DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: September 8, 2011

From: Foods Program Science and Research Steering Committee

Subject: Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods

To: Foods Program Executive Committee

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

As a regulatory agency tasked with ensuring the safety of the nation's food supply, it is imperative that the analytical methods our laboratories employ for surveillance, compliance and outbreak investigations continue to meet the highest standards of review and performance. Development of standardized validation requirements for all regulatory methods used to detect microbial pathogens and chemical and radiological contaminants used in our laboratories is a critical step in ensuring that we continue to meet the highest standards possible.

The attached document, now formally adopted by the SRSC, establishes those requirements that must be fulfilled in the evaluation of microbiological methods to be used in our testing laboratories. It also establishes performance evaluation criteria (verification and validation) necessary for the use of commercially-available microbiological diagnostic kits and platforms. In the near future, these guidelines will be posted in the appendices of the on-line version of the Bacteriological Analytical Manual. Additional venues for publication and dissemination of these guidelines are being explored and will be announced when they become available. Please share this microbial methods validation standard operating procedure with anyone who may be conducting or supervising microbial methods validation projects or otherwise needs to be aware of these new requirements.

Shortly, a method validation sub-committee will be constituted and charged with providing guidance and oversight to all validation studies. In the interim, all inquiries pertaining to method validation for microbial food-borne contaminants should be addressed directly to your representative on the SRSC.

Thank you,

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Center for Food Safety and Applied Nutrition
Office of Regulatory Science
Office of Food Safety
Office of Applied Research and Safety Assessment

Center for Veterinary Medicine
Office of Research

National Center for Toxicological Research
Division of Microbiology

Office of Regulatory Affairs
Division of Field Science
ORA Cadre of Microbiology Subject Matter Experts

FDA Methods Validation Guidelines for Microbial Pathogens

US Food & Drug Administration
Office of Foods

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

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I. INTRODUCTION

Purpose

The Foods Program within the U.S. Food & Drug Administration is responsible for ensuring the safety of the nation's food supply. FDA accomplishes this through education; inspection; data collection; standards setting; prompt investigation of outbreaks; and, enforcement actions when appropriate. The effectiveness of the Foods Program 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 Office of Foods (OF) through the Science and Research Steering Committee (SRSC) has established these criteria by which all Food Program microbiological methods shall be evaluated and validated.

Scope

These criteria apply to all FDA laboratories that develop and participate in the validation of analytical food methods for Agency-wide implementation in a regulatory capacity. This includes all research laboratories, and field labs where analytical methods may be developed or expanded for potential regulatory use. At the time of final approval by OF and SRSC, this document will supersede all other intra-agency documents pertaining to food-related method validation criteria for microbial analytes.

Administrative Authority and Responsibilities

All criteria established in this document for analytical method validation have been adopted and approved by the OF and the SRSC.

The Method Validation Subcommittee

Upon approval of these guidelines, the SRSC will authorize the formation of a Methods Validation Subcommittee (MVS) to serve as the governing body for all method validation concerns. The overarching responsibility of the MVS is to provide guidance and oversight to all Foods Program laboratories during the method development and validation process and will be the responsible authority for recommendations, evaluations and final approval of all validation studies.

Under SRSC guidance, a specific set of charges will be established and will include provisions for:

- Representation from CFSAN, ORA, CVM, and NCTR
- A biostatistician as a permanent member of the MVS to provide expertise for all statistical needs.
- A mandate to coordinate with the FDA Bacteriological Analytical Manual Council (BAM Council) for all method validations studies intended to support adoption of a method by the BAM.

Upon enactment of the charges, the MVS will immediately consult with Center QA/QC managers, to adopt SOP's for governing all administrative processes needed for emergency and non-emergency method validation proposals and studies.

General Responsibility of the Originating Laboratory

The originating (developing) laboratory must work in close consultation with the MVS throughout the validation process. It will be the responsibility of the originating laboratory to include their respective QA/QC manager in all aspects of the validation process and to ensure proper adherence to all criteria described in this document.

Method Validation Definition

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

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

Applicability

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

- Qualitative assays
- Analyte
    - Bacteriological, *e.g.*
        - *Salmonella* spp.
        - Pathogenic *Escherichia coli*
        - *Listeria monocytogenes*

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

Requirements

Method validation shall be required for:

- Submission of a new or original method.
- Expansion of the scope of an existing method to include additional analytes.
- Changes in intended use *i.e.* screening or confirmatory.
- Platform extensions or significant parameter changes *e.g.* enrichment times, adaptation to another real-time PCR thermal cycler).
- Matrix extensions. The verification of method performance with a new matrix is intended to assure that the new matrix will produce neither high false positive rates (matrix is free from cross reactive substances) nor high false negative

rates (matrix is free of inhibitory substances)as defined for the level of validation required and the intended use of the method.

- Modification of a method that may alter its performance specifications. This includes: changes to the fundamental science of an existing method, equivalence issues such as substitutions of reagents/apparatus, changes to some instrument/platform parameters, changes to time/temperature incubation periods, or enrichment media. All but the most minor of changes should be evaluated for effects on method performance.
- In cases where the sample preparation and/or the extraction procedure/analytical method is modified from the existing test procedure and protocol, the new method should demonstrate that the modifications do not adversely affect the precision and accuracy or bias of the data obtained.
- Modification of a method's performance range (*e.g.* specificity, sensitivity) beyond previously validated levels.

II. CRITERIA AND GUIDANCE FOR THE VALIDATION OF FDA-DEVELOPED METHODS

This section provides validation criteria and guidance for FDA-developed or extensively modified, existing methods (see *Section I-Requirements,* for general examples). All methods will be validated to one of four distinct levels that range from "urgent usage" (Level 1) to full collaborative (Level 4). Based on the criteria described below; however not all methods will or should be validated to meet the requirements of a Level 4: full collaborative study.

Definitions

The Reference Method

The reference method is defined as that method by which the performance of an alternate method is measured or evaluated. Validation studies must include comparison to a recognized reference method to demonstrate equivalence or increased performance, the significance of which must be determined statistically. For bacterial analytes, reference methods are generally culture-based and result in a pure isolate. The FDA Bacteriological Analytical Manual (BAM), the USDA Microbiology Laboratory Guidebook (MLG) and ISO culture methods contain recognized reference culture methods. 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 MVC will be necessary to define the most appropriate reference method. ***All*** new methods ***must*** be validated against the agreed-upon reference method.

Originating Laboratory

The laboratory that developed the and has initiated the validation effort.

Collaborating Laboratory

Laboratory (or laboratories) involved in method validation studies.

Method Validation Levels

Within the FDA foods program, method validation exercises confirm by examination (and the provision of objective evidence) that the particular requirements for a method have been fulfilled. All methods used by the FDA in support of its regulatory and compliance roles must be validated by the accompanying criteria. These criteria serve to demonstrate that a method can detect, identify and potentially quantify an analyte or analytes to a defined standard of performance. Four levels of performance are defined. The hierarchy of scrutiny will provide general characteristics on the method's utility and insights for its intended use, the assessed risk, and the food-borne illness potential for an analyte-matrix pairing.

Level One
This level has the lowest level of validation, with all the work done by one lab. The inclusivity and exclusivity has been tested, but by a limited number of strains. The analyte was tested at a level based on the intended use of the method, with just normal background flora. There is no aging of the artificially-inoculated samples and no comparison to an existing reference culture method. The expectation would be for the originating lab to continue to conduct further testing to eventually elevate the method to a higher level of validation.

> **Intended Use:** Emergency needs. A method developed for the detection of an analyte, or a matrix not previously recognized or identified as a threat to food safety or public health. As the first level in the development of any method designed for regulatory use; performance of the method at this level of scrutiny 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, Level 1 criteria should be followed as closely as the situation will allow. Representatives of the MVS and Agency subject matter experts (SMEs) should be in close consultation with the originating laboratory. Once the crisis has past and it has been determined that there is a need for further validation, procedures outlinde in this document will be followed.*

Level Two
This is a more robust study. The originating lab has done a more comprehensive initial study, with inclusivity/exclusivity levels at the AOAC Collaborative Study level. If possible, a comparison has been done to an existing reference culture method. One other independent laboratory has participated in the collaborative study. Some of the criteria of the study are at a lower level than the AOAC Collaborative Study, but still appropriate for the developing method at this stage.

> **Intended Use:** Emergency needs. Slightly higher false-positive rates may be acceptable as all samples analyzed with methods validated to this level will require confirmatory testing.

Level Three
Most of the criteria followed by the originating lab are at the AOAC level, including inclusivity/exclusivity, analyte levels, competitor strains, aging, and comparison to existing method when available. The additional collaborating labs follow many of the criteria of an AOAC collaborative study.

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

Level Four
This validation level has criteria that currently exceed AOAC Collaborative Study levels adopted in 2006. Though a revised set of AOAC validation guidelines are expected to be published in 2011, any FDA-developed method reaching this level of validation ***should be*** eligible for recognition and adoption by the AOAC.

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

Criteria

Tables 1-4 highlight validation study parameters for the four defined validation levels and include distinctions in requirements for originating (Table 1 and 3) and collaborating laboratories (Table 2 and 4).

Tables 1 and 2 pertain principally to conventional bacterial pathogens (and other pathogenic microorganisms) that meet the following general characteristics:

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

Tables 3 and 4 highlight validation scenarios for microbial pathogens characterized as difficult to isolate, limited resources for extensive inclusivity and exclusivity studies, and either non-culturable for enrichment purposes or, enrichment cannot be accomplished in a timely manner.

FDA Methods Validation Guidelines for Microbial Pathogens

Table 1- General Qualitative Guidelines for Microbial Analytes-Originating Laboratory Requirements*

Originating Laboratory Criteria	Level One: Urgent usage	Level Two: Independent lab validation	Level Three: Multiple lab collaborative	Level Four: Full collaborative study	†AOAC Collaborative Study
# of target organism (inclusivity)	10 (20 for Salmonella)[a]	50 (unless 50 aren't available)[a,b]	50 (unless 50 aren't available)[a,b]	50 (unless 50 aren't available)[a,b]	50[a,b]
# of non-target organism (exclusivity)	10 strains[c]	30 strains[c]	30 strains[c]	30 strains[c]	30 strains[c]
# of foods	1 or more[d]	1 or more[d]	1 or more[d]	1 or more[d]	Up to 20 foods[e]
# of analyte levels/food matrix[f]	set level based on intended use and negative control	One inoculated level[f] and uninoculated level	One inoculated level[f] and uninoculated level	One inoculated level[f] and uninoculated level	One inoculated level[f] and uninoculated level
Replicates per food at each level tested	20	20	20	20	20
Aging of inoculated samples prior to testing	No	Yes	Yes[g]	Yes[g]	Yes[g]
Addition of competitor strain[h]	Normal background flora	In 1 food at +1 log>analyte at fractional positive[f] analyte level	In 1 food at +1 log>analyte at fractional positive[f] analyte level	In 1 food at +1 log>analyte at fractional positive[f] analyte level	In 1 food at +1 log>analyte at fractional positive[f] analyte level
Comparison to recognized method	No	Yes, if available	Yes, if available	Yes, if available	Yes, if available

*Derived from microbiology method validation criteria originally developed by the BAM Council, 2007-2008.
[a]Each at 10^3 CFU/ mL (g) for molecular methods following the method protocol (1 $\log_{10}$ above the LOD for other methods).
[b]100 serotypes for Salmonella testing
[c]At 10^3 CFU/mL for non-target organisms grown in a non-selective rich medium
[d]For FDA regulatory use, methods are only valid for foods that have been tested; validation can be extended to other foods by further testing
[e]Depends on applicability of method
[f]Must be adjusted to achieve fractional positive results (one or both methods give 50%±25% of tests positive), advisable to use one additional level at +1 log
[g]Period of aging depends on food being tested. Perishable foods should be aged under refrigeration for 48 – 72 h. Frozen and shelf stable foods should be aged for a minimum of 2 weeks at -20°C or at room temperature, respectively.
[h]An appropriate competitor is one that gives similar reactions in enrichment and detection systems
†Included for comparative purpose to Level Four Criteria; criteria under this heading are derived from guidelines published in 2007-2008. A revised set of AOAC validation guidelines are expected to be published in 2011.

Table 2 - General Qualitative Guidelines for Microbial Analytes-Collaborating Laboratory Requirements*

Collaborating Laboratory Criteria	Level Two: Independent lab validation	Level Three: Multiple lab collaborative	Level Four: Full collaborative study	AOAC Collaborative Study
# of laboratories providing usable data	2	**5**	10	10
# of foods	1 or more[a]	1 or more[a]	1 or more[a]	1 to 6 foods[b]
# of strains of organism	1 per food	1 per food	1 per food	1 per food
# of analyte levels/food matrix[c]	2 levels: One inoculated level[c] and uninoculated level	3 levels: One inoculated level[c] one at 1 log higher and uninoculated level	3 levels: One inoculated level[c], one at 1 log higher and uninoculated level	3 levels: One inoculated level[c] one at 1 log higher and uninoculated level
# of replicate samples/food	**8 per analyte level**	**8 per analyte level**	**8 per analyte level**	6 per analyte level †
Aging of inoculated samples prior to testing	No[e]	Yes[d]	Yes[d]	Yes[d]
Comparison to Recognized Method	Yes, if available	Yes, if available	Yes, if available	Yes, if available

[*] Derived from microbiology method validation criteria originally developed by the BAM Council, 2007-2008.
[a] For FDA regulatory use, methods are only valid for foods that have been tested; validation can be extended to other foods by further testing
[b] Depends on applicability of method
[c] Must be adjusted to achieve fractional positive results (one or both methods give 50%±25% of tests positive), advisable to use one additional level at +1 log
[d] Period of aging depends on food being tested. Perishable foods should be aged under refrigeration for 48 – 72 h. Frozen and shelf stable foods should be aged for a minimum of 2 weeks at -20°C or at room temperature, respectively.
[e] Aging would occur during shipment or holding at the originating lab

Table 3 - General Qualitative Guidelines for Food-borne Microbial Pathogens That Present Unique Isolation and/or Enrichment Challenges†- Originating Laboratory Criteria

Originating Laboratory Criteria	Category One: Urgent usage	Category Two: Independent lab validation	Category Three: Multiple lab collaborative	Category Four: Full collaborative study
Replicates per strain	3	3	8	8
Comparison to recognized method[a]	No	Yes, if available	Yes, if available	Yes, if available

Table 4 - General Qualitative Guidelines for Food-borne Microbial Pathogens That Present Unique Isolation and/or Enrichment Challenges† - Collaborating Laboratory Criteria

Collaborating Laboratory Criteria	Level One: Urgent usage	Level Two: Independent lab validation	Level Three: Multiple lab collaborative	Level Four: Full collaborative study
# of laboratories providing usable data[b]	n/a	2	5	10
Replicates per strain	n/a	3	8	6
Comparison to Recognized Method[a]	n/a	Yes, if available	Yes, if available	Yes, if available

†Such examples include but are not limited to RNA food-borne viruses, and protozoan parasites. See Appendix 3 sections V and VI.

[a] If no validated PCR-based method is available then a biochemical and/or serological method will be used.

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

Operational Considerations

MVS approval of methods validation plans is required prior to initiating any methods validation work. The following elements must be addressed in all proposals for method validation studies (in non-emergency use situations).

- Intended use statement for the method being validated.

- Study Design-Applicability of paired v. unpaired sampling and the statistical analyses to be employed. Statistical methods must be employed to verify equivalent or statistically-significant improvement of performance between the new method and the reference method (or in some cases, the originally validated method) to include but not limited to sample means and the degree of accuracy. The MVS biostatistician will provide guidance on applicable statistical tools that will be employed on a case-by-case basis (see ***Assessment*** for additional details).

- For methods intended to be applied to purified organisms as confirmation of organism identity, specific virulence or other characteristics-In this situation the organism has been enriched and recovered from a food so the matrix itself will not be a factor in the analysis at this point. These methods need to meet all applicable inclusivity and exclusivity requirements for originating laboratory and also in a multi-laboratory validation study. Food testing is not applicable in this case, however, strains should represent those that were naturally incurred and recovered from as many varied food sources as possible.

- Use of an appropriate reference method as determined in consultation with the MVS.

- Strain selection for inclusivity and exclusivity testing-To assess the reliability of all methods considered for use in the analysis of regulatory food samples, it is necessary to establish levels of specificity and sensitivity through inclusivity (sensitivity) and exclusivity (specificity) testing.

    - The FDA Foods Program maintains a database of in-Agency microbial analyte collections. Within these collections, strains and serovars derived from food surveillance programs, food-borne outbreak investigations, and clinical specimens, are available to all Agency scientists. Access is governed by “U.S. Food and Drug Administration Foods Program Internal Strain Sharing Standard Operating Procedure”

    - 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. For all organisms, other than *Salmonella* spp, 10-50 strains of the target analyte(s) are required (dependent on the validation level) for inclusivity testing. For *Salmonella* methods, the number of target organisms is

increased to a minimum of 100 serotypes. These serotypes should reflect the genotypic diversity of the organism and should include all of the subspecies as well as the majority of somatic groups found within the genus *Salmonella*.

- The choice of exclusivity strains should closely reflect related, potentially cross-reactive organisms. Other factors such as virulence, frequency of occurrence and availability should be considered. .

- Species/strains specified for use in inclusivity and exclusivity panels must be traceable to the source. The source and origin of each species/strain should be documented. See Appendix 3 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 MVC or its designee will work in concert with the originating laboratory to test their methods with the maximum number of available strains when the developer is unable to comply with the requirements of this document.

- Sample size for an alternative method is at the discretion of the method developer but the reference method sample size must always be as specified by the reference method. Sample sizes for BAM reference methods are routinely 25 g unless otherwise specified. The method developer must always confer with the MVS for guidance when validating a new method that has a different sample size than the reference method.

- Suitability and availability of incurred samples in the proposed validation study.

- Inoculum preparation (when artificially-contaminated samples will be used).

- Sample preparation, naturally-occurring microflora, and the requirement for aerobic plate counts (APC) to verify background microflora.

- Need for inclusion of competitive microflora. For food matrices that exhibit low naturally-occurring microflora background (as determined by APC), validation studies will adhere to AOAC-established parameter *i.e.*1 log greater than microbial analyte being tested. Selection of competitive microflora to be used will be done in consultation with the MVS.

- Selection of spiking levels (when artificially-contaminated samples will be used).

- Method(s) for spiking and uniformity of contamination; assessing the need of an independent resource for sample preparation and distribution.

- Matrix aging to assess method robustness.

- Microbial analyte stress, cell injury, and matrix-derived inhibition of analyte enrichment/growth.

- Selection of appropriate foods. Food matrices will be validated individually based on the historical outbreak record and epidemiological link between matrix, pathogen, and illness. Some examples are provided in Appendix 2. Extension of a method to include additional food matrices will require additional validation studies. See Sections IV and V.

- Fractional positive samples for both the reference method and the test method must achieve 50%±25% positive results (See ***Appendix 1: Definition of Terms***, page xx, for a complete description of fractional recovery)

  NOTE: *Depending on the parameters being evaluated, method ruggedness and robustness should be evaluated independently.*

Assessment

- Acceptable false negative and false positive rates will be established in consultation with the MVS. Factors that will influence this decision may include but not be limited to the replicate number and intended use (emergency, screening, confirmatory). False negative rates must be ≤5% (Ideally, false negative rates should be ≤2% in all instances though this may not be feasible without using inordinately large replicate numbers).

- Acceptable false positive rates will be based on the method's intended use *e.g.* screening methods may exhibit a range not to exceed ≤10%-15%. Methods classified as confirmatory must have false positive rates ≤5%.

- False positive and false negative rates will be evaluated in total (across all labs/data sets)

- Appropriate statistical measures will be included in all studies. Statistical algorithms must also be employed to test for significance differences (superiority or equivalence) and for data disqualification *(see below),* These may include but are not limited to Chi square or a variant of Chi square. 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 MVS during the planning phase of any validation study.

- Disqualification of data sets. Data sets derived from a validation exercise can be disqualified. Examples include but may not be limited to:

- Negative controls (un-inoculated controls) yield a positive outcome-an indicator of lab/operator error.
- Evidence for non-homogeneity of bulk-inoculated material.
- Statistically -supported outliers

In most instances, a proficiency test (PT) will be required following completion of a validation study prior to the implementation of the method in a regulatory setting. Participation by all appropriate FDA regulatory laboratories will be necessary. The MVS will work in concert with ORA Division of Field Science staff and field lab microbiology management to assessed the need, and if warranted, develop and conduct all PT exercises.

III. CRITERIA AND GUIDANCE FOR THE VALIDATION OF FDA-DEVELOPED MOLECULAR-BASED ASSAYS

These criteria and guidelines are intended to support method validation efforts for developers of molecular-based assays, *e.g.* PCR to be used to confirm the identity or exclusion of isolated colonies.

This validation scheme is for both conventional and real time PCR assays. If validating a real time assay, the platform and chemistry must be specified. It is strongly recommended that a real time assay be validated on two to three other platforms i.e. thermal cyclers or workstations. Other molecular methods should also state chemistry and platform requirements and include multiple platforms where possible.

The criteria necessary to determine four levels of validation for qualitative PCR assays for bacteria are the following:

Inclusivity and exclusivity

The inclusivity and exclusivity requirements described above apply here. The amount of template, whether using bacterial cells or purified nucleic acid, should be comparable for both inclusivity and exclusivity panels.

It is expected from the originating laboratory that all primer and/or probe sequences would initially be screened for uniqueness by searching a bacterial genomic database for homology. It is recommended that a BLAST search be performed against the GenBank non-redundant database.

Target gene(s) and controls (positive and negative).

Molecular-based assays to target gene(s) from a specific microbial analyte, whether to a virulence factor or taxonomic identifier (e.g. 16S DNA), must have demonstrable specificity (inclusivity and exclusivity) for that particular pathogen. Positive and negative control strains and reactions should be incorporated into the assay evaluation. Internal amplification controls for real-time PCR assays ***are required*** for regulatory food or environmental sample analyses.

Comparison to the Reference Method

When possible, the originating laboratory will compare the PCR-based method to another PCR-based reference identification method. If a PCR-based method is not available, then bacteriological, biochemical, and/or serological reference methods may be used.

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

Definitions

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.

Verification: The confirmation by examination and the provision of objective evidence that specified requirements have been fulfilled.

Criteria

For Kits Fully Validated in a Collaborative Study Monitored by an Independent Accrediting Body

1. For commercially-available microbiological diagnostic kits whose performance parameters have been fully validated in a collaborative study monitored and evaluated by an independent accrediting body e.g. AOAC-OMA, AFNOR, etc.

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

Verification Requirements

- Six replicates of the inoculated matrix and six replicates of the un-inoculated matrix are tested and confirmed by both the alternative and the reference method.
- If no false positive or false negative results are obtained, then the new matrix is verified.
- Each commodity to be tested should be spiked with a level close to the detection limit, usually <30 cfu of analyte per 25 g food sample to determine if there is any interference from the matrix.
- If unacceptable false positive or false negative results are observed (as defined for the intended use of the method), then the study must be expanded to a full SLV (Table1) to define the operating characteristics of the method with the new matrix. Consult Section V: Food Matrix Extension for more detailed information.

  NOTE: The criteria above apply only for foods which were part of the collaborative study by an independent accrediting body. The use of such kits for food matrices that were not included in the original collaborative

study must be preceded by a food matrix extension study. (See Section V: Food Matrix Extension)

2. For commercially-available microbiological diagnostic kits whose performance parameters are supported by data obtained through an abbreviated validation protocol and evaluated by an independent accrediting body e.g. AOAC-RI.

All methods fitting into this description ***must*** be validated per standards defined for Agency-developed (FDA) microbiology methods defined in Section II.

V. FOOD MATRIX EXTENSION FOR VALIDATED MICROBIOLOGY METHODS

The validation of a method for a food matrix not previously included in a validation study is necessary to assure that the new matrix will produce neither high false positive (matrix is free from cross reactive substances) nor high false negative rates (matrix is free of inhibitory substances)

Methods used in FDA field laboratories for regulatory purposes must be validated for a specific food item. Though it is generally assumed that the more closely related a new food matrix is to a previously validated matrix for a defined analyte, the greater the probability that the new matrix will behave similarly, new matrices must be validated individually.

Two general approaches may be employed for the inclusion of addition food matrices for a validated method. Consultation between laboratory managers, QMS managers and the MVS will aid in determining which approach is more applicable for any given situation.

1. Guidance to Support Field Laboratory Expedience

Due to the nature of the foods programs in FDA, ORA microbiology labs analyze a huge variety of food matrices. Very often validation studies can not address all the varied matrices-at specific levels of validation-that may be part of normal ORA operations. In many cases (particularly during outbreak investigations) samples must be analyzed immediately; there may not always be a method available that has been fully validated for the specific food matrix.

For such situations, ORA laboratories will analyze the matrix in question concurrently with a matrix spike. The matrix spike will consist of a 25 gram sample of the product spiked with an inoculum of 30 cells or less of the target analyte. Negative spike results invalidate the analysis and the sample must be analyzed using the conventional culture procedure.

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

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

NOTE: Depending on the intended use of the method, it may be deemed necessary (Consultation between laboratory managers, QMS managers and the MVS) to

proceed to a more extensive matrix extension protocol as outlined below (Section V, part 2)

2. Guidance for the Formal Expansion of Validated Food Matrices

Table 5 - Food Matrix Extension Protocol for Microbiology Methods

Laboratory Criteria	Matrix Extension, Single Laboratory Validation (SLV)	Matrix Extension, Multi-Laboratory Validation (MLV)	Matrix Extension, Harmonized Collaborative Validation (HCV)
# of strains of target organism (inclusivity)	1[a]	1[a]	1[a]
# of non-target organism (exclusivity)	None	None	None
# of foods	1 food type	1 food type	1 food type
# analyte level/food matrix	2 levels: One inoculated level[b] and matrix blank	3 levels: Two inoculated levels[b] and matrix blank	3 levels: Two inoculated levels[b] and matrix blank
Replicates per food at each level tested	3-6 sources[c]	6 sources[c]	6 sources[c]
Aging of inoculated samples prior to testing	No	Yes[d]	Yes[d]
Interference evaluations	Normal background	Normal background	Normal background
Comparison to recognized method	Yes[e]	Yes[e]	Yes[e]
# of laboratories providing usable data	1 - Originating laboratory	5[f]	10[f]

[a] Use analyte used for original collaborative study of method validation.
[b] Depends on applicability of method: refer to originating laboratory study for suggested levels.
[c] Different food sources are defined as different commercial brands. If only one food source is available, the food matrix extension can be valid only for that source.
[d] Period of aging depends on food being tested. Perishable foods should be aged under refrigeration for 48 – 72 h. Frozen and shelf stable foods should be aged for a minimum of 2 weeks at -20°C or at room temperature, respectively.
[e] Food matrix being tested should have parallel positive controls run consisting of an already validated food matrix spiked at the same levels as the matrix being tested.
[f] A Food Matrix SLV should be completed prior to initiation of a Food matrix MLV of HCV.

VI. PLATFORM EXTENSION

Expanding the use of a validated method to include another instrument or platform requires validation using a process similar to that used for the original microbial analyte evaluation. Such instances include the use of an instrument or platform similar in scope and function to that currently validated and approved for use however it may differ by commercial manufacturer, use proprietary reagents and diagnostic software, etc.

Platform extension validation must be done by comparing the proposed new platform to the previously validated platform. The platform extension validation must be performed by analyzing multiple matrices, using 3 spike levels (fractional, one log higher and blank) in 6 replicates and running the new platform and the old platform side-by-side. In planning platform extension validation, one must determine what aspect of the technology will be compared in order to determine how the study should proceed.

> ***Example:***
> Method A is a validated method for the screening/detection of EHEC and other STECs using a qPCR assay developed for the Cepheid SmartCycler II. The performance of this application was validated against the existing culture method (the reference standard). The ABI7500 Fast instrument will be compared to the Cepheid SmartCycler II (Method A).

NOTE: *For situations that involve upgrades (or reformulations) of currently-available commercial kits, and updated software packages that may influence method performance, only method verification will be needed.*

VII. REFERENCES AND SUPPORTING DOCUMENTS

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Foundations of Clinical Research, Applications to Practice, Leslie Gross Portney, Mary Watkins, Appleton & Lange, 1993.

Harmonized Guidelines for Single-laboratory Validation of Methods of Analysis, Pure and Applied Chemistry, 2002, 74, 835 - 855.

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International ANS/ISO/IEC Guide 8402, Quality Management and Quality Assurance Vocabulary, 1994.

International ANS/ISO/IEC Standard 17025, Second edition 2005-05-15.

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VIII. APPENDIX 1
Definition of Terms

Action level: Level of concern for an analyte that must be reliably 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.

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 validated analytical method provides data that can resolve a particular scientific issue in a specified matrix.

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

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

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

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

> ***NOTE:*** *The term "Standard Reference Material" (SRM) is the name of a certified reference material (CRM), which is the trademark name of a certified reference material that has been certified and is distributed by the National Institute of Standards and Technology (NIST).*

Detection limit: A detection limit is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. It is often called the limit of detection (LOD) which is the lowest concentration level that can be determined

statistically different from a blank at a specified level of confidence. It is determined from the analysis of sample blanks and samples at levels near the expected LOD (see ISO 11843, CLSI EP17).

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

Food category: A group of specific related foods. Appendix 2 lists nine recommended food categories: meat products, poultry, fish and seafood products, fruit- and vegetable-based products, dairy products, chocolate/bakery products, animal feeds, pasta, and miscellaneous.

Food matrix: Components that comprise the food sample.

Food product: Any substance usually composed primarily of carbohydrates, fats, water and/or proteins that can be eaten or drunk by an animal or human for nutrition or pleasure. See Appendix 2 for examples of representative food products.

Food type: An item that is processed, partially processed or unprocessed for consumption. Appendix 2 lists various types such as raw, heat processed, frozen, fermented, cured, smoked, dry, low moisture, etc.

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

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

Incurred Samples: Naturally-contaminated test samples.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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).

Selectivity: The capability of a method to discriminate between the analyte of interest and other components of the sample including matrix components.

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. For assays that

will be used to test human clinical specimens, the method's analytical sensitivity is distinct from the method's clinical diagnostic sensitivity. Clinical diagnostic sensitivity is the percentage of persons who have a given condition who are identified by the method as positive for the condition (high analytical sensitivity does not guarantee acceptable diagnostic sensitivity).

Sensitivity: The change in the response of a measuring instrument divided by the corresponding change in the stimulus.

Source : The origin of a test sample. A sample matrix may have variability due to its source. For example, a water sample may have variable characteristics, and therefore, may show method results variability, depending on whether the sample source is drinking water, ground water, surface water, or waste water.

[a] Different food sources are defined as different commercial brands. Different water sources could be from different areas of a reservoir. Different plant or soil sources could be samples from the different areas of a plot or field. Different sediment sources could be samples from different areas of a water body.

Source: The number of sources for a food method validation study may be determined by the number and selection of matrices analyzed in the method validation study. For example, if a variety of food matrices with differing physical and chemical properties are selected, the number of sources for each food sample matrix may be one or more. For a method validation study analyzing one food matrix, 3-5 sources of the food matrix are recommended.

Specificity: Analytical specificity is the ability of a method to measure one particular analyte in the presence of components which may be expected to be present. For methods that will be used to test human clinical specimens, the method's analytical specificity is distinct from the method's clinical diagnostic specificity. Clinical diagnostic specificity is the percentage of persons who do not have a given condition who are identified by the method as negative for the condition.

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

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

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

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

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

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

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

IX. APPENDIX 2
Examples of Food Types and Associated Microbiological Contaminants

Table 1-Food Categories Relevant to Foodborne Pathogenic Bacteria

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

Food type	Yersinia	Clostridium perfringens	Listeria mono	E. coli O157	Staph aureus	Campy	Salmonella	B. cereus
Meats								
raw	x		x	x		X	x	x
heat processed			x	x	x		x	
frozen			x	x			x	
fermented			x	x			x	
cured		x	x		x		x	
other		dishes / gravy	pate					
Poultry								
raw	x					X	x	
heat processed							x	
frozen							x	
other		dishes / gravy						
Seafood								
raw	x		x	x		X	x	
heat processed							x	
frozen			x	x			x	
shellfish	x			x		X	x	
smoked		x	x		x		x	
other							x	
Fruits & Vegetables								
unpasteurized juice				x			x	
raw	x		x	x		X	x	
heat processed		x						
frozen			x				x	
dry								x
juice/concentrate				x			x	
low moist							x	
nut meats			x	x			x	
others								
Dairy								
raw	x		x	x	x	X	x	x
heat processed			x					x
frozen			x	x	x		x	x
Fermented?			x	x	x		x	
dry					x		x	x

ice cream			x				x	
cheese			x	x			x	
Chocolate / bakery								
low moist							x	
dry powder							x	
milk chocolate							x	
other					pastry			custard
Animal feed								
low moist							x	
pet food							x	
Pasta								
uncooked							x	
Misc								
dressings			x	x			x	
spices		x					x	
mayonnaise			x	x		X	x	
flour			x			X	x	
egg / derivatives				x			x	
cereal/rice								x

Table 2 - AOAC Food Categories Relevant to Non-pathogenic Microorganisms

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

dressings	x	x	x	X	x
spices			x		x
mayonnaise	x	x	x		x
egg / derivatives			x	X	
cereal / rice			x	X	

FDA Methods Validation Guidelines for Microbial Pathogens

Representative Food Products in Categories

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

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

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

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

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

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

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

Uncooked Pasta:
Uncooked noodles, macaroni, spaghetti

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

X. APPENDIX 3
Strains and Serovars for Inclusivity and Exclusivity Panels
(*abridged)*

- Panels of strains and serovars present in Appendix 3 are not intended to be exhaustive. It is meant to serve as a guide or starting point for the method developer as they construct exclusive and inclusive panels for method validation.
- Access to microbial analyte strains and serovars as listed in the FDA Office of Foods Strain Collection Catalog is governed by "U.S. Food and Drug Administration Foods Program Internal Strain Sharing Standard Operating Procedure"

I. E. coli O157:H7

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

	Serotype	Genotype		
		***stx*1**	***stx*2**	***uid*A-O157:H7/H-**
Shigella dysenteriae		+	-	-
Hafnia alvei		-	-	-
Morganella morganii		-	-	-
Citrobacter fruendii		-	-	-
Lectercia adecarboxylata		-	-	-
Hafnia alvei		-	-	-
Shigella sonnei		-	-	-
Shigella boydii		-	-	-
Shigella flexneri		-	-	-
Citrobacter fruendii		-	-	-
Salmonella Grp. 30		-	-	-
Salmonella lansing Grp.P		-	-	-
Klebsiella pneumoniae		-	-	-
Listeria monocytogenes		-	-	-
Listeria innocua		-	-	-
Listeria ivanovii		-	-	-
Listeria seeligeri		-	-	-
Listeria welshimeri		-	-	-
Vibrio cholerae	*O1 Inaba*	-	-	-
Vibrio parahaemolyticus	*O4*	-	-	-
Vibrio vulnificus		-	-	-
Staphylococcus aureus		-	-	-
Rhodococcus equi		-	-	-
Lactobacillus sp.		-	-	-
Lactobacillus sp.		-	-	-
Salmonella typhimurium		-	-	-
Streptococcus pyogenes		-	-	-
Algaligenes faecalis		-	-	-
Salmonella choleraesuis		-	-	-
Yersinia entercolitica		-	-	-
Yersinia entercolitica		-	-	-
Enterobacter cloacae		-	-	-

Salmonella (inclusivity)

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

IIa. *Salmonella*: Subspecies Set

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

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

IIb. *Salmonella*: Outbreak Cluster Set

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

IIc. *Salmonella*: Food Set

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

III. Listeria spp.

Organism	Isolate #	Isolate Information	Serology
		Food Isolates	
L. monocytogenes	15b42	cucumber	4
	3365	mackerel	4b6
	3312	cheese	1a1
	15b27	radish	1
	2388	coleslaw	1
	2478	raw milk	1
	3313	shrimp	1a1
	3326	roast beef	1a1
	3358	milk product	1a2
	3363	cook snow crab	1a2
	3756	beef & gravy Rh-	1
	15b72	apple juice	1
	15b85	cream ch. & veg	1
	15c14	avocado pulp	1
	15c22	fontina cheese	1
	15a90	turkey ham	3b
	2450	veg. mix	1
	2475	cold cut sand.	1
	2492	ice cream	1
	3291	popsicle	1a1
	3318	lobster	1a2
	3321	raw shrimp	4b6
	3332	mex-style cheese	4b6
	3359	surimi scallops	1a1
	3362	Pollack	1a1
	3558	cheese	4b
	3644	red bean ice bar	4b6
	3662	cheese	4b6
	15b70	cheddar cheese	4
L. monocytogenes	2369	**Patient Isolates**	1
	2370		1
	15b55		1
	15b65		1
	3555		4
	3664		1a1
	3666		4b6
	3668		4b6
	15a82		4
	15b56		4
	15b58		4
	15b81		1
	15b82		4
L. monocytogenes	3315	**Environmental Isolates (swab)**	1a1
	3286		1a2
	3308		1a2
	3360		1a1

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

Organism	Isolate #	Organism	Isolate #
L. innocua	3107	L. welshimeri	2230
	3124		2231
	3516		3425
	3654		3441
	3758		3659
	6273		15b05
	3181		15b06
	3270		15b16
	3390		15b46
	3392		15b48
	3552		15b50
	3757	Hafnia alvei	6410
	15a93	E. coli	6365
	15a94	Morganella morganii	13b67
	15a95	Shigella dysenteriae	13c94
	15b30	Citrobacter freundii	13d26
	15b31	E. coli	13d64
	15b51	Leclercia adecarboxylata	13d65
	15a92	Hafnia alvei	13d66
	ATCC 33090	Shigella sonnei	13g01
L. ivanovii	2244	Shigella boydii	13g18
	3106	Shigella flexneri	13g19
	3417	Citrobacter freundii	6251
	6274	Salmonella Grp. 30	6269
L. ivanovii	15a96	Salmonella lansing Grp. P	6270
	15a97	Klebsiella pneumonia	6271
	15a98	Vibrio cholerae	6277
	15b24	Vibrio parahaemolyticus	6278
	ATCC 19119	Vibrio vulnificus	6279
L. seeligeri	2232	Staphylococcus aureus	ATCC 25923
	2233	Rhodococcus equi	6281
	2243	Lactobacillus sp.	6282
	2302	Lactobacillus sp.	6286
	3110	Salmonella typhimurium	6290
	3126	Streptococcus pyogenes	ATCC 19615
	3389	Alcaligenes faecalis	ATCC 8750
	3423	Salmonella choleraesuis	ATCC 6539
	3439	Yersinia entercolitica	1269
L. seeligeri *(continued)*	3451	Yersinia entercolitica	1270
	3517	E. coli	13a80

3531	Enterobacter cloacae	18g53
3656		
6275		
15b07		
15b08		
15b09		
15b26		
15b28		
15b49		

IV.Shigella

Inclusive Panel

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

IV. *Shigella* *(continued)*

Bacteria strain	Strain no.	Source*
Acinetobacter baumannii	19606	ATCC
Aeromonas caviae	15468	ATCC
Aeromonas hydrophila	7966	ATCC
Bacillus licheniformis	12759	ATCC
Bacillus sphaericus	4525	ATCC
Bacillus stearothermophilus	12016	ATCC
Bacillus subtilis	6633	ATCC
Bordetella bronchiseptica	10580	ATCC
Burkholderia cepacia	25608	ATCC
Citrobacter freundii	255	PRLSW
Citrobacter freundii	food isolate	PRLSW
Citrobacter freundii	68	MNDAL
Citroabcter younger	food isolate	PRLSW
Clostrodium sporogenes	11437	ATCC
Edwardsiella tarda	254	PRLSW
Enterobacter aerogenes	13048	ATCC
Enterobacter aerogenes	11	VADCLS
Enterobacter cancerogenus	food isolate	PRLSW
Enterobacter cloacae	260	PRLSW
Enterobacter cloacae	71	MNDAL
Enterococcus durans	6056	ATCC
Enterococcus faecalis	7080	ATCC
Erysipelothrix rhusiopathiae	19414	ATCC
Enterotoxgenic *E. coli*	H10407	CFSAN
Enterotoxgenic *E. coli*	C600/pEWD299	CFSAN
Enterotoxgenic *E. coli*	65	MNDAL
Escherichai coli O157:H7	43890	ATCC
Escherichai coli O157:H7	43888	ATCC
Escherichai coli O157:H7	43895	ATCC
Escherichai coli O157:H7	68-98	CDC
Escherichai coli O157:H7	24-98	CDC
Escherichai coli O157:H7	20-98	CDC
Escherichai coli O157:H7	16-98	CDC
Escherichai coli O157:H7	63	MNDAL
Escherichai coli O157:H7	4	VADCLS
Escherichai coli O157:H44	26	VADCLS
Escherichia coli O111:NM	04.SB.00067	OCPHL
Escherichia coli O143:H4	05.SB.00141	OCPHL
Escherichia coli	8739	ATCC
Escherichia coli	25922	ATCC
Escherichia coli (hemo +)	food isolate	PRLSW
Escherichia coli (hemo +)	28	VADCLS
Escherchia coli (sorbitol –)	food isolate	PRLSW
Escherchia coli (sorbitol –)	food isolate	PRLSW
Escherchia coli	64	MNDAL
Escherchia coli	74	MNDAL
Escherichi coli	8	VADCLS
Klebsiella pnenumoniae	13883	ATCC
Klebsiella pnenumoniae	75	MNDAL
Klebsiella oxytoca	66	MNDAL
Leclercia adecarboxylata	23216	ATCC
Leclercia adecarboxylata	73	MNDAL

Listeria innocua	33090	ATCC
Listeria ivanovii	19119	ATCC
Listeria monocytogenes	19115	ATCC
Listeria monocytogenes	H2446	CDC
Listeria monocytogenes	H8393	CDC
Listeria monocytogenes	H8494	CDC
Listeria monocytogenes	H8395	CDC
Listeria seeligeri	35967	ATCC
Morganella morganii	257	PRLSW
Paenibacillus polymyxa	7070	ATCC
Pantoea agglomerans	food isolate	PRLSW
Pasteurella aerogenes	27883	ATCC
Plesiomonas shigelloides	51903	ATCC
Proteus mirabilis	7002	ATCC
Proteus mirabilis	food isolate	PRLSW
Proteus kauseri	13315	ATCC
Proteus vulgaris	69	MNDAL
Providencia alcalifaciens	51902	ATCC
Providencia rettgeri	76	MNDAL
Providencia stuartii	257	PRLSW
Pseudomonas aeruginosa	27853	ATCC
Pseudomonas aeruginosa	9027	ATCC
Pseudomonas aeruginosa	67	MNDAL
Pseudomonas mendocina	food isolate	PRLSW
Rhodococcus equi	6939	ATCC
Salmonella Gaminara	8324	ATCC
Salmonella diarizonae	12325	ATCC
Salmonella Abortusequi	9842	ATCC
Salmonella diarizonae	29934	ATCC
Salmonella diarizonae	252	PRLSW
Salmonella Mbandaka	253	PRLSW
Salmonella Tennessee	249	PRLSW
Salmonella Lexington	248	PRLSW
Salmonella Havana	241	PRLSW
Salmonella Baildon	61-99	CDC
Salmonella spp.	78-99	CDC
Salmonella spp.	87-03	CDC
Salmonella spp.	98-03	CDC
Salmonella Braenderup	H 9812	CDC
Salmonella Enteritidis	59	MNDAL
Salmonella Heidelberg	60	MNDAL
Salmonella Kentucky	61	MNDAL
Salmonella Newport	62	MNDAL
Salmonella Typhimurium	30	VADCLS
Serratia liquefaciens	27592	ATCC
Serratia liquefaciens	70	MNDAL
Sphingomonas paucimobilis	72	MNDAL
Staphylococcus aureus	6538	ATCC
Staphylococcus aureus	25923	ATCC
Staphylococus epidermidis	14990	ATCC
Staphylococcus xylosus	29971	ATCC
Streptococcus equi subsp. equi	9528	ATCC
Streptococcus gallolyticus	9809	ATCC
Streptococcus pyogenes	19615	ATCC
Vibrio cholerae	14035	ATCC
Vibrio cholerae	14033	ATCC

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

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

V. Food-borne RNA Viruses

These panels were developed and adopted by the FDA BAM council, 200-2008

Inclusivity requirements

Target	Level One	Level Two	Level Three	Level Four
Norovirus	1 Strain Genogroup I 1 Strain Genogroup II	2 Strains - Genogroup I 5 Strains - Genogroup II	5 Strains – Genogroup I 10 Strains – Genogroup II	10 Strains – Genogroup I 20 Strains – Genogroup II
Hepatitis A	HM175/18f (subgenotype 1B) ATCC #VR-1402	5 Strains[a]	10 Strains[b]	20 Strains[b]
Enterovirus	Poliovirus 1 (attenuated) ATCC #VR-1562	5 Strains[c]	15 Strains[d]	30 Strains[d]

Hepatitis A Panels

Level Two ([a]should include the following strains):

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

Levels Three and Four *([bs]hould include the following strains)*:

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

Enterovirus Panels

Level Two *([c]should include the following strains):*

Poliovirus 1 (attenuated)	ATCC #VR-1562
Coxsackievirus A3	ATCC #VR-1007
Echovirus 1	ATCC #VR-1038

Levels Three and Four *([d]should include the following strains):*

Poliovirus 1 (attenuated)	ATCC #VR-1562
Poliovirus 3 (attenuated)	ATCC #VR-63
Coxsackievirus A3	ATCC #VR-1007
Echovirus 1	ATCC #VR-1038
Echovirus 21	ATCC #VR-51

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

Exclusivity Panel

Target	Level One	Level Two	Level Three	Level Four
Norovirus	10 strains[a]	20 strains[b]	30 strains[b]	40 strains[b]
Hepatitis A	10 strains[c]	20 strains[d]	30 strains[d]	40 strains[d]
Enterovirus	10 strains[e]	20 strains[f]	30 strains[f]	40 strains[f]

Norovirus Panels

Level One *([a]must include)*:

Panel A

HM175/18f (subgenotype 1B)	ATCC #VR-1402 (or equivalent)
Poliovirus 1 (attenuated)	ATCC #VR-1562 (or equivalent)
Feline calicivirus	ATCC #VR-2057
Murine calicivirus	

Levels Two, Three and Four *([b]must include):*

Panel A representatives ***plus:***

Panel B

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

Hepatitis A Panels

Level One *([c]must include):*

Panel C

norovirus genogroup I	
norovirus genogroup II	
Poliovirus 1 (attenuated);	ATCC #VR-1562 (or equivalent)
Coxsackievirus A3	ATCC #VR-1007 (or equivalent)

Levels Two, Three and Four *([d]must include)*:

Panel C representatives ***plus***

Panel D

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

Enterovirus Panels:

Level One *([e]must include):*

Panel E

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

Levels Two, Three and Four *([f]must include)*:

Panel E representatives ***plus***

Panel F

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

VI.Protozoan Parasites

A. *Cyclospora cayetanensis*

a. Inclusive Panel

As many geographic and outbreak isolates as are available

b. Exclusive Panel

***Cyclospora* spp.**

C. cercopitheci
C. colobi
C. papionis

***Eimeria* spp.**

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

Additional Microorganisms

Cryptospordium spp
Apicomplexa
Bacterial isolates

B. *Cryptosporidium* spp.

Inclusive Panel

C. hominis
C. parvum (multiple strains available)

Exclusive Panel

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

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FDA Methods Validation Guidelines for Microbial Pathogens

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