference and/or alternative methods must yield 50%±25% of tests positive) at this level; the high-level inoculum should be approximately 1 log greater than that used to achieve fractional results. All 5 replicates at the high inoculum should yield positive results. Consult MMVS for inoculation of processed and low moisture food types.

^kIndependent laboratory and multi-laboratory test portions are blind coded randomly by the study coordinator or someone not involved in analyses.

^lPeriod of aging depends on the food or environmental sample 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. Consult with the MMVS for aging and storage of environmental sample types. This requirement may be waived or modified at the discretion of the MMVS if the pathogen is unstable in the matrix. Determination of MPN after aging is encouraged.

^mAn appropriate competitor is one that gives similar reactions in enrichment and detection systems. Natural background microflora can fulfill this requirement if it is present in the matrix at a level 1 log greater than the fractional positive level of the target analyte. Perform aerobic plate counts on all foods tested to determine levels of background microflora. If needed, the competitor should be added to the food/environmental sample prior to splitting into test portions/samples and inoculation with target analyte. This requirement may be waived or modified at the discretion of the MMVS if the analyte is unstable in the matrix.

ⁿValidation studies should use the BAM reference method if available. Other reference methods can be used at the discretion of the MMVS.

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Table 2: General guidelines for validation of qualitative detection methods for microbial analytes with unique isolation and/or enrichment challenges^{a,b}

Criteria	Single-Laboratory Validation Study	Independent Laboratory Validation Study	Multi-Laboratory Validation Study
Participating Laboratory	Originating Laboratory	Collaborator	Collaborators
# of Target Organism (Inclusivity) ^c	TBD ^{d,e}	NA ^f	NA
# of Non-Target Organism (Exclusivity) ^c	TBD ^{d,g}	NA	NA
# of Collaborators Providing Usable Data ^h	NA	1	Minimum of 5 ⁱ
# of Foods ^{i,j,k}	1 or more	1 or more	1 or more
# of Analyte Levels/Food Matrix	3 levels: Minimum of two inoculated levels (one fractional ^l and one 1 log higher) and one uninoculated level	3 levels: Minimum of two inoculated levels (one fractional ^l and one 1 log higher) and one uninoculated level	3 levels: Minimum of two inoculated levels (one fractional ^l and one 1 log higher) and one uninoculated level
Replicates per Food at Each Level Tested	Non-culturable ≥5 Culturable ≥10 for the fractional level (5 each for the uninoculated and high levels)	Non-culturable ≥5 ^{m,n} Culturable ≥10 for the fractional level (5 each for the uninoculated and high levels) ^{m,n}	8 per level ^{m,n}
Aging of Inoculated Samples Prior to Testing ^o	Yes	Yes	Yes
Addition of Competitor Strain ^p	In 1 food at +1 log>target analyte at fractional positive analyte level	In 1 food at +1 log>target analyte at fractional positive analyte level	In 1 food at +1 log>target analyte at fractional positive analyte level
BAM Reference Method Comparison Requirement ^q	Yes, if available	Yes, if available	Yes, if available

^aRecommend method developers consult with the MMVS biostatistician to determine appropriate statistics.

^bSuch examples include but are not limited to RNA food-borne viruses and protozoan parasites.

^cUsing purified analytes tested individually without a food matrix. See [Appendix 4](#) (4.5 and 4.6) for suggested strains. Inclusivity/exclusivity testing is only necessary for new methods and when deemed necessary by the MMVS. Inclusivity/exclusivity testing is unnecessary for method extensions to new matrices, and minor modifications to existing reference methods.

^dTo be determined in consultations with the originating laboratory, the MMVS, and subject matter experts. The originating laboratory must consult with the MMVS prior to initiating study.

^eThe target concentration for testing is 10 to 100 times the LOD₅₀ of the alternative method.

^fNot Applicable.

^gAt growth limit for non-target organisms. Exclusivity non-target organisms are grown in a rich, non-selective medium, where applicable.

^hLabs providing data are required to run study on the same instrument platform.

ⁱWhere circumstance and resources permit.

^jIncludes environmental samples ([Appendix 3](#)). For FDA regulatory use, methods are only valid for matrices that have been tested; the MMVS may require that a new method be validated for 3 foods within a food category (See [Appendix 3](#)). See [Section 5.0](#) for further guidance on matrix extension criteria.

^kFor the numbers of multi-ingredient foods (e.g., salsa) to be tested, consultation with the MMVS is required prior to initiation of the study. Certain types of complex environmental samples may also fall under this category and require MMVS consultation.

^lMust be adjusted to achieve fractional positive results (i.e., the reference and/or alternative methods with preferably 50%±25% of tests positive) at this level; the high-level inoculum should be approximately 1 log greater than that used to achieve fractional results. All 5 replicates at the high inoculum should yield positive results.

^mNumber of required replicates is at the discretion of MMVS. Consult with the MMVS.

ⁿIndependent laboratory and multi-laboratory test portions are blind coded randomly by the study coordinator or someone not involved in analyses.

^oPeriod of aging depends on the food or environmental sample 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. Consult with the MMVS for aging and storage of environmental sample types. This requirement may be waived or modified at the discretion of the MMVS if the pathogen is unstable in the matrix.

^pWhere applicable, an appropriate competitor is one that gives similar reactions in enrichment and detection systems. Natural background microflora can fulfill this requirement if it is present in the matrix at a level 1 log greater than the fractional positive level of the target analyte. Perform aerobic plate counts on all foods tested to determine levels of background microflora. If needed, the competitor should be added to food/environmental sample prior to splitting into test portions/samples and inoculating with target analyte. This requirement may be waived or modified at the discretion of the MMVS if the analyte is unstable in the matrix.

^qValidation studies should use the BAM reference method if available. Other reference methods can be used at the discretion of the MMVS.

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 provides validation criteria for identification/characterization methods for microbial analytes.

Table 3: General guidelines for validation of identification 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,c}	Variable ^{d,e}	16	16
# of Non-target Organism (Exclusivity) ^{b,c}	Variable ^{f,g}	8	8
# of Collaborators Providing Usable Data ^h	NA ⁱ	1	10
Reference Method Comparison Requirement ^j	Yes, if available	Yes, if available	Yes, if available

^aRecommend method developers consult with the MMVS biostatistician to determine appropriate statistics.

^bUsing purified analytes tested individually. See [Appendix 4](#) for suggested strains.

^cIndependent laboratory and multi-laboratory test portions are blind coded randomly by the study coordinator or someone not involved in analyses.

^dThe target concentration for testing is 10 to 100 times the LOD₅₀ of the alternative method. Inclusivity organisms are grown in a rich, non-selective medium.

^e**Inclusivity for non-Salmonella:** Family level, 200 target strains; Genus-level, 150 target strains; Species level, 100 target strains; (Sub)type level, 25 target strains. **Inclusivity for Salmonella:** Genus or species level, 150 serotypes covering all species, subspecies, and major serogroups; *Salmonella* serotype level, at least 5 strains per serotype claimed.

^fAt growth limit (e.g., 10⁹ CFU/ml) for non-target organisms. Exclusivity non-target organisms are grown in a rich, non-selective medium.

^g**Exclusivity for non-Salmonella:** Family level, at least 100 non-target strains; Genus level, at least 100 non-target strains; Species, 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, 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). **Exclusivity for 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.

^hLabs providing data are required to run study on the same instrument platform.

ⁱNot Applicable.

^jStrains used in ID studies will be fully characterized (e.g., biochemically, serologically and/or genetically in sufficient detail for their identity to be known) before use. The reference method can use historical data and should be repeated if discrepancy in alternative method and reference method.

2.3.3 Validation Criteria for Quantitative Detection Methods for Conventional Microbial Analytes

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 provides validation criteria for quantitative methods for microbial analytes.

Table 4: General guidelines for validation of quantitative 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 are not available) ^{c,d}	NA ^e	NA
# of Non-target Organism (Exclusivity) ^b	30 strains ^f	NA	NA
# of Collaborators Providing Usable Data ^g	NA	1	10
# of Foods ^{h,i}	3 or more	1 or more	1 or more
# of Analyte Levels/Food Matrix	4 levels: low, medium, and high inoculum levels ^j and one uninoculated level	4 levels: low, medium, and high inoculum levels ^j and one uninoculated level	4 levels: low, medium, and high inoculum levels ^j 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 ^k	2 replicates per level for a total of 8 replicates ^k
Aging of Inoculated Samples Prior to Testing ^l	Yes	Yes	Yes
Addition of Competitor Strain ^m	In 1 food at +1 log>target analyte at highest analyte level	In 1 food at +1 log>target analyte at highest analyte level	In 1 food at +1 log>target analyte at highest analyte level
BAM Reference Method Comparison Requirement ⁿ	Yes, if available	Yes, if available	Yes, if available
Confirmation of Test Portions ^o	Varies	Varies	Varies

^aRecommend method developers consult with the MMVS biostatistician to determine appropriate statistics.

^bUsing purified analytes tested individually without a food matrix. See [Appendix 4](#) for suggested strains.

^cThe target concentration for testing is 10 to 100 times the LOD₅₀ of the alternative method.

^d100 serotypes for *Salmonella* testing.

^eNot applicable.

^fAt growth limit (i.e., 10⁹ CFU/ml) for non-target organisms. Exclusivity non-target organisms are grown in a rich, non-selective medium.

^gLabs providing data are required to run study on the same instrument platform.

^hWhere circumstance and resources permit. Includes environmental samples ([Appendix 3](#)). For FDA regulatory use, methods are only valid for matrices that have been tested; the MMVS may require that a new method be validated for 3 foods within a food category (See [Appendix 3](#)). See Section 5 for further guidance on matrix extension criteria.

ⁱFor the numbers of multi-ingredient foods (e.g., salsa) to be tested, consultation with the MMVS is required prior to initiation of the study. Certain types of complex environmental samples may also fall under this category and require MMVS consultation.

^jThe 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 alternative method. Consult MMVS for inoculation of heat processed and low moisture food types.

^kIndependent laboratory and multi-laboratory test portions are blind coded randomly by the study coordinator or someone not involved in analyses.

^lPeriod of aging depends on the food or environmental sample 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. Consult with the MMVS for aging and storage of environmental sample types. This requirement may be waived or modified at the discretion of the MMVS if the pathogen is unstable in the matrix. Determination of MPN after aging is encouraged.

^mAn appropriate competitor is one that gives similar reactions in enrichment and detection systems. Natural background microflora can fulfill this requirement if it is present in the matrix at a level 1 log greater than the fractional positive level of the target analyte. Perform aerobic plate counts on all foods tested to determine levels of background microflora. If needed, the competitor should be added to food/environmental sample prior to splitting into test portions/samples and inoculating with target analyte. This requirement may be waived or modified at the discretion of the MMVS if the pathogen is unstable in the matrix.

ⁿValidation studies should use the BAM reference method if available. Other reference methods can be used at the discretion of the MMVS.

^oRepresentative isolates must be confirmed if a specific claim is made. If, for example, 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.

2.3.4 Validation Criteria for Emergency Usage

2.3.4.1 Definition

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 (e.g., surveillance).

This level has the lowest level of validation. One or more labs will have done all the work. Sensitivity and specificity (inclusivity and exclusivity) have 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 is over 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.*

2.3.4.2 Criteria for Qualitative Methods

Table 5 provides validation criteria for qualitative emergency use for microbial analytes.

Table 5: General guidelines for validation of qualitative emergency methods for microbial analytes^a

Criteria	Emergency Use Validation Study
Participating Laboratory	Originating Laboratory
# of target organism (inclusivity) ^{b,c}	TBD ^{d,e}
# of non-target organism (exclusivity) ^c	TBD ^f
# of laboratories providing usable data	1
# of foods	1 or more ^g
# of analyte levels/food matrix	TBD ^d
Replicates per food at each level tested	TBD ^d
Aging of inoculated samples prior to testing	No
Addition of competitor strain	No
BAM Reference Method Comparison Requirement ^h	TBD ^d

^aRecommend method developers consult with the MMVS biostatistician to determine appropriate statistics.

^bAnalytes include conventional and those that present unique isolation/enrichment challenges (examples include but are not limited to RNA food-borne viruses, and protozoan parasites).

^cUsing purified analytes tested individually without a food matrix.

^dTo be determined in consultation with the originating laboratory, the MMVS, and subject matter experts. The originating laboratory must consult with the MMVS prior to initiating study.

^eThe target concentration for testing is 10 to 100 times the LOD₅₀ of the alternative method.

^fAt growth limit (e.g., 10⁹ CFU/ml) for non-target organisms. Exclusivity non-target organisms are grown in a rich, non-selective medium.

^gWhere circumstances and resources permit. Includes environmental samples ([Appendix 3](#)). For FDA regulatory use, methods are only valid for matrices that have been tested.

^hValidation studies should use the BAM reference method if available. Other reference methods can be used at the discretion of the MMVS 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 to the MMVS Chair (listed on the internal [MDVIP Organizational Structure and Membership](#) SharePoint page). External correspondence should be submitted to Microbiology.MVS@fda.hhs.gov.
- As defined in the RSSC SOP Methods Development, Validation, and Implementation Program ([Appendix 2](#)), all multi-laboratory 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 the FDA Food Programs Application for Multi-Laboratory Validation (MLV) Proposal and Final Report Review ([Appendix 2](#)) 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 in MLVs, since a variety of unforeseen circumstances can cause individual collaborator data sets to be rejected.
- The following elements must be addressed in all proposals for method validation studies (in non-emergency use situations).
 - o Intended use or applicability statement for the method being validated.
 - o Applicability of paired vs. unpaired sampling/testing.
 - **Paired study (paired/matched data):** The qualitative reference method and alternative method have a common first enrichment step. In this case only one test portion of a sample is used to obtain a result with the reference method and the alternative method. The incubated broth is then used in the second procedure step of both the reference method and the alternative method. The results from both methods are strongly dependent upon each other.
 - **Unpaired study (unpaired/unmatched data):** The qualitative reference method and alternative method have no common first enrichment step. In this case both the reference and alternative method use their own test portions from a sample. These test portions originate from the same sample. The resulting data are called unpaired but are matched at the level of the sample. The expected variation between results is larger than for a paired study.
 - o Statistical methods must be employed to verify equivalence or improvement of performance between the alternative method and the reference method (or in some cases, the originally validated method). The MMVS 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).
 - o 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.**
 - o Strain selection for inclusivity and exclusivity testing – This facet of the validation

study is to assess the reliability and specificity of the alternative method.

- Individual laboratories within the FP enterprise maintain their own inventories of microbial analyte collections. These collections, strains, and serovars are derived from food surveillance programs, food-borne outbreak investigations, and clinical specimens, and may be available to FP scientists upon request.
 - 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 tested individually. The choice of exclusivity strains should closely reflect related, potentially cross-reactive organisms. Other factors such as virulence, frequency of co-occurrence with the target analyte, and availability should be considered. Exclusivity testing is performed on purified cultures tested individually.
 - Species/strains specified for use in inclusivity and exclusivity panels must be traceable to the source. The source and origin of each species/strain must be documented. Strains do not have to originate from national collections such as ATCC. See [Appendix 4](#) 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 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.
- o Suitability and availability of naturally contaminated samples in the proposed validation study.
 - o Inoculum preparation, spiking methodology, and uniformity of contamination (when artificially contaminated samples will be used).
 - o Sample preparation, naturally occurring microflora, and the requirement for aerobic plate counts (APC) to verify background microflora. **APCs should always be performed on test sample batches.**
 - o 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 the AOAC International-established parameter of one log greater than microbial analyte being tested. Selection of competitive microflora to be used will be done in consultation with the MMVS.
 - o Selection of spiking levels (when artificially contaminated samples will be used).
 - o Matrix aging to assess method robustness.
 - o Microbial analyte stress, cell injury, and matrix-derived inhibition of analyte enrichment/growth.
 - o 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 3](#). Extension of a method to include additional categories of food matrices (matrix extension) will require additional validation studies. See [Section 5.0](#).

- o 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 the reference method (inoculated x g) to composite (inoculated x g + uninoculated x g).
- o Inocula designed to yield fractional positive results. Samples for either the reference method or the alternative 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). False positive and false negative rates will not be determined during the SLV, because the number of replicates is too small.
- Statistical analyses must be employed to test for equivalence or significant differences (superiority) and for data disqualification (see *below*). 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 biostatistician 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:
 - o Negative controls (uninoculated controls) yield a positive outcome—an indicator of lab/operator error.
 - o Deviation from the prescribed method.
 - o Quality control deficiencies (e.g., homogeneity and stability). Statistically supported outliers (quantitative methods).
 - o Failure to achieve fractional results within specified ranges (across all labs/data sets).

3.0 CRITERIA AND GUIDANCE FOR 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 the guidelines for the validation of nucleic acid sequence-based methods located on the FDA [Foods Program Methods Validation Processes and Guidelines](#) page. These criteria apply to all FP laboratories that develop and participate in the validation of targeted nucleic acid sequence-based analytical methods for human and animal food, cosmetics, and veterinary products for Agency-wide implementation in a regulatory capacity.

4.0 CRITERIA AND GUIDANCE FOR VALIDATION AND VERIFICATION OF COMMERCIALY 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 specific use requirements 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 (See [Section 5.1](#)).

4.2 Criteria

4.2.1 *Validated Commercially Available Microbiological Diagnostic Kits*

Commercially available microbiological diagnostic kits are considered validated for use in FDA HFP ORTS laboratories if they have been fully validated against the BAM reference method, or in a full validation (SLV and MLV) against a U.S. reference method monitored and evaluated by a third-party methods validation body (e.g., AOAC). AOAC validated methods must be confirmed to have been validated against a U.S. reference method (MMVS can assist if needed). Commercially available kits validated against non-U.S. reference methods must undergo full validation (See section 4.2.2). AFNOR and MicroVal are examples of validation bodies that use non-U.S. reference methods.

Each lab must perform an in-house verification for the "*first use*" of a validated alternative

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 First-Use Method Verification Requirements (*per FDA ORTS lab*)

- Six replicate test portions of the inoculated matrix and six replicate test portions of the uninoculated 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 alternative method is verified to function in the user's laboratory on any matrix included in the scope of the method. Each commodity to be tested should be spiked with a level close to the detection limit, usually <30 colony forming units (CFU) of bacterial analyte per 25 g of matrix test portion (or inoculum level appropriate for non-culturable analytes) or any other specified sample size (e.g., <30 CFU/375 g composite test portion) 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 ([Table 1](#)).

Note: The matrix chosen for verification should be one routinely tested by the user laboratory. The verification criteria described above apply only for foods and environmental samples which are included in the scope of the method and were part of the SLV and MLV validation studies by an independent accrediting body. The use of such kits for food matrices that were not included in the original validation studies must be preceded by a food matrix verification or extension study ([Section 5.1](#)). 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.

4.2.2 Unvalidated Commercially Available Microbiological Diagnostic Kits

Commercially available microbiological diagnostic kits are not considered validated for use in ORTS laboratories if they have not been fully validated against the BAM reference method, or against a U.S. reference method monitored and evaluated by a third-party methods validation body (see [Section 4.2.1](#)).

Unvalidated methods **must** be validated according to the criteria defined for Agency-developed (FDA) microbiology methods (see [Section 2.2.1](#); 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 be

considered minor and only necessitate verification against the original method. It is recommended that statistical analyses be performed on the verified performance specification to support implementation of the modification. Testing a new matrix in the same sample category ([Appendix 3](#)) as a matrix included in the original or subsequent validation studies is an example of a minor modification ([Section 5.1.1](#), matrix verification).

- More extensive modifications that may affect method performance of the validated original method may be considered major and require either a limited study (SLV or ILV, [Section 2.2.1](#)) or an MLV Study as described in [Table 1](#). Examples of major modifications include (but aren't limited to) changes in sample preparation, media, incubation (time/temperature), or molecular methods (primer/probe, chemistry). Testing a new matrix in a different category ([Appendix 3](#)) than the matrices included in the original or subsequent validation studies is an example of a major modification ([Section 5.1.2](#), matrix extension).

The MMVS will determine the level of validation required for the modifications. The originating laboratory should consult with the MMVS prior to initiating studies.

Specific criteria for method extensions of matrices and platforms to existing methods are described in greater detail in [Sections 5.1](#) and [5.2](#).

5.1 Matrix Extension Validation and Verification

FDA HFP microbiology ORTS laboratories analyze a wide variety of food and environmental samples. Methods used in ORTS laboratories for regulatory purposes must be evaluated for *each* food and environmental sample type ([Appendix 3](#)). FDA regulatory methods are only validated for the foods or environmental samples that were evaluated in the SLV study but it is not possible for an SLV to include all possible matrices or anticipate what matrix or matrices will be involved in emergency situations or outbreak investigations, two scenarios where samples must be analyzed immediately. Methods must be verified for all new matrices. This is to ensure that the new matrix will not produce false positive (matrix is free from cross reactive substances) or false negative (matrix is free of inhibitory substances) results.

As described below, either a verification process or additional validation studies will be required before any given validated method can be used to test foods or environmental samples not included in the original method validation. Close consultation between method developers, laboratory managers, QMS managers and the MMVS 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 a matrix verification or extension is accompanied by additional modifications to the method, an SLV, ILV, or MLV (as described in [Table 1](#)) will be required. This decision will be at the discretion of the MMVS.

5.1.1 Matrix Verification Guidance for New Matrices in the Same Category as Matrices in the Original or Subsequent Validation Studies

Method verification of a new matrix, commonly referred to as matrix verification, refers to instances where a method will be used to test a matrix (or matrices) from the same

category of food or environmental sample types (See [Appendix 3](#)) included in the original or subsequent validation studies. ORTS laboratories will analyze the matrix in question concurrently with a single matrix spike. The matrix spike will consist of 25-375 grams of product spiked with a level close to the detection limit, usually <30 colony forming units (CFU) of bacterial analyte per 25 g of matrix test portion (or inoculum level appropriate for non-culturable analytes) or any other specified sample size (e.g., <30 CFU/375 g composite test portion). Quality control procedures must be followed to ensure the spike is representative of the analytical testing of an actual sample.

Situations where a spike control sample 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. Matrix spikes of 25 g test portions will be sufficient in most cases. Negative results from spiked test portions invalidate the analysis, and the sample must be analyzed using the conventional culture procedure.

FDA ORTS laboratories may continue to perform individual matrix test portion spikes for matrices that have not been fully validated for the method. Matrix spike results will be entered into the field accomplishment computerized tracking system (FACTS), or the automated laboratory information system (ALIS) and data will be evaluated and classified according to specific food and matrix spike results. When a specific matrix has yielded at least seven consecutive positives and no negative results using matrix spikes or a $\geq 95\%$ confidence level (19 of 20 positives) over time, that matrix will be considered verified for the method. The method can then be used by all ORTS laboratories for that specific matrix without further positive spike controls.

ORTS will maintain and update lists detailing the expansion of sample matrices for methods used by ORTS laboratories; matrix lists for *Salmonella* and *Listeria* methods will be posted on the ORTS [Matrix Spike Extension Database](#) SharePoint site.

5.1.2 Matrix Extension Guidance for New Matrices in a Different Category Than Matrices in the Original or Subsequent Method Validation Studies

Method extension to a new matrix, commonly referred to as matrix extension, refers to instances where a method will be used to test a matrix (or matrices) in a different category of food or environmental sample types (See [Appendix 3](#)) included in the original or subsequent validation studies. An SLV and possibly an ILV will be required as described in Tables 1 and 2 to officially extend the scope of the method in the Official Method Compendium.

5.2 Platform Extension

Method extension to a new platform, commonly referred to as platform extension, refers to the proposed use of a new, similarly functioning instrument on an approved method that differs from the platform used in the original validation study. Such platform differences include (but aren't limited to) being of similar function and capacity but from a different manufacturer; from the same manufacturer but with significantly different performance parameters (e.g., 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 first-use method verification process ([Section 4.2.1.1](#)). Other instances, however, may necessitate an SLV or ILV as described in [Table 1](#). For molecular methods, frozen extracts from the original validation study may be used for platform extensions after consultation with 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, refer to internationally recognized Microbiological Validation Guidelines such as AOAC International's Appendix J (<https://doi.org/10.1093/9780197610145.005.010>) or the ISO 16140 series. The validation protocols found in these internationally recognized guidelines do not supersede the requirements of FDA's microbiological methods validation guidelines but can provide methods developers and laboratorians with useful information. Contact MMVS for more information.

APPENDICES

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.

Alternative 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.

Association Française de Normalisation (AFNOR): The AFNOR Group designs and deploys solutions based on voluntary standards around the world. The Group serves the general interest in its standardization activities and provides services in such competitive sectors as training, professional and technical information and intelligence, assessment, and certification.

AOAC International: Originally the Association of Official Agricultural Chemists (AOAC), now known as AOAC International. Including the AOAC Official Methods Analysis (OMA) program, and AOAC Research Institute (AOAC RI). AOAC International's global science and professional support programs support analytical excellence by developing and validating standards and methods, certifying technical performance and proficiency, and providing training and capacity-building programs.

Batch (sample batch): A group of food or environmental samples collected from the same source, produced under identical conditions, and within a single defined time period.

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).

Colony-Forming Unit (CFU): A unit of measure used to estimate the number of viable (living and reproducing) microbial cells in a sample or pure culture, where each unit is defined as a cell or cluster of cells capable of forming a visible colony under specific growth conditions, as determined by serial dilution and plating. This represents viable, culturable units, rather than total cell count.

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).

Environmental Samples: Samples from a surface of equipment or from the production environment, or from water used in the manufacturing process. Examples include (but aren't limited to) swabs and dusts collected from food production facilities, retail establishments, primary production facilities, such as poultry houses and cattle feed lots, and agricultural environments, many kinds of agricultural waters, wastewater, soils, sediments, and animal feces. Environmental samples are listed as a category in [Appendix 3](#).

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 3](#) lists recommended food categories: meat products, poultry, fish and seafood products, fruit- and vegetable-based products, dairy products, chocolate/bakery products, animal feeds, pasta, multi-component foods, environmental samples, 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 3](#) for examples of representative food products.

Food type: An item that is processed, partially processed or unprocessed for consumption. [Appendix 3](#) 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% ($\pm 25\%$) of the total number of replicates in the set. A set of replicate analyses are those replicates analyzed by a method (either reference or alternative). In the context of the entire data set, values outside the prescribed fractional range ($50\% \pm 25\%$) 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.

Independent laboratory validation (ILV) study: An ILV is a study to determine if a method can be successfully performed by a laboratory other than the originating laboratory. An ILV study may be required for method extensions and method modifications that do not require an MLV.

Inoculation: The process of introducing a known amount of the target analyte into the sample matrix.

Bulk inoculation: The target analyte is added into a large, pooled quantity of the sample matrix all at once, aged, and then subdivided into individual test portions for analysis.

Seeded inoculation: The target analyte is added into individual test portions, aged, and then analyzed.

Spiked inoculation: The target analyte is added into individual test portions then analyzed without an aging phase.

In silico: Computational or computer simulated testing that involves searching databases (e.g., GenBank) to compare primer, probe, or sequence designs against known genetic sequences to predict reactivity.

In vitro: Outside of a living organism (e.g., test tube, petri dish), including microbiological living cultures and genetic material (DNA, RNA). Latin for “in glass”.

International Organization for Standardization (ISO): An independent, non-governmental, international standard development organization composed of representatives from the national standards organizations of member countries.

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.

Limit of detection (LOD): See method detection limit below. Example: LOD₅₀ is the level of detection for which 50 % of tests give a positive result.

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 extension: a validation of a food or environmental sample type not included in the original method validation.

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.

MicroVal: an international certification organization for the validation and approval of alternative methods for the microbiological analysis of food and beverages. MicroVal facilitates validation and certification against ISO 16140-2:2016, ISO 16140-6:2019 and other validation standards.

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.

Most Probable Number (MPN): A statistical estimate of the density of viable microorganisms in a sample, derived from the pattern of positive (growth) and negative (no growth) results across

a series of multiple-tube serial dilutions. It represents the most likely concentration of the target analyte based on the Poisson distribution. See [BAM Appendix 2: Most Probable Number from Serial Dilutions for more information](#).

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.

Paired study (paired/matched data): The qualitative reference method and alternative method have a common first enrichment step. In this case only one test portion of a sample is used to obtain a result with the reference method and the alternative method. The incubated broth is then used in the second procedure step of both the reference method and the alternative method. The results from both methods are strongly dependent upon each other.

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.

Quantitative 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 affect 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 (RLOD): The limit of detection of the alternative 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.

Single-laboratory validation (SLV) study: An SLV study is carried out by the originating laboratory to determine if the alternative method performs as well or better than the reference method, if available. This is the first step in the validation process for methods designed for routine regulatory applications.

Source: The origin of a sample batch. 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 wastewater. 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 from different areas of a plot or field. Different sediment sources could be 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 specific 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 that have 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.

Test portion: A representative quantity taken from a test unit (which was selected from the sample batch) for use in the analysis.

Test unit: A specific quantity taken from a sample batch for use in the validation experiment.

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 (International Vocabulary of Basic and General Terms in Metrology; VIM, 1993).

Unpaired study (unpaired/unmatched data): The qualitative reference method and alternative method have no common first enrichment step. In this case both the reference and alternative method use their own test portions from a sample. These test portions originate from the same sample. The resulting data are called unpaired but are matched at the level of the sample. The expected variation between results is larger than for a paired study.

Validation method: The confirmation by examination and the provision of objective evidence that the 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 (first-use method verification, [Section 4.2.1.1](#)). Also, the means used to demonstrate that the method functions (without any adaptation) in the user's laboratory on new matrices in the same category as matrices in the original or subsequent method validation studies (matrix verification, [Section 5.1.1](#)).

Appendix 2: Links to Reference Documents

The reference documents below are available internally on the FDA SharePoint page [MDVIP Charter, SOPs, Guidelines, Forms & Other Documents](#)

Method Validation Subcommittee Charter

FDA Food Programs Application for Multi-laboratory Validation (MLV) Proposal and Final Report Review

Method Development, Validation, and Implementation Program (MDVIP) Standard Operating Procedure

Guidelines for the Validation of Chemical Methods

Guidelines for the Validation of Analytical Methods for Nucleic Acid Sequence-Based Analysis of Food, Feed, Cosmetics and Veterinary Products

Guidelines on the Replacement of Laboratory Consumable Products Prescribed in the FDA Laboratory Analytical Methods Used within the Foods Program

**FDA Microbiology Method Validation Subcommittee
Justification for following food validation guidelines for environmental samples collected from food production and adjacent areas**

Appendix 3: Examples of Food and Environmental Sample Types and Associated Microbiological Analytes

Appendix 3.1: Food Categories Relevant to Pathogenic Microbial Analytes

(Adapted from Table A1 in *Feldsine et al., 2002 J. AOAC Int. 85:1187-1200* and Table A1 in ISO 16140-2:2016)

Food category & type	<i>Yersinia</i> spp.	<i>Clostridium perfringens</i>	<i>Listeria monocytogenes</i>	<i>E. coli</i> O157/ STEC	<i>Staphylococcus aureus</i>	<i>Campylobacter</i> spp.	<i>Salmonella</i> spp.	<i>Bacillus cereus</i>	<i>Cyclospora cayetanensis</i>	<i>Cryptosporidium parvum/ hominis</i>	Hepatitis A Virus/ Norovirus
Meats											
Raw	x			x		x	x	x			
Heat processed			x	x	x		x				
Frozen			x	x			x				
Fermented			x	x							
Cured		x	x		x		x				
Other		Dishes/gravy	Pâté				x				
Poultry											
Raw	x					x	x				
Heat processed							x				
Frozen							x				
Others		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 juices				x			x			x	
Raw	x		x	x		x	x		x	x	x
Heat processed		x									
Frozen			x				x		x	x	x
Dry								x			
Juice/concentrates				x			x				
Low moisture							x				
Nut meats			x	x			x				
Others											
Dairy products											
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 moisture							x				
Dry powder							x				
Milk chocolate							x				
Others					Pastry				Custards		
Pasta											
Uncooked noodles							x				
Multi-component foods*											
Raw	x		x	x	x	x	x	x			

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Food category & type	<i>Yersinia</i> spp.	<i>Clostridium perfringens</i>	<i>Listeria monocytogenes</i>	<i>E. coli</i> O157/ STEC	<i>Staphylococcus aureus</i>	<i>Campylobacter</i> spp.	<i>Salmonella</i> spp.	<i>Bacillus cereus</i>	<i>Cyclospora cayentanensis</i>	<i>Cryptosporidium parvum/ hominis</i>	Hepatitis A Virus/ Norovirus
Heat processed		X	X	X	X		X	X			
Frozen			X	X	X		X	X			
Dry					X		X	X			
Mayonnaise-based salads/spreads			X	X		X	X				
Condiments, dips, dressings			X	X			X				
Animal feeds											
Low moisture							X				
Pet food							X				
Environmental											
Production area (equipment, surfaces, drains)	X	X	X	X	X	X	X	X			X
Water (processing/agricultural)		X	X	X	X	X	X	X	X		X
Soils/sediments			X	X		X	X				X
Animal Feces			X	X		X	X				
Miscellaneous											
Spices		X					X				
Flour			X	X		X	X				
Egg and derivatives				X			X				
Cereals/rice								X			

*Validation of multi-component foods may require the testing of multiple brands/formulations, consult MMVS.

Appendix 3.2: AOAC Food Categories Relevant to Non-pathogenic Microbial Analytes (Adapted from Table A2 in Feldsine et al., (2002) Journal AOAC Int. 85(5) 1198-1199)

Food category & type	Yeasts and molds	Lactic acid bacteria	Total viable counts	Coliforms	<i>Escherichia coli</i>
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
Others					
Seafood					
Raw	X	X	X	X	X
Heat processed		X	X	X	
Frozen	X		X	X	X
Smoked	X	X	X	X	
Fruits and 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		

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Food category & type	Yeasts and molds	Lactic acid bacteria	Total viable counts	Coliforms	<i>Escherichia coli</i>
Juice/concentrates	x	x	x		
Low moisture	x				
Dairy products					
Raw	x	x	x	x	x
Heat processed			x	x	
Frozen	x		x	x	x
Fermented	x				x
Dry			x	x	
Chocolate/bakery products					
Low moisture/IMF	x		x	x	
Dry			x	x	
Milk chocolate	x		x	x	
Animal feeds					
Low moisture	x		x	x	
Dry pet food	x			x	x
Pasta					
Uncooked noodles	x		x	x	
Miscellaneous					
Dressings	x	x	x	x	x
Spices			x		x
Mayonnaise	x	x	x		x
Egg and derivatives			x	x	
Cereals/rice			x	x	

Representative Food Types 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

Environmental:

Production facility areas/equipment, retail establishments, poultry houses, cattle feed lots, agricultural water, processing water, waste water, soils, sediments, dusts, and animal feces.

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 4: Strains and Serovars for Inclusivity and Exclusivity Panels

- This appendix is meant to serve as a guide or starting point for the method developer as they construct exclusivity and inclusivity 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 to their exact identity. Strains are not restricted to national culture collections, such as ATCC, but they must be fully traceable to the original source.

Appendix 4.1: *Escherichia coli* O157:H7 inclusivity and exclusivity panels

E. coli O157:H7 Inclusivity Panel

<i>E. coli</i>	Serotype	Genotype		
		stx1	stx2	uidA-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			
	<i>E. coli</i> , stx1 insert			
	O14:H19	-	+	-
	O28:H35			
	O48:H21			
	O55:H7			
	O104:H21			

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<i>E. coli</i>	Serotype	Genotype		
		stx1	stx2	uidA-O157:H7/H-
	O121:H19			
	O165:H25			
	<i>E. coli</i> , stx2 insert			
Non-toxigenic <i>E. coli</i>	Non-O157:H7	-	-	-
	O55:H7			
	O157:H16			
	O157:H45			

E. coli O157:H7 Exclusivity Panel (Source ATCC and SEAHAFL)

	Isolate #	Serotype	Genotype		
			stx1	stx2	uidA-O157:H7/H-
<i>Alcaligenes faecalis</i>	ATCC 8750		-	-	-
<i>Aeromonas hydrophila</i>	ATCC 7965		-	-	-
<i>Citrobacter freundii</i>	ATCC 8090		-	-	-
<i>Enterobacter cloacae</i>	6288		-	-	-
<i>Hafnia alvei</i>	ATCC 13337		-	-	-
<i>Klebsiella pneumoniae</i>	ATCC 13883		-	-	-
<i>Lactobacillus sp.</i>	6286		-	-	-
<i>Leclercia adecarboxylata</i>	ATCC 23216		-	-	-
<i>Listeria innocua</i>	6273		-	-	-
<i>Listeria ivanovii</i>	6274		-	-	-
<i>Listeria monocytogenes</i>	6272		-	-	-
<i>Listeria seeligeri</i>	6275		-	-	-
<i>Listeria welshimeri</i>	6276		-	-	-
<i>Morganella morganii</i>	13b67		-	-	-
<i>Pasteurella aerogenes</i>	ATCC 27883		-	-	-
<i>Proteus mirabilis</i>	ATCC 7002		-	-	-
<i>Rhodococcus equi</i>	6281		-	-	-
<i>Salmonella enterica</i>	2571	Lansing	-	-	-
<i>Salmonella enterica</i>	2644	Typhimurium	-	-	-
<i>Salmonella enterica</i>	18q11	Urbana	-	-	-
<i>Shigella boydii</i>	13g18		-	-	-
<i>Shigella dysenteriae</i>	ATCC 29026		+	-	-
<i>Shigella flexneri</i>	13g19		-	-	-
<i>Shigella sonnei</i>	13g01		-	-	-
<i>Staphylococcus aureus</i>	ATCC 25923		-	-	-
<i>Streptococcus pyogenes</i>	ATCC 19615		-	-	-
<i>Vibrio cholerae</i>	ATCC 15748		-	-	-
<i>Vibrio parahaemolyticus</i>	ATCC 17802		-	-	-
<i>Vibrio vulnificus</i>	6279		-	-	-
<i>Yersinia enterocolitica</i>	1269		-	-	-

Appendix 4.2: *Salmonella* Inclusivity Panels

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

Salmonella: Subspecies Set

SAFE Designation	Original Designation	Serotype	Subspecies
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

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SAFE Designation	Original Designation	Serotype	Subspecies
42	ST-16	IV 16:z4,z32:-	IV
43	ST-21	IV 40:g,z51:-	IV
44	ST-22	IV 40:z4,z24:-	IV
45	94-0708	48:i:-	<i>S. bongori</i> (formerly subsp V)
46	95-0123	40:z35:-	<i>S. bongori</i> (formerly subsp V)
47	96-0233	44:z39:-	<i>S. bongori</i> (formerly subsp V)
48	CNM-256	60:z41:-	<i>S. bongori</i> (formerly subsp V)
49	CNM-262	66:z41:-	<i>S. bongori</i> (formerly subsp V)
50	95-0321	48:z35:-	<i>S. bongori</i> (formerly subsp V)
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

Salmonella enterica: 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

***Salmonella enterica*: 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

Appendix 4.3: *Listeria monocytogenes* inclusivity and exclusivity Panels***Listeria monocytogenes* Inclusivity Panel**

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

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Isolate #	Isolate information	Serology
3315	Environmental Isolates (swab)	1a1
3360		1a1
3286		1a2
3308		1a2
ATCC 15313	Other Isolates	1
V-7		1a1
ATCC 19114		4a
KC 1710		4a7,9
Scott A		4b6
ATCC 19116		4c
ATCC 19115		

Listeria monocytogenes Exclusivity Panel

Organism	Isolate #	Organism	Isolate #
<i>Listeria innocua</i>	3107	<i>Listeria welshimeri</i>	2230
	3124		2231
	3516		3425
	3654		3441
	3758		3659
	6273		15b05
	3181		15b06
	3270		15b16
	3390		15b46
	3392		15b48
	3552		15b50
	3757		ATCC 8750
	15a93		13d26
	15a94		6251
	15a95		18g53
	15b30		6365
<i>Listeria ivanovii</i>	15b31	<i>Alcaligenes faecalis</i>	13a80
	15b51		13d64
	15a92		13d66
	ATCC 33090		6410
	2244		6271
	3106		6282
	3417		6286
	6274		13d65
	15a96		13b67
	15a97		6281
<i>Listeria seeligeri</i>	15a98	<i>Citrobacter freundii</i>	ATCC 6539
	15b24		SAFE 80
	ATCC 19119		6270
	2232		6290
	2233		13g18
	2243		13c94
	2302	<i>Enterobacter cloacae</i>	13g19
	3110		13g01

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Organism	Isolate #	Organism	Isolate #
	3126	<i>Staphylococcus aureus</i>	ATCC 25923
	3389	<i>Streptococcus pyogenes</i>	ATCC 19615
	3423	<i>Vibrio cholerae</i>	6277
	3439	<i>Vibrio parahaemolyticus</i>	6278
	3451	<i>Vibrio vulnificus</i>	6279
	3517	<i>Yersinia enterocolitica</i>	1269
	3531	<i>Yersinia enterocolitica</i>	1270
	3656		
	6275		
	15b07		
	15b08		
	15b09		
	15b26		
	15b28		
	15b49		

Appendix 4.4: Shigella Inclusivity and Exclusivity Panels

Shigella Inclusivity Panel

Species (Group)	Serotype
<i>Shigella boydii</i> (C)	1
	2
	3
	4
	5
	6
	7
	8
	9
	10
	11
	12
	14
	15
	16
	17
	18
	19
<i>Shigella dysenteriae</i> (A)	1
	2
	3
	4
	5
	6
	7
	8
	9
	10
	11
	12
	13
	14
	15
<i>Shigella flexneri</i> (B)	1a
	1b
	1d
	2a
	2b
	3a
	3b

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Species (Group)	Serotype
	4a
	4av
	4b
	5a
	5b
	6
	X
	Y
<i>Shigella sonnei</i> (D)	1

Shigella Exclusivity Panel

Bacteria Strain	Isolate #	Source*
<i>Acinetobacter baumannii</i>	19606	ATCC
<i>Aeromonas caviae</i>	15468	ATCC
<i>Aeromonas hydrophila</i>	7966	ATCC
<i>Bacillus licheniformis</i>	12759	ATCC
<i>Bacillus sphaericus</i>	4525	ATCC
<i>Bacillus stearothermophilus</i>	12016	ATCC
<i>Bacillus subtilis</i>	6633	ATCC
<i>Bordetella bronchiseptica</i>	10580	ATCC
<i>Burkholderia cepacia</i>	25608	ATCC
<i>Citrobacter freundii</i>	255	IRVLHAFL
<i>Citrobacter freundii</i>	food isolate	IRVLHAFL
<i>Citrobacter freundii</i>	68	MNDAL
<i>Citrobacter youngi</i>	food isolate	IRVLHAFL
<i>Clostridium sporogenes</i>	11437	ATCC
<i>Edwardsiella tarda</i>	254	IRVLHAFL
<i>Enterobacter aerogenes</i>	13048	ATCC
<i>Enterobacter aerogenes</i>	11	VADCLS
<i>Enterobacter cancerogenus</i>	food isolate	IRVLHAFL
<i>Enterobacter cloacae</i>	260	IRVLHAFL
<i>Enterobacter cloacae</i>	71	MNDAL
<i>Enterococcus durans</i>	6056	ATCC
<i>Enterococcus faecalis</i>	7080	ATCC
<i>Erysipelothrix rhusiopathiae</i>	19414	ATCC
Enterotoxigenic <i>E. coli</i>	H10407	OAMT
Enterotoxigenic <i>E. coli</i>	C600/pEWD299	CFSAN
Enterotoxigenic <i>E. coli</i>	65	MNDAL
<i>Escherichia coli</i> O157:H7	43890	ATCC
<i>Escherichia coli</i> O157:H7	43888	ATCC
<i>Escherichia coli</i> O157:H7	43895	ATCC
<i>Escherichia coli</i> O157:H7	68-98	CDC
<i>Escherichia coli</i> O157:H7	24-98	CDC

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Bacteria Strain	Isolate #	Source*
<i>Escherichia coli</i> O157:H7	20-98	CDC
<i>Escherichia coli</i> O157:H7	16-98	CDC
<i>Escherichia coli</i> O157:H7	63	MNDAL
<i>Escherichia coli</i> O157:H7	4	VADCLS
<i>Escherichia coli</i> O157:H44	26	VADCLS
<i>Escherichia coli</i> O111:NM	04.SB.00067	OCPHL
<i>Escherichia coli</i> O143:H4	05.SB.00141	OCPHL
<i>Escherichia coli</i>	8739	ATCC
<i>Escherichia coli</i>	25922	ATCC
<i>Escherichia coli</i> (hemo +)	food isolate	IRVHAFL
<i>Escherichia coli</i> (hemo +)	28	VADCLS
<i>Escherichia coli</i> (sorbitol –)	food isolate	IRVHAFL
<i>Escherichia coli</i> (sorbitol –)	food isolate	IRVHAFL
<i>Escherichia coli</i>	64	MNDAL
<i>Escherichia coli</i>	74	MNDAL
<i>Escherichia coli</i>	8	VADCLS
<i>Klebsiella pneumoniae</i>	13883	ATCC
<i>Klebsiella pneumoniae</i>	75	MNDAL
<i>Klebsiella oxytoca</i>	66	MNDAL
<i>Leclercia adecarboxylata</i>	23216	ATCC
<i>Leclercia adecarboxylata</i>	73	MNDAL
<i>Listeria innocua</i>	33090	ATCC
<i>Listeria ivanovii</i>	19119	ATCC
<i>Listeria monocytogenes</i>	19115	ATCC
<i>Listeria monocytogenes</i>	H2446	CDC
<i>Listeria monocytogenes</i>	H8393	CDC
<i>Listeria monocytogenes</i>	H8494	CDC
<i>Listeria monocytogenes</i>	H8395	CDC
<i>Listeria seeligeri</i>	35967	ATCC
<i>Morganella morganii</i>	257	IRVHAFL
<i>Paenibacillus polymyxa</i>	7070	ATCC
<i>Pantoea agglomerans</i>	food isolate	IRVHAFL
<i>Pasteurella aerogenes</i>	27883	ATCC
<i>Plesiomonas shigelloides</i>	51903	ATCC
<i>Proteus mirabilis</i>	7002	ATCC
<i>Proteus mirabilis</i>	food isolate	IRVHAFL
<i>Proteus hauseri</i>	13315	ATCC
<i>Proteus vulgaris</i>	69	MNDAL
<i>Providencia alcalifaciens</i>	51902	ATCC
<i>Providencia rettgeri</i>	76	MNDAL
<i>Providencia stuartii</i>	257	IRVHAFL
<i>Pseudomonas aeruginosa</i>	27853	ATCC
<i>Pseudomonas aeruginosa</i>	9027	ATCC
<i>Pseudomonas aeruginosa</i>	67	MNDAL

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Bacteria Strain	Isolate #	Source*
<i>Pseudomonas mendocina</i>	food isolate	IRVHAFL
<i>Rhodococcus equi</i>	6939	ATCC
<i>Salmonella</i> Gaminara	8324	ATCC
<i>Salmonella</i> IIIb 47:i:z53:z57	12325	ATCC
<i>Salmonella</i> Abortusequi	9842	ATCC
<i>Salmonella</i> IIIb 60:r:e,n,x,z15	29934	ATCC
<i>Salmonella</i> II 4,12:b:-	17	SAFE
<i>Salmonella</i> Mbandaka	253	IRVHAFL
<i>Salmonella</i> Tennessee	249	IRVHAFL
<i>Salmonella</i> Lexington	248	IRVHAFL
<i>Salmonella</i> Havana	241	IRVHAFL
<i>Salmonella</i> Baidon	61-99	CDC
<i>Salmonella</i> II 48:d:z6	12	SAFE
<i>Salmonella</i> IIIa 62:g,z51:-	24	SAFE
<i>Salmonella</i> IV 50:g,z51:-	35	SAFE
<i>Salmonella</i> Braenderup	H 9812	CDC
<i>Salmonella</i> Enteritidis	59	MNDAL
<i>Salmonella</i> Heidelberg	60	MNDAL
<i>Salmonella</i> Kentucky	61	MNDAL
<i>Salmonella</i> Newport	62	MNDAL
<i>Salmonella</i> Typhimurium	30	VADCLS
<i>Serratia liquefaciens</i>	27592	ATCC
<i>Serratia liquefaciens</i>	70	MNDAL
<i>Sphingomonas paucimobilis</i>	72	MNDAL
<i>Staphylococcus aureus</i>	6538	ATCC
<i>Staphylococcus aureus</i>	25923	ATCC
<i>Staphylococcus epidermidis</i>	14990	ATCC
<i>Staphylococcus xylosus</i>	29971	ATCC
<i>Streptococcus equi</i> subsp. <i>equi</i>	9528	ATCC
<i>Streptococcus gallolyticus</i>	9809	ATCC
<i>Streptococcus pyogenes</i>	19615	ATCC
<i>Vibrio cholerae</i>	14035	ATCC
<i>Vibrio cholerae</i>	14033	ATCC
<i>Vibrio parahaemolyticus</i>	17802	ATCC
<i>Vibrio vulnificus</i>	27562	ATCC
<i>Yersinia enterocolitica</i>	51871	ATCC
<i>Yersinia enterocolitica</i>	27729	ATCC
<i>Yersinia kristensenii</i>	33639	ATCC

*ATCC: American Type Culture Collection

CDC: Centers for Disease Control and Prevention

IRVHAFL: Irvine Human and Animal Food Laboratory, FDA

MNDAL: Minnesota Department of Agriculture Laboratory

OCPHL: Orange County Public Health Laboratory, CA

OAMT: Office of Applied Microbiology and Technology, FDA

SAFE: See Appendix 4.2

VADCLS: Virginia Division of Consolidated Laboratory Services

Appendix 4.5: Food-borne RNA Viruses Inclusivity and Exclusivity Panels

These panels were developed and adopted by the FDA BAM Council, 2009-2015. Updated by MMVS 2026.

Food-borne RNA Virus Inclusivity/Exclusivity Recommendations^a

Target	Panel	Number of Strains
Norovirus	Inclusivity ^b	30
	Exclusivity ^c	40
Hepatitis A	Inclusivity ^d	20
	Exclusivity ^e	40
Enterovirus	Inclusivity ^f	30
	Exclusivity ^g	40

^aFollowing Table 2 of the guidelines above

Norovirus Panels

Human Norovirus Inclusivity Panel (^bshould include the following strains):

10 strains Genogroup I
20 strains Genogroup II
Strains of Genogroups IV, VIII, and IX if available

Norovirus Exclusivity Panel (^cshould include the following strains or equivalents):

Animal Norovirus Genogroups if available	such as Genogroups III, GIV, GV, GVI, GVIII, and GIX
Hepatitis A HM175/18f (subgenotype 1B)	ATCC #VR-1402
Hepatitis A HAS-15 (subgenotype 1A)	ATCC #VR-2281
Hepatitis E (synthetic RNA)	ATCC #VR-3258SD
Human adenovirus 11	ATCC #VR-849
Human enterovirus 71	ATCC #VR-1432
Human sapovirus (synthetic RNA)	ATCC #VR-3237SD
Feline calicivirus	ATCC #VR-2057
Murine calicivirus (Murine norovirus 1)	ATCC #VR-1937
Coxsackievirus A	ATCC #VR-1007 (or equivalent such as ATCC VR-850)
Echovirus 1	ATCC #VR-1038
Rotavirus;	ATCC #VR-2018
Astrovirus	ATCC #VR-1936 or #VR-3238SD

Campylobacter spp. (1)
Escherichia coli (1)
Salmonella enterica (1)
Shigella spp. (1)
Vibrio spp. (1)
Listeria spp. (1)

Hepatitis A Virus Panels

Hepatitis A Inclusivity Panel (^dshould 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

Hepatitis A Virus Exclusivity Panel (^eshould include the following strains or equivalents):

Norovirus genogroup I	
Norovirus genogroup II	
Hepatitis E (synthetic RNA)	ATCC #VR-3258SD
Human adenovirus 11	ATCC #VR-849
Human enterovirus 71	ATCC #VR-1432
Human sapovirus (synthetic RNA)	ATCC #VR-3237SD
Coxsackievirus A	ATCC #VR-1007 (or equivalent such as ATCC VR-850)
Echovirus 1	ATCC #VR-1038
Rotavirus	ATCC #VR-2018
Feline calicivirus	ATCC #VR-2057
Murine calicivirus (Murine norovirus 1)	ATCC #VR-1937
Astrovirus	
<i>Escherichia coli</i> (1)	
<i>Campylobacter</i> spp. (1)	
<i>Salmonella enterica</i> (1)	
<i>Shigella</i> spp. (1)	
<i>Vibrio</i> spp. (1)	
<i>Listeria</i> spp. (1)	

Enterovirus Panels

Enterovirus Inclusivity Panel (^fshould include the following strains):

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

Enterovirus Exclusivity Panel (^gshould include the following strains or equivalents):

norovirus genogroup I	
norovirus genogroup II	
Hepatitis A HM175/18f (subgenotype 1B)	ATCC #VR-1402 (or equivalent)
Hepatitis A HAS-15 (subgenotype 1A)	ATCC #VR-2281 (or equivalent)
Hepatitis E (synthetic RNA)	ATCC #VR-3258SD
Human adenovirus 11	ATCC #VR-849
Human enterovirus 71	ATCC #VR-1432
Human sapovirus (synthetic RNA)	ATCC #VR-3237SD
Coxsackievirus A	ATCC #VR-1007 (or equivalent such as ATCC VR-850)
Echovirus 1	ATCC #VR-1038
Rotavirus	ATCC #VR-2018 (or equivalent)
Feline calicivirus	ATCC #VR-2057
Murine calicivirus (Murine norovirus 1)	ATCC #VR-1937
<i>Campylobacter</i> spp. (1)	
<i>Escherichia coli</i> (1)	
<i>Salmonella enterica</i> (1)	
<i>Shigella</i> spp. (1)	
<i>Vibrio</i> spp. (1)	
<i>Listeria</i> spp. (1)	

Appendix 4.6: Protozoan Parasites

A. *Cyclospora cayetanensis*

Inclusivity Panel

As many geographic and outbreak isolates as are available

Exclusivity Panel

Cyclospora spp.

C. cercopitheci

C. colobi

C. papionis

Eimeria spp.

E. acervulina

E. bovis

E. brunetti

E. maxima

E. mitis

E. mivati

E. necatrix

E. nieschulzi

E. praecox

E. tenella

Additional Microorganisms

Cryptosporidium spp.

Apicomplexa

Bacterial isolates

B. *Cryptosporidium* spp.

Inclusivity Panel

C. hominis

C. parvum (multiple strains available)

Exclusivity Panel

C. baileyi

C. canis

C. cuniculus

C. felis

C. meleagridis

C. muris

C. serpentis

Cyclospora spp.

Apicomplexa

Bacterial isolates