BUREAU OF CANNABIS CONTROL

DISCIPLINARY GUIDELINES

NOVEMBER 2017

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I. INTRODUCTION

Pursuant to Business and Professions Code section 26011.5, the protection of the public is of the highest priority for the Bureau of Cannabis Control (Bureau). In keeping with its mandate to protect the public, the Bureau has adopted these recommended uniform guidelines in order to promote consistency in disciplinary orders for similar offenses on a statewide basis. This document is intended for use by those involved in the administrative disciplinary process (e.g., Administrative Law Judges (ALJ), Deputy Attorneys General (DAG), Bureau licensees and their legal counsel, and other interested parties), and may be revised from time to time, and distributed to interested parties upon request.

The Bureau requests that the suggested disciplinary orders contained in these guidelines be levied consistently and appropriately, based on the nature and seriousness of the violation(s) confirmed in an administrative action. The Bureau recognizes that mitigating or aggravating circumstances, in addition to other factors, may necessitate departure from these recommended orders and terms of probation. If there are any deviations from the guidelines, the Bureau requests that the ALJ hearing the matter include an explanation in the Proposed Decision so that the circumstances can be better understood and evaluated by the Bureau before final action is taken.

Additionally, these guidelines only apply to formal administrative disciplinary processes. These guidelines do not apply to other alternatives available to the Bureau, such as administrative citations and fines, except in cases where an Accusation has been filed against a registrant or licensee for failure to pay an assessed administrative fine and/or comply with an order of abatement issued by the Bureau.

II. FACTORS TO BE CONSIDERED IN DETERMINING PENALTIES

In determining whether revocation, suspension, probation, fine, or a combination is to be imposed in a given case, factors such as the following should be considered:

1. Nature and severity of the act(s), offenses, or crime(s) under consideration.
2. Actual or potential harm to the public.
3. Actual or potential harm to any patient.
4. Prior disciplinary record.
5. Number and/or variety of current violations.
6. Mitigating evidence.
7. Rehabilitation evidence, including but not limited to, a statement of rehabilitation containing any evidence that demonstrates fitness for licensure, or a certificate of rehabilitation under Penal Code section 4852.01.
8. In case of a criminal conviction, compliance with conditions of sentence and/or court-ordered probation.
9. Overall criminal record.
10. Time passed since the act(s) or offense(s) occurred.
11. If applicable, evidence of expungement proceedings pursuant to Penal Code Section 1203.4.

III. DISCIPLINARY GUIDELINES

The Medicinal and Adult-Use Cannabis Regulation and Safety Act (MAUCRSA) specifies the offenses for which the Bureau may take disciplinary action. Following are samples of the codes and regulation numbers, titles of the offenses and the associated Bureau determined disciplinary recommendations. When filing an accusation, the Bureau or Office of the Attorney General are not limited to the violations listed herein. They may also cite any and all additional related statutes and regulations violated not listed below. The following is ***not*** a comprehensive list of potential violations and in no way, should limit the Bureau or the Attorney General's Office from asserting any relevant and applicable violation. The Bureau suggests that for cases with multiple violations, suspensions or other disciplines run concurrently. All standard terms of probation as stated herein shall be included for all probations.

As used herein, statutes and regulations are referenced as follows:
Business and Professions Code: (B&P)
Title 16, California Code of Regulations: (CCR)
Penal Code: (PC)

California Code of Regulations Disciplinary Order Guidelines - Tier 1

Minimum: revocation stayed, 5 to 15-day suspension, a fine (as determined by the "Fine Formula" below), or a combination of a suspension and fine.

Maximum: revocation

Tier 1 discipline is recommended for:

- violations which are potentially harmful

Violations of the following codes are representative of this category:

Violation Description	Authority
Failure to Surrender License	B&P § 119 (d) CCR § 5022
Failure to Notify the Bureau of Changes	CCR § 5023
Unauthorized Modification of Licensed Premises	B&P § 26055(c) CCR § 5027
Prohibited Commercial Cannabis Activity Between Medicinal and Adult-Use Licensees	CCR § 5032(b)
Unauthorized Storage of Inventory	CCR § 5033
Failure to Maintain Records	B&P § 26160 CCR §§ 5037(a), 5310, 5426, 5505-5506
Allowing the Unauthorized Use of the Track and Trace System and Failing to Maintain Track and Trace System Requirements	CCR §§ 5048-5050 and 5052
Failure to Properly Display and Post License	CCR § 5039

Failure to Comply with Advertising and Marketing Requirements	B&P §§ 26151- 26152 CCR §§ 5040-5041
Failure to Ensure Restricted Access to Limited-Access and Other Restricted Areas	B&P § 26070 CCR §§ 5042 and 5401
Failure to Comply with Security Requirements	CCR §§ 5043-5047 and 5403(b)(1)-(2)
Failure to Comply with Proper Cannabis Destruction and Waste Management	CCR §§ 5054-5055 and 5410(e)
Unauthorized Storage of Cannabis Goods and Storage-only Services	CCR §§ 5033 and 5300-5302
Failure to Comply with Packaging and Labeling Requirements	B&P § 26070 CCR §§ 5303 and 5412
Failure to Comply with Insurance Requirements	CCR § 5308
Failure to Comply with Inventory Documentation and Reconciliation Requirements	CCR §§ 5051, 5309 and 5423-5424
Failure to Comply with Transportation Requirements of Cannabis Goods	B&P § 26070 CCR §§ 5311-5312
Failure to Comply with Transport Personnel Requirements	CCR § 5313
Unauthorized Use of Distributor Transport Only License	CCR § 5315
Failure to Comply with Shipping Manifest Requirements	B&P §§ 26067 and 26070 CCR § 5314
Unauthorized Hours of Operation	CCR § 5403(a) and (b)(3), and 5422(b)
Unauthorized Sale of Cannabis Plants	CCR § 5408(a)-(b)
Use of Pesticide on Live Plants	CCR § 5408(c)
Unauthorized Furnishing of Free Cannabis Goods	CCR § 5411
Failure to Comply with Exit Packaging Requirements	B&P § 26070.1 CCR § 5413
Failure to Comply with Delivery Requirements	CCR §§ 5415-5418 and 5421
Failure to Provide Delivery Request Receipts	B&P § 26090 CCR § 5420
Unauthorized Receipt of Inventory Shipment	CCR § 5422
Failure to Record Sales to Customer	CCR § 5425
Failure to Comply with Requirements for Temporary Cannabis Event License	CCR § 5600 et seq.
Non-Permitted Use of License	B&P § 119(b)-(f)
Failure to Comply with Local Ordinance Regulating Commercial Cannabis Activity	B&P § 26030(f)
Failure to Comply with Operating Procedures	B&P § 26030(j)
Sale of Alcohol or Tobacco Products	B&P § 26054(a)
Failure to Record Commercial Cannabis Activity on Sales Invoice or Receipt	B&P § 26161
Failure to Exercise Care for Safety of Self or Others Due to Being Under the Influence of an Intoxicating Substance	PC § 647(f)

California Code of Regulations Disciplinary Order Guidelines - Tier 2

Minimum: revocation stayed, 15 to 30-day suspension, a fine (as determined by the "Fine Formula" below), or a combination of a suspension and fine.

Maximum: revocation

Tier 2 discipline is recommended for:

- Violations with a serious potential for harm
- Violations which involve greater risk and disregard of public safety

Violations of the following codes are representative of this category:

Violation Description	Authority
Exceeding License Privileges for Commercial Cannabis Activity	B&P §§ 26050 and 26053
Unauthorized Use and Operation of Designated Premises	CCR § 5025
Subletting of Premises	CCR § 5028
Failure to Comply with Track and Trace Reporting and System Reconciliation Requirements	CCR §§ 5049-5051
Failure to Comply with Video Surveillance System Requirements	CCR § 5044
Failure to Comply with Security Personnel Requirements	CCR § 5045
Failure to Verify Age of Customers and Unauthorized Access to Retail Areas	B&P § 26140 CCR §§ 5400 and 5402
Failure to Comply with Employee Age Restrictions	B&P § 26140 CCR § 5031
Sale or Furnish of Adult-use Cannabis Goods to Minors	B&P §§ 26030(g) and 26140 CCR § 5404
Consumption of Cannabis Goods by a Minor on Licensed Premises	B&P § 26200
Failure to Properly Display Cannabis Goods	CCR § 5405
Unauthorized Sale of Non-Cannabis Goods on Premises	CCR § 5407
Exceeding Daily Limits of Cannabis Goods Sales	CCR § 5409
Unauthorized Return of Cannabis Goods	CCR §§ 5053 and 5410
Consumption of Cannabis Goods During Delivery	CCR § 5419
Failure to Ensure Laboratory Testing Arrangements and Quality Assurance	CCR §§ 5304-5307
Failure to Comply with Microbusiness Operations Requirements	CCR § 5500
Failure to Comply with Laboratory Testing Requirements	CCR § 5700 et seq.
False or Misleading Declaration of Correction in a Notice to Comply	CCR § 5801
Prohibited Attire and Conduct	CCR § 5806

Prohibited Entertainers and Conduct	CCR § 5807
Allowing for the Copy or Display of a Fictitious License or a License that is Canceled, Revoked, or Altered	B&P § 119
Misdemeanor Offenses by Licensees	B&P § 125
Discipline by Another Agency	B&P § 141
Failure to Provide Safe Conditions for Inspection	B&P §§ 26030(i)

California Code of Regulations Disciplinary Order Guidelines - Tier 3

Minimum: revocation stayed, 45-day suspension, a fine (as determined by the "Fine Formula" below), or a combination of a suspension and fine.

Maximum: revocation

Tier 3 discipline is recommended for:

- Knowing or willfully violating laws or regulations pertaining to commercial cannabis activity
- Fraudulent acts relating to the licensee's commercial cannabis business

Violations of the following codes are representative of this category:

Violation Description	Authority
Failure to Notify the Bureau of a Change in Ownership	CCR § 5023 and 5024
Obtaining a License for Premises in Restricted Location	B&P § 26054 CCR § 5026
Conducting Commercial Cannabis Activity with Non-Licensees	CCR § 5032(a)
Failure to Notify the Bureau of Criminal Acts, Civil Judgments, and Revocation of a Local License, or Other Authorization after Licensure	CCR § 5035
Failure to Notify the Bureau of Significant Discrepancy, Theft, Loss, and Criminal Activity	B&P § 26070 (k) CCR § 5036
Restricting or Hindering the Examination of Books and Records	B&P §§ 26160-26161 CCR § 5037(b)-(c)
Obstruction of Inspections, Investigations, or Audits	CCR § 5800
Delivery or Transport of Cannabis Goods Outside of California or to a Publicly Owned or Leased Location	B&P § 26080 CCR § 5416(b)-(c)
Failure to Correct Any Objectionable Conditions on Premises	CCR § 5808(a)-(b)
Illegal Sale of Dangerous Drugs, or Other Controlled Substances	CCR § 5808(d)
Failure to Pay Fine	B&P § 125.9(b)(5)
Engage in Conduct that is Grounds for Denial of Licensure	B&P § 480(a)
False Statement in Application	B&P § 480(d)
Securing License by Fraud, Deceit, or Misrepresentation.	B&P § 498

Fine Formula

In instances where the Bureau allows a fine to be paid, the following method will be used to calculate the fine.

Gross Cannabis Sales divided by **Number of Days Open in Calculation Period = Average Daily Sale Amount**

Average Daily Sale Amount multiplied by **Number of Days of the Suspension = Potential Fine Amount**

The books and records of the licensee shall be kept in such a manner that the average daily sale amount and/or the loss of profits from commercial cannabis activity that the licensee would have suffered from a suspension can be determined with reasonable accuracy therefrom, and such books, records, and information shall be accessible to the Bureau to make an accurate and complete determination of any fine amount.

Minimum and Maximum Fine Amounts

The minimum and maximum fine amount is based on the tier the licensee falls into on annual license fee schedule listed in 16 CCR § 5015.

License Type	Operations ($Million Max. Per License)	Minimum Fine to Maximum Fine
Testing Laboratory	Up to 50 Million	$1,000 to $40,000
	Greater than 50 million to 500 Million	$2,000 to $90,000
	Greater than 500 Million	$4,000 to $180,000
Distributor	Up to 2 million	$1,000 to $2,400
	Greater than 2 million to 8 million	$2,000 to $10,000
	Greater than 8 million to 80 million	$4,000 to $72,000
	Greater than 80 million	$8,000 to $250,000
Distributor Transport Only Self-Distribution	Up to 2 million	$1,000 to $2,400
	Greater than 2 million to 8 million	$2,000 to $4,000
Distributor Transport Only	Up to 2 million	$1,000 to $2,400
	Greater than 2 million to 8 million	$2,000 to $5,000

Retailer	Up to .5 million	$1,000 to $8,000
	Greater than .5 million to 1.5 million	$2,000 to $24,000
	Greater than 1.5 million to 4.5 million	$4,000 to $72,000
	Greater than 4.5 million	$8,000 to $144,000
Microbusiness	Up to .5 million	$1,000 to $10,000
	Greater than .5 million to 1.5 million	$2,000 to $30,000
	Greater than 1.5 million to 4.5 million	$4,000 to $84,000
	Greater than 4.5 million	$8,000 to $240,000

IV. STANDARD CONDITIONS OF PROBATION

The protection of the public is the highest priority of the Bureau. In disciplinary matters where probation as been imposed, the Bureau believes conditions should be imparted to ensure public protection and to allow the probationer the opportunity to demonstrate rehabilitation. The following conditions of probation provide for consumer protection and establish a mechanism to monitor the rehabilitation progress of a probationer. Generally, the Bureau recommends a minimum of three (3) years' probation.

Introductory Language and Conditions 1-7 are required as follows:

1. OBEY LAWS

   Respondent shall obey all state and local laws. A full and detailed account of any and all violations of law shall be reported by the respondent to the Bureau in writing within seventy-two (72) hours of occurrence. To permit monitoring of compliance with this condition, respondent shall submit completed fingerprint forms and fingerprint fees within 45 days of the effective date of the decision, unless previously submitted as part of the licensure application process.

   CRIMINAL COURT ORDERS: If respondent is under criminal court orders, including probation or parole, and the order is violated, this shall be deemed a violation of these probation conditions, and may result in the filing of an accusation and/or petition to revoke probation.

2. SUBMIT WRITTEN REPORTS

Respondent, during the period of probation, shall submit or cause to be submitted such written reports/declarations and verification of actions under penalty of perjury, as required by the Bureau, but no more frequently than once each calendar quarter. These reports/declarations shall contain statements relative to respondent's compliance with all the conditions of the Bureau's Probation Program. Respondent shall immediately execute all release of information forms as may be required by the Bureau or its representatives.

3. REPORT IN PERSON

Respondent, during the period of probation, shall appear in person at interviews/meetings as directed by the Bureau or its representatives.

4. COMPLY WITH CONDITIONS OF PROBATION

Respondent shall fully comply with the conditions of probation established by the Bureau and cooperate with representatives of the Bureau in its monitoring and investigation of the respondent's compliance with the Bureau's Probation Program. Respondent shall inform the Bureau in writing within no more than 15 days of any address change. Upon successful completion of probation, respondent's license shall be fully restored.

5. POSTING OF SIGN

During the period of suspension, Respondent shall prominently post a sign or signs, provided by the Bureau, indicating the beginning and ending dates of the suspension and indicating the reason for the suspension. The sign or signs shall be conspicuously displayed in a location or locations open to and frequented by customers. The location(s) of the sign(s) shall be approved by the Bureau and shall remain posted during the entire period of actual suspension.

Additionally, the Respondent shall circulate a notice of the conditions of probation to all employees, and post the notice in a conspicuous place where notices to employees are posted or available to employees. New employees shall also be provided a copy of the notice of the conditions of probation.

6. MAINTAIN VALID LICENSE

Respondent shall, at all times while on probation, maintain a current and valid license with the Bureau, including any period during which suspension or probation is tolled.

7. COST RECOVERY

Respondent shall pay to the Bureau costs associated with its investigation and enforcement pursuant to Business and Professions Code Section 26031 in the amount of $_____ . Respondent shall be permitted to pay these costs in a payment plan approved by the Bureau, with payments to be completed no later than three months prior to the end of the probation term.

If respondent has not complied with this condition during the probationary term, and respondent has presented sufficient documentation of his or her good faith efforts to comply with this condition, and if no other conditions have been violated, the Bureau, in its discretion, may grant an extension of the respondent's probation period up to one year without further hearing in order to comply with this condition. During the one year extension, all original conditions of probation will apply.

8. LICENSE SURRENDER

During respondent's term of probation, if he or she ceases business or is otherwise unable to satisfy the conditions of probation, respondent may surrender his or her license to the Bureau. The Bureau reserves the right to evaluate respondent's request and to exercise its discretion whether to grant the request, or to take any other action deemed appropriate and reasonable under the circumstances, without further hearing. Upon formal acceptance of the tendered license, respondent will no longer be subject to the conditions of probation. Surrender of respondent's license shall be considered a disciplinary action and shall become a part of respondent's license history with the Bureau.

9. VIOLATION OF PROBATION

If a respondent violates the conditions of his or her probation, the Bureau after giving the respondent notice and an opportunity to be heard, may set aside the stay order and impose the stayed discipline (revocation/suspension) of the respondent's license. If during the period of probation, an accusation or petition to revoke probation is filed against respondent's license, or the Bureau has served the respondent a notice of intent to set aside the stay, the Bureau shall have continuing jurisdiction, and the probationary period shall automatically be extended and shall not expire until final resolution of the matter.

VI. INTRODUCTORY LANGUAGE AND OPTIONAL TERMS AND CONDITIONS OF PROBATION

The following introductory language and all standard probation conditions are to be included in probationary decisions/orders. For applicants, cost recovery conditions do not apply. For licensees, all standard probation conditions apply. Optional terms and conditions may be included in orders of probation based upon violations.

INTRODUCTORY LANGUAGE FOR ALL ORDERS

IT IS HEREBY ORDERED that License Number _____ issued to Respondent_____ is [revoked/suspended/fined] [for/in the amount of] [days/amount], [however, the revocation is stayed] and respondent is placed on probation for _____ years on the following conditions.

SEVERABILITY CLAUSE – Each condition of probation contained herein is a separate and distinct condition. If any condition of this Order, or any application thereof, is declared unenforceable in whole, in part, or to any extent, the remainder of this Order, and all other applications thereof, shall not be affected. Each condition of this Order shall separately be valid and enforceable to the fullest extent permitted by law.

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

2nd Edition

US Food & Drug Administration
Office of Foods and Veterinary Medicine

April 2015

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

Center for Veterinary Medicine
Office of Research

National Center for Toxicological Research
Division of Microbiology

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

Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 2nd Ed.

APPROVAL PAGE

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

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ORA, Member of the ORA Scientific Advisory Council

US Food & Drug Administration
Office of Foods and Veterinary Medicine

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

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1.0 INTRODUCTION

1.1 Purpose

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

1.2 Scope

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

1.3 Administrative Authority and Responsibilities

All criteria established in this document for analytical method validation have been adopted and approved by the OFVM and the SRSC. As stated in the Methods Development, Validation and Implementation Program SOP (APPENDIX 3), The Method Validation Subcommittee (MVS) will have oversight responsibility for all collaborative validation studies (See Section 2.2.2.3).

1.4 The Method Validation Subcommittee

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

otherwise stated, most correspondence between the method developer(s) and the MMVS will be by email using the following address: Microbiology.MVS@fda.hhs.gov.

1.5 General Responsibility of the Originating Laboratory

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

1.6 Method Validation Definition

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

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

1.7 Applicability

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

- Qualitative assays *i.e.* detection assays
- Quantifiable assays *i.e.* real-time PCR
- Analyte-specific

- Bacteriological, *e.g.*
  - *Salmonella* spp.
  - Pathogenic *Escherichia coli*
  - *Listeria monocytogenes*
  - *Shigella* spp.
  - *Vibrio* spp.
  - *Campylobacter* spp.
- Microbial toxins *(excluding marine biotoxins. See Chemistry method validation guidelines)*
- Viral pathogens, *e.g.*
  - Hepatitis A virus
  - Norovirus
  - Enterovirus
- Parasitic protozoan pathogens, *e.g.*
  - *Cryptosporidium*
  - *Cyclospora cayetanensis*
- Indicator organisms

- Bioengineered analytes, *e.g.*
  - Genetically-modified foods (GMOs)
- Applications
  - Pre- and selective enrichment
  - Microbial analyte recovery and concentration
  - Screening, high-throughput, confirmation
- Procedures
  - Phenotypic, *e.g.*
    - Biochemical characterization for identification
    - Antibiotic resistance traits for identification
    - Antigenic characterization for identification
  - Genetic, *e.g.*
    - Nucleic acid isolation/concentration/purification
    - Polymerase Chain Reaction
      - Conventional
      - Real-time
      - Reverse transcription
    - Sequencing, *e.g.*
      - Whole genome
      - Selective sequencing
      - Single nucleotide polymorphism (SNP) analysis
    - Strain-typing applications
- Immunological
  - Antibody capture
  - ELISA
  - Flow cytometry

1.8 Requirements

Method validation shall be required for:

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

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

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

2.1 Validation Definitions

2.1.1 The Reference Method

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

2.1.2 The Alternate Method

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

2.1.3 The Originating Laboratory

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

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

2.1.4 The Collaborating Laboratory

The collaborating laboratory refers to the laboratory (or laboratories) other than the originating laboratory involved in multi-laboratory method validation studies.

2.2 The Method Validation Process

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

2.2.1 Emergency Usage (Level One)

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

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

NOTE: *Under emergency situations where the rapid development and deployment of a method is needed to immediately address an outbreak event, Level 1 - Emergency Use criteria should be followed as closely as the situation will allow.*

2.2.2 Method Validation Levels (for Non-Emergency Use Methods)

2.2.2.1 Single-laboratory Validation (Level Two - Part a)

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

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

2.2.2.2 Independent Laboratory Validation (Level Two - Part b)

One other independent laboratory has participated in the validation study using the method of the originating lab and criteria described in Table 1. Successful completion of this level of scrutiny and the approval of the MMVS are prerequisite steps prior to any collaborative validation study.

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

2.2.2.3 Collaborative Validation Study (Level Two – Part c)

A Collaborative 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 (W. Horwitz, IUPAC, 1987). 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.

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

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

2.3 Validation Criteria

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

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

2.3.1.1 Definition

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

2.3.1.2 Criteria

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

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

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

	Emergency	Non-Emergency Validation Processes		
Criteria	**Emergency Use**	**Single Laboratory Validation Study**	**Independent Laboratory Validation Study**	**Collaborative Validation Study**
Participating Laboratory	Originating Laboratory	Originating Laboratory	Collaborating Laboratory	Collaborating Laboratories
# of target organism (inclusivity)[a]	‡TBD	50 (unless 50 aren't available)[b,c]	⁴NA	⁴NA
# of non-target organism (exclusivity) [a]	‡TBD	30 strains[d]	⁴NA	⁴NA
# of laboratories providing usable data	1	1	1	10
# of foods	1or more[e]	1or more[e]	1or more[e]	1or more[e]
# of analyte levels/food matrix	‡TBD	Two inoculated levels[f] and one uninoculated level	Two inoculated levels[f] and one uninoculated level	3 levels: One inoculated level[f], one at 1 log higher[g] and one uninoculated level
Replicates per food at each level tested	‡TBD	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)	8
Aging of inoculated samples prior to testing	No	Yes[h]	Yes[h]	Yes[h]
Addition of competitor strain[i]	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
Reference Method Comparison Requirement[j]	‡TBD	Yes, if available	Yes, if available	Yes, if available

[a]Using pure cultures without a food matrix.
[b]Each at 10^3 CFU/mL following the method protocol (1 $\log_{10}$ above the LOD for other methods); or 10^3 CFU/reaction for molecular methods *e.g.* PCR.
[c]100 serotypes for Salmonella testing.
[d]At 10^3 CFU/mL for non-target organisms grown in a non-selective rich medium.
[e]For FDA regulatory use, methods are only valid for foods that have been tested; the MMVS may require that a new method be validated for 3 foods within a food category (See APPENDIX 5). See Section 6 for further guidance on matrix extension criteria.
[f]Must be adjusted to achieve fractional positive results (one or both methods *i.e.* the reference and alternate methods must yield 50%±25% of tests positive) at this level; the high level inoculum should be approximately1 log greater than that used to achieve fractional results. All 5 replicates at the high inoculum should yield positive results.
[g]All test samples inoculated at this level must yield 100% positive results
[h]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.
[i]An appropriate competitor is one that gives similar reactions in enrichment and detection systems. Natural background microflora can fulfill this requirement as long as it present in the matrix at a level 1 log greater than the target analyte.
[j]Independent Laboratory and Collaborative Validation Studies should use the most effective reference method available.
‡TBD to be determined in consultations with the originating laboratory, the MMVS, and subject matter experts.
⁴ Not Applicable

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

Tables 2 provides validation criteria for microbial pathogens characterized as difficult to isolate, limited resources for extensive inclusivity and

exclusivity studies, and either non-culturable for enrichment purposes or, enrichment cannot be accomplished in a timely manner.

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

	Emergency	Non-Emergency Validation Processes		
Criteria	**Emergency Use**	**Single Laboratory Validation Study**	**Independent Laboratory Validation Study**	**Collaborative Validation Study**
Participating Laboratory	Originating Laboratory	Originating Laboratory	Collaborating Laboratory	Collaborating Laboratories
# of target organism (inclusivity)[a]	‡TBD	‡TBD	¥NA	¥NA
# of non-target organism (exclusivity)[a]	‡TBD	‡TBD	¥NA	¥NA
# of laboratories providing usable data[b]	1	1	1	5[¥]
# of foods	1 or more[¥]	1 or more[¥]	1 or more[¥]	1 or more[¥]
# of analyte levels/food matrix	‡TBD	One inoculated level[c] and one uninoculated level	One inoculated level[c] and one uninoculated level	3 levels: One inoculated level[c], one at 1 log higher[d] and one uninoculated level
Replicates per food at each level tested	‡TBD	3	3	8[¥]
Reference Method Comparison Requirement[e]	‡TBD	Yes, if available	Yes, if available	Yes, if available

[a]Using pure cultures without a food matrix.
[b]Labs providing data are required to run study on same PCR platform.
[c]Must be adjusted to achieve fractional positive results (one or both methods *i.e.* the reference and alternate methods must yield 50%±25% of tests positive) at this level, advisable to include when possible one additional level at +1 log.
[d]All test samples inoculated at this level must yield 100% positive results.
[e] Independent Laboratory and Collaborative Validation Studies should use the most effective reference method available.
†Such examples include but are not limited to RNA food-borne viruses, and protozoan parasites. See APPENDIX 3 Sections V and VI.
‡TBD to be determined in consultations with the originating laboratory, the MMVS, and subject matter experts.
¥ Not Applicable.
[¥]Where circumstance and resources permit.

2.3.2 Validation Criteria for Identification Methods

2.3.2.1 Definition

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

2.3.2.2 Criteria

Table 3- General Guidelines for the Validation of Identification Methods for Microbial Analytes

	Non-Emergency Validation Processes		
Criteria	**Single Laboratory Validation Study**	**Independent Laboratory Validation Study**	**Collaborative Validation Study**
Participating Laboratory	**Originating Laboratory**	**Collaborating Laboratory**	**Collaborating Laboratories**
# of target organism (inclusivity)[a]	≥50 (unless 50 aren't available)[b,c]	1[c]	12[c]
# of non-target organism (exclusivity)[a]	≥30 strains[b,c]	1[c]	12[c]
# of laboratories providing usable data	1	1	10
Replicates[d]	3	3	3
Reference Method Comparison Requirement	Yes, if available	Yes, if available	Yes, if available

[a]At 10^3 CFU/mL for target organisms and non-target organisms grown in a non-selective rich medium. 10^3 CFU/reaction for molecular methods *e.g.* PCR.
[b]100 serotypes for *Salmonella* testing.
[c]Should be evaluated together in one single study; inclusive and exclusive samples should be intermingled and blinded
[d]All replicates must yield the correct answer

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

2.3.3.1 Definition

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

2.3.3.2 Criteria

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

	Non-Emergency Validation Processes		
Criteria	Single Laboratory Validation Study	Independent Laboratory Validation Study	Collaborative Validation Study
Participating Laboratory	Originating Laboratory	Collaborating Laboratory	Collaborating Laboratories
# of target organism (inclusivity)	50 (unless 50 aren't available)	NA[f]	NA[f]
# of non-target organism (exclusivity)	30 strains	NA[f]	NA[f]
# of laboratories providing usable data	1	1	10
# of foods	1 or more[a]	1 or more[a]	1 or more[a]
# of analyte levels/food matrix[b]	4 levels: Low medium and high inoculum levels[b] and one uninoculated level	4 levels: Low medium and high inoculum levels[b] and one uninoculated level	4 levels: Low medium and high inoculum levels[b] and one uninoculated level
Replicates per food at each level tested	5 replicates per level for a total of 20 replicates per method	5 replicates per level for a total of 20 replicates per method	Two test portions per level for a total of 8 test portions
Aging of inoculated samples prior to testing	Yes[c]	Yes[c]	Yes[c]
Addition of competitor strain[d]	In 1 food at +1 log>analyte at highest analyte level	In 1 food at +1 log>analyte at highest analyte level	In 1 food at +1 log>analyte at highest analyte level
Reference Method Comparison Requirement	Yes, if available	Yes, if available	Yes, if available
Confirmation of Test Portions	NA[f]	NA[f]	Yes, follow the reference method

[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. See section 5.1
[b]The low level should be at or near the limit of detection; medium and high levels should be chosen to span the analytical range of the alternate method.
[c]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.
[d]An appropriate competitor is one that gives similar reactions in enrichment and detection systems. Natural background microflora can fulfill this requirement as long as it present in the matrix at a level 1 log greater than the target analyte.
[f] Not Applicable

2.4 Method Validation Operational Aspects

2.4.1 General Considerations

- All correspondence *e.g.* proposals, validation reports etc., with the MMVS will be initiated via email using the following address: Microbiology.MVS@fda.hhs.gov.
- As defined in the SRSC Document titled "Method Development, Validation and Implementation SOP (See APPENDIX 3), all method validation plans must be

submitted to and approved by the MMVS prior to initiating any methods validation work beyond the single lab validation stage. See APPENDIX 4 for proposal formatting.

- The number of laboratories submitting usable data in all the above tables represents the minimum number allowable for a successful validation study. It is suggested that 4 additional labs be considered for participation, since a variety of unforeseen circumstances can cause data sets to be rejected.
- The following elements must be addressed in all proposals for method validation studies (in non-emergency use situations).

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

    - Individual laboratories within the FVM research enterprise maintain their own inventories of microbial analyte collections. These collections, strains and serovars derived from food surveillance programs, food-borne outbreak investigations, and clinical specimens, are available to all Agency scientists. Access is governed by "U.S. Food and Drug Administration Foods Program Internal Strain Sharing Standard Operating Procedure" (http://inside.fda.gov:9003/downloads/OC/OfficeofFoods/UCM353743.pdf).
    - The choice of inclusivity strains should reflect the genetic, serological, and/or biochemical diversity of the organisms involved, as well as other factors such as virulence, frequency of occurrence and availability. Inclusivity testing is performed on purified cultures.
    - The choice of exclusivity strains should closely reflect related, potentially cross-reactive organisms. Other factors such as virulence, frequency of occurrence and availability should be considered. Exclusivity testing is performed on purified cultures.

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

- Suitability and availability of naturally-contaminated samples in the proposed validation study.
- Inoculum preparation, spiking methodology, and uniformity of contamination (when artificially-contaminated samples will be used).
- Sample preparation, naturally-occurring microflora, and the requirement for aerobic plate counts (APC) to verify background microflora.
- Need for inclusion of competitive microflora. For food matrices that exhibit low naturally-occurring microflora background (as determined by APC), validation studies will adhere to AOAC-established parameter *i.e.*1 log greater than microbial analyte being tested. Selection of competitive microflora to be used will be done in consultation with the MMVS.
- Selection of spiking levels (when artificially-contaminated samples will be used).
- Matrix aging to assess method robustness.
- Microbial analyte stress, cell injury, and matrix-derived inhibition of analyte enrichment/growth.
- Selection of appropriate foods. 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 5. Extension of a method to include additional food matrices will require additional validation studies. See Sections IV and V.
- Formation of composited samples. In some instances, it may be necessary to validate composited samples. 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.
- Inocula designed to yield fractional positive results. Samples for both the reference method and the test method must achieve 50%±25% positive results (See APPENDIX 1: Glossary of Terms, for a complete description of fractional recovery).

2.4.2 Assessment of Validation Results

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

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

3.0 CRITERIA AND GUIDANCE FOR THE VALIDATION OF FDA-DEVELOPED 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 guidance is intended to govern validation studies for either conventional or 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 provide detailed chemistry and platform prerequisites and include multiple platforms where possible.

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

3.1 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.

3.2 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.

3.3 Comparison to the Reference Method

The originating laboratory will compare the PCR-based method to bacteriological, biochemical, and/or serological reference methods. PCR-based methods may only be compared to PCR-based reference identification methods when bacteriological, biochemical, and/or serological reference methods are unavailable.

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

4.1 Definitions

4.1.1 Validation of an Alternative Method

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

4.1.2 Verification

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

- The confirmation by examination and the provision of objective evidence that specified requirements have been fulfilled.
- To assess the performance of a method in the user's laboratory against the specifications of the method established during the validation.

- To assess the method performance on items included in the scope of the method and tested routinely by the user laboratory.
- To demonstrate that the method functions (without any adaptation) in the user's laboratory on matrices not included in the original method validation.

4.2 Criteria

4.2.1 Commercially-available Microbiological Diagnostic Kits Whose Performance Parameters Have been Fully Validated in a Multi-laboratory 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.

4.2.1.1 Verification Requirements *(per lab)*

- Six replicates of the inoculated matrix and six replicates of the un-inoculated matrix are tested and confirmed by both the alternative and the reference method.
- If no false positive or false negative results are obtained, then the 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 or any other specified 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 (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 verification criteria described 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 5: Food Matrix Extension)

4.2.2 Commercially-available Microbiological Diagnostic Kits Whose Performance Parameters are Supported by Data Obtained Through an Independent Laboratory Validation Protocol and Evaluated by an Independent Accrediting Body *e.g.* AOAC-RI.

All methods fitting into this description ***must*** be validated according to the criteria defined for Agency-developed (FDA) microbiology methods (*See* Section 2).

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

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

Some modifications (*e.g.* ease-of-use capabilities, availability/substitution of reagents or instrumentation, sample handling/sample processing adaptations, etc.) may only necessitate verification against the original method according to criteria detailed in Section 4.2.1.1., whereas other modifications may require significant validation data to support their use. It is recommended that statistical analyses be performed on the verified performance specifications to support implementation of the modification. These include:

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

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

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

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

5.1 Matrix Extension

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

Very often however, validation studies can neither address all the varied matrices nor fully anticipate what matrix or matrices will be involved in emergency situations

or outbreak investigations – two scenarios where samples must be analyzed immediately.

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

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

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

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

In instances where a method will be used to test a food (or foods) from the same category of food (See APPENDIX 5) included in the original validation study, ORA laboratories will analyze the matrix in question concurrently with a matrix spike. The matrix spike will consist of a 25 gram 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 matrices that have not been fully validated for the method. Matrix spike results will be entered into Field Accomplishment Computerized Tracking System (FACTS) and data will be evaluated and classified according specific food, and matrix spike results. When a specific food has yielded at least seven positive and no negative results using matrix spikes; or, a >95% confidence level (19 of 20 positives), the method will be considered verified for that food product. The method can then be used for that food without further positive spike controls.

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

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

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

5.2 Platform Extension

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

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

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

In planning platform extension validation, the method developer and the MMVS, must determine what aspect of the technology will be compared in order to determine how the study should proceed. In some instances a platform extension study may require only a simple verification process. Other instances, however, may necessitate an SLV or Independent Validation Study as described in Table 1.

APPENDIX 1
Glossary of Terms

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

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

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

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

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

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

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

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

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

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

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

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

Collaborative study: A Collaborative 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.

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

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

Food category: A group of specific related foods. Appendix 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 5 for examples of representative food products.

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

Fractional recovery: Validation criterion that is satisfied when a common set of samples (e.g., inoculation level), yields a partial number of positive determinations and a partial number of negative determinations within a replicate set of samples. The proportion of positive samples should approximate 50% (±25%) of the total number of replicates in the set. A set of replicate analyses are those replicates analyzed by on method (either reference or alternate). In the context of the entire data set, values outside the prescribed

fractional range (50%±25%) may be considered. For example, for studies where a larger number of test portions were analyzed, (i.e., 60), a larger fractional range may be acceptable. Other parameters may be considered on an individual basis.

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

Incurred samples: Naturally-contaminated test samples.

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

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

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

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

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

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

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

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

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

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).

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

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

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

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

Specificity: 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.

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

Specificity: 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.

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 the specific use of a method are fulfilled.

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

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

APPENDIX 2
SRSC Method Validation Subcommittee Charter

SRSC Method
Validation Subcommit

APPENDIX 3
Method Development, Validation and Implementation SOP

Methods
Development-Validatio

APPENDIX 4
FVM Microbiology Method Validation Study Application

FVM Micro Method
Validation Study Appli

APPENDIX 5

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	

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

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

- This appendix is meant to serve as a guide or starting point for the method developer as they construct exclusive and inclusive panels for method validation and is not intended to be exhaustive.
- Access to microbial analyte strain and serovar and collections within the FVM research enterprise is governed by "U.S. Food and Drug Administration Foods Program Internal Strain Sharing Standard Operating Procedure"

	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 I. E.

coli O157:H7

	Serotype	Genotype		
		stx1	*stx2*	*uidA*-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		-	-	-

II. *Salmonella* (inclusivity)

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

IIa. *Salmonella*: Subspecies Set

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

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

IIb. *Salmonella*: Outbreak Cluster Set

SAFE Designation	Original Designation	Serotype
56	AM04695	Typhimurium / DT' 04b
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 / DT 104
71	AM01797	Typhimurium / DT 104
72	AM03759	Typhimurium / DT 104
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
	2386	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
Staphylococcus 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 (*[b]should include the following strains*):

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

Enterovirus Panels

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

Panel E

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

Levels Two, Three and Four *([d]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

Guidelines for the Validation of Chemical Methods for the FDA FVM Program

2nd Edition

US Food & Drug Administration
Office of Foods and Veterinary Medicine

April 2015

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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
Office of New Animal Drug Evaluation

Office of Regulatory Affairs
Office of Regulatory Science
ORA Laboratories

APPROVAL PAGE

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

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ORA, Member of the ORA Scientific Advisory Council

US Food & Drug Administration
Office of Foods and Veterinary Medicine

Guidelines for the Validation of Chemical Methods for the FDA FVM Program, *2nd Edition*

TABLE OF CONTENTS

1.0 INTRODUCTION

1.1 Purpose

The U.S. Food and Drug Administration (FDA) is responsible for ensuring the safety of approximately 80% of the nation's food supply. FDA laboratories contribute to this mission through routine surveillance programs, targeted regulatory analyses, and emergency response when contaminated food or feed is detected or suspected in a public health incident. The effectiveness of these activities is highly dependent on the quality and performance of the laboratory methods needed to support regulatory compliance, investigations and enforcement actions. To ensure that the chemical methods employed for the analysis of foods and feeds meet the highest analytical performance standards appropriate for their intended purposes, the FDA Office of Foods and Veterinary Medicine (OFVM) through the Science and Research Steering Committee (SRSC) has established criteria by which all Foods and Veterinary Medicine (FVM) Program chemical methods shall be evaluated and validated. This document defines four standard levels of performance for use in the validation of analytical regulatory methods for chemical analytes in foods and feeds.

1.2 Scope

These criteria apply to FDA laboratories as they develop and participate in the validation of analytical regulatory methods for chemical analytes in anticipation of Agency-wide FVM Program implementation. These criteria do not apply to methods developed by or submitted to FDA under a codified process or official guidance (e.g., in the Code of Federal Regulations, CPGs, etc.) such as for veterinary drug approval. For such studies, the appropriate Center for Veterinary Medicine (CVM) or other Program guidance documents should be followed. This guidance is a forward-looking document; the requirements described here will only apply to *newly*-developed methods and significant modifications to existing methods (see Requirements). Once a method has been validated at the appropriate level, it can be implemented according to OFVM document, FDA-OFVM-3, "Methods Development, Validation, and Implementation Program", which establishes a standard operating procedure for the methods development, validation and implementation process [1]. For example, for a multi-laboratory validated method to be used in a widespread regulatory application, it can be implemented by other FDA laboratories following the method verification process. However, method verification is normally part of a local laboratory's quality control procedures and is not considered within the scope of this validation document.

1.3 Administrative Authority and Responsibilities

All criteria established in this document for analytical method validation have been adopted and approved by the OFVM and the SRSC. The OFVM document, FDA-OFVM-3, establishes the standard operating procedure for the approval and tracking of method development and validation activities within the FVM Program [1]. Single laboratory validation (SLV) studies (including both Level 1 and Level 2 validations) can be managed wholly by the respective Center and Office line management structure. Oversight and coordination of multi-laboratory validation (MLV) studies (including both Level 3 and Level 4 validations) are the responsibility of the Methods Validation Subcommittees (MVS).

1.4 The Method Validation Subcommittee

Under the charge of the SRSC, the Chemistry Methods Validation Subcommittee (CMVS) will have oversight responsibility for MLV studies involving chemical methods associated

with the FVM Program which are intended for use in a regulatory context. The CMVS is a subcommittee of the Chemistry Research Coordinating Group (CRCG), which reports directly to the SRSC. The CMVS is governed by the organizational structure, roles and responsibilities as detailed in its charter [2]. Briefly, the CMVS will oversee and coordinate, in collaboration with the originating laboratory, all MLV studies for chemical methods developed within the FDA OFVM Enterprise to support regulatory analytical needs. This includes the evaluation and prioritization of proposed MLV studies as well as evaluation of completed MLV studies and reports. Submissions of chemical validation proposals, reports, questions, *etc.* can be directed to the CMVS through a central email account:

Chemistry.mvs@fda.hhs.gov

However, where possible, MLVs should be discussed in appropriate Technical Advisory Groups or with the CRCG to ensure the broadest possible consideration of factors before committing resources to an MLV.

1.5 General Responsibility of the Originating Laboratory

It is the responsibility of the originating laboratory to ensure proper adherence to all criteria described in this document. The originating laboratory should work in consultation with the CMVS and/or its designated Technical Advisory Group (TAG) throughout the multi-laboratory 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.

1.6 Overview of Method Validation

Method validation is the process of demonstrating or confirming that a method is suitable for its intended purpose. The purpose of these methods may include but is not limited to qualitative analysis, quantitative analysis, screening analysis, confirmatory analysis, limit tests, matrix extensions, platform extensions, and emergency/contingency operations. Validation includes demonstrating performance characteristics such as accuracy, precision, sensitivity, selectivity, limit of detection, limit of quantitation, linearity, range, and ruggedness, to ensure that results are meaningful and appropriate to make a decision. Method validation is a distinct phase from method development/ optimization and should be performed *subsequent* to method development. Methods may be validated for one or more analytes, one or more matrices, and one or more instruments or platforms. The method is validated by conducting experiments to determine the specific performance characteristics that serve to define and quantify method performance.

1.7 Applicability

This document establishes validation criteria for regulatory methods that are to be widely used to detect chemical analytes in food, feed and other FDA regulated products covered by the FVM Program including, but not limited to, the following:

Chemotherapeutic Residues
Color Additives
Decomposition Products
Dietary Supplement Ingredients/Adulterants
Elemental and Metals
Food and Feed Additives and Preservatives
Food Allergens
Gluten

Intentional Adulterants/Poisons
Mycotoxins
Nutrients
Persistent Organic Pollutants
Pesticides
Seafood and plant toxins
Toxic Elements
Veterinary Drug Residues

Please note that although these guidelines mainly cover multi-laboratory validations, criteria for several validation levels are discussed and are differentiated from full MLVs. There are situations where a method is being extended to handle what is likely to be a very limited (perhaps one time) use by one laboratory and is therefore not intended for Agency-wide regulatory use, thus would be validated at a lower level. For example, when a single pesticide laboratory receives several new food matrices for multi-residue analyses that were not covered in the previous validation of the method, these guidelines would not generally be required and a more abbreviated validation/verification within the pesticide program's guidelines may be acceptable.

1.8 Requirements

Method validation is required for:

- Submission of a new or original method.
- Expansion of the scope of an existing method to include additional analytes.
- Expansion of the scope of an existing method to include additional matrices.
- Changes in the intended use of an existing method (*e.g.*, screening vs. confirmatory).
- Modifications to a method that may alter its performance specifications (*e.g.*, modifications that could significantly affect the precision and accuracy, changes to the fundamental science of an existing method, significant changes to reagents, apparatus, instrumental parameters, sample preparation and/or extraction, or modification of a method's range beyond validated levels). Some examples of allowable modifications that would not require further validation are provided in the document, ORA-LAB.5.4.5 Attachment A-Modification Criteria [3].

2.0 CRITERIA AND GUIDANCE FOR THE VALIDATION OF CHEMICAL METHODS

2.1 General Validation Tools and Protocol Guidance

There are a number of excellent references and guides available providing further information on method validation for chemical methods [3-20]. The following provides some general guidelines/tools that should be used to assess method performance:

General Protocol: Prepare and analyze method blanks, matrix blanks, reference materials (if available) and matrix spikes (using matrix blanks if available) of known concentration as generally described under the Methods Validation Levels section and Table 1 below. Accuracy or bias and precision are calculated from these results. Data will also be used to evaluate matrix effects and ruggedness/robustness of the method resulting from changes in the sample matrix.

The following general validation tools should be used to generate method performance characteristics as described in the Performance Characteristics section below.

Blanks: Use of various types of blanks enables assessment of how much of the result is attributable to the analyte in relation to other sources. Blanks are useful in the determination of limit of detection.

Reference materials and certified reference materials: The use of known reference materials (when available and applicable) should be incorporated to assess the accuracy or bias of the method, as well as for obtaining information on interferences.

Matrix Blank: This type of blank is a substance that closely matches the samples being analyzed with regard to matrix components. Matrix blanks are used to establish background level (presence or absence) of analyte(s) and to verify that sample matrix and equipment used does not interfere with or affect the analytical signal.

Matrix Spikes (Laboratory Fortified Matrix): Recovery determinations can be estimated from fortification or spiking with a known amount of analyte and calculation of spike recoveries. (Note: spike recovery may not be accurately representative of recovery from naturally incurred analytes.) Matrix effects can also be assessed with these samples. Accuracy or bias and precision are calculated from these results. The data can also be used to evaluate robustness of the method resulting from changes in the sample matrix.

Incurred Samples: This type of sample contains (not laboratory fortified) the analyte(s) of interest (if available) and can be used to evaluate precision and bias (if analyte concentration(s) are reliably known). Analyte recovery can also be evaluated through successive extractions of the sample and/or comparison to another analytical procedure with known bias.

Reagent Blank: This type of blank incorporates all reagents used in the method and is subjected to all sample processing operations. It serves to verify that reagents are analyte free and the equipment used does not interfere with or affect the analytical signal.

Replicate Analyses: The precision of the analytical process can be evaluated using replicate analyses. The originating laboratory should assure that adequate sample replicates are

performed and that results from replicate measurements of each analyte are compared. Minimally, the method repeatability should be evaluated.

Interferences: Spectral, physical, and chemical interferences can be evaluated by analyzing samples containing various suspected interferences. Carryover should be evaluated using the incorporation of blanks immediately following standards and samples.

Statistics: Statistical techniques are employed to evaluate accuracy, trueness (or bias) precision, linear range, limits of detection and quantitation, and measurement uncertainty.

2.2 Reference Method

A reference method is a method by which the performance of an alternate or new method may be measured or evaluated. For chemical analytes, an appropriate reference method is not always identifiable or available. However, there are some instances in which the use of a reference method is appropriate such as when replacing a method specified for use in a compliance program. Consultation between the originating laboratory and the CMVS and the Program Office is suggested when deciding if the use of a reference method will be necessary.

2.3 Performance Characteristics

Performance characteristics that should be evaluated in order to validate a method will vary depending on the intended use of the method, the type of method (*e.g.*, quantitative vs. qualitative), and the degree to which it has been previously validated (e.g., matrix extension, analyte extension, platform extension). Although definitions of these characteristics are included in Appendix 1, this document is not meant to address the various ways of calculating characteristics such as method detection level, limit of detection or limit of quantitation.

Performance Characteristics for Validation of New Quantitative Methods: Validation of new quantitative methods should include at a minimum evaluation of the following performance characteristics: accuracy, precision, selectivity, limit of detection, limit of quantitation, linearity (or other calibration model), range, measurement uncertainty, ruggedness, confirmation of identity and spike recovery.

Performance Characteristics for Validation of New Qualitative Methods: Validation of new qualitative methods should include at a minimum evaluation of the following performance characteristics: sensitivity, selectivity, false positive rate, false negative rate, minimum detectable concentration, ruggedness, and confirmation of identity.

Performance Characteristics for Validation of Method Extensions: Validating the extension of methods that have previously been validated requires a careful evaluation of the intended purpose of the extension. In cases where the sample preparation and/or the extraction procedure/analytical method is modified from the existing test procedure, it should be demonstrated that the modifications do not adversely affect the precision and accuracy of the data obtained. In order to implement the modified method, generally the standard or existing method is first performed. The modified method performance then is verified by comparison with that of the original method.

2.4 Confirmation of Identity

Confirmation of identity for each analyte must be performed as part of the method validation for regulatory enforcement for both qualitative and quantitative methods. Unambiguous confirmation of identity usually requires analytically identifying key features of each analyte in the scope of the new method being validated such as with mass spectral fragmentation patterns or by demonstration of results in agreement with those obtained using an independent analysis.

FDA has issued guidance documents on the development, evaluation, and application of mass spectrometric methods for confirming the identity of target analytes including: CVM Guidance for Industry 118: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues [4] and ORA-LAB.010, Guidance for the Analysis and Documentation to Support Regulatory Action on Pesticide Residues [5]. Following the CVM guidance is required for veterinary drug residue methods. The ORA-LAB.010 document was written specifically for pesticide analyses. For other types of chemical contaminants in food (e.g. food additives, mycotoxins, etc.), the CVM document should be followed because it was written as a Guidance for Industry and therefore has been more widely internally and externally reviewed and distributed. In addition, OFVM is currently drafting a supplement to CVM Guidance for Industry 118 specifically addressing the use of high resolution mass spectrometry and the evaluation of exact mass measurement data.

2.5 Method Validation Levels

The following describes the four standard levels of performance defined for method validation of analytical regulatory methods for chemical analytes in foods. This approach is based on the Food Emergency Response Network (FERN), SOP No: FERN-ADM.0008.00, FERN Validation Guidelines for FERN Chemical, Microbiological, and Radiological Methods [6], as well as AOAC guidelines for single-laboratory validation [7] and collaborative studies [8]. Key validation parameters for each level are summarized in Table 1. It is the responsibility of the originating (developing) laboratory to determine the appropriate level of validation required up to and through single laboratory validations. It is highly recommended that originating laboratories work with the appropriate Technical Advisory Group when determining the appropriate level of validation.

NOTE: *Not all methods will or should be validated to the highest level.*

Level One

This is a single laboratory validation level with the lowest level of validation requirements and is appropriate for emergency/limited use. Performance of the method at this initial level of scrutiny will determine, in part, whether further validation is useful or warranted.

Intended Use: emergency/limited use/matrix extension/analyte extension/ platform extension. Examples of where Level One validation would be acceptable include, isolated consumer complaints, single-occurrence samples, and application of a method developed for a specific analyte(s) to a matrix, not previously validated in response to a real or perceived threat to food safety or public health. Validation of method performance with a new matrix is intended to assure that the new matrix will produce accurate and reliable results for all the analytes in the scope of the method. Generally, all targeted analytes still must be included in matrix spikes at this level, if widespread use in this matrix is anticipated for regulatory purposes. As the first level of validation of methods for matrix, analyte or platform extension/emergency use, it would be expected that a

more rigorous single laboratory validation at least equivalent to Level Two below would be performed before more widespread non-emergency regulatory use.

Level Two

This is a single laboratory validation level. The originating lab has conducted a comprehensive validation study, with performance criteria similar to an AOAC Single Laboratory Validation study. If appropriate, a comparison with an existing reference method has been performed. Some of the criteria of the study may be at a lower level than the AOAC Single Laboratory Validation study, but are appropriate for the developing method at this stage.

Intended Use: Routine regulatory testing, emergency needs, minor method modifications, analyte and matrix extensions of screening methods. If a method validated at this level is expected to have use that is widespread, long term, of high public visibility or potentially involved in international trade conflicts, its validation should be extended to at least Level Three below.

Level Three

This is a multi-laboratory validation level. Level Three validation employs a minimum of one collaborating laboratory in addition to the originating laboratory. Most of the criteria followed by the originating lab are at a level similar to the AOAC full collaborative study level with comparison to an existing reference method when available and appropriate. The additional collaborating laboratories follow many of the criteria found in an AOAC collaborative study. The main differences are that Level Three validation employs at least one additional collaborating laboratory instead of the eight to ten used by AOAC and requires fewer replicates for each food matrix/spike level.

Intended Use: Methods validated to this level of scrutiny are acceptable for use in all regulatory circumstances including screening analyses, confirmatory analyses, regulatory surveys, and compliance support. If the method is expected to have use that is widespread, long term, of high public visibility or involved in international trade conflicts, it may be appropriate to have its validation extended to Level Four.

Level Four

This validation level has criteria equivalent to a full AOAC or ISO Collaborative Study. Any method reaching this level of validation should be able to be submitted for adoption by the AOAC as a fully collaborated method.

2.6 Acceptability Criteria

There are various acceptability ranges for method validation performance criteria that may be appropriate depending on the application or intended use of the methodology and especially the levels of concern, action levels or tolerance for the chemical analyte. Some examples of acceptability ranges used by various national and international organizations and their sources are provided in Appendix 2. Acceptable spike recoveries vary with analyte concentration as indicated in Appendix 2 (e.g., recoveries may fall in approximately the 80-120% range for quantitative methods at the 1 µg/g (ppm) concentration). Repeatability and reproducibility also vary with analyte concentration. The acceptability ranges in Appendix 2 provide approximate target ranges for method developers and the MVS and are not rigid binding guidelines. It is recognized that for some situations such as with difficult matrices, extremely low analyte concentrations (e.g., chlorinated dioxins, persistent organic

pollutants), multi-residue methods and with emergency situations these general acceptability ranges may not be achievable or required.

Table 1. Key Validation Parameter Requirements for Chemical Methods

	Level One: Emergency/ Limited Use	Level Two: Single Laboratory Validation	Level Three: Multi-Laboratory Validation	Level Four: Full Collaborative Study
Number participating labs	1	1	≥ 2	8 (quantitative) 10 (qualitative)
Number of matrix sources per matrix*	≥1	≥3 recommended where available	≥3 recommended where available	≥3 recommended where available
Number of analyte(s) spike levels for at least one matrix source**	≥2 spike levels + 1 matrix blank	≥3 spike levels + 1 matrix blank	≥3 spike levels + 1 matrix blank	≥3 spike levels + 1 matrix blank
Replicates required per matrix source at each level tested per laboratory	≥2 (quantitative) ≥2 (qualitative)	≥2 (quantitative) ≥3 (qualitative)	≥2 (quantitative) ≥3 (qualitative)	≥2 (quantitative) ≥3 (qualitative)
Replicates required at each level tested per laboratory if only one matrix source used	≥4 (quantitative) ≥6 (qualitative)	≥6 (quantitative) ≥9 (qualitative)	≥3 (quantitative) ≥6 (qualitative)	≥2 (quantitative) ≥6 (qualitative)

*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, but if only one food matrix is studied then ≥3 sources are recommended where available. The number of matrix sources may be reduced, particularly if it is difficult to obtain blank matrix sources, as long as the total number of spike levels and matrix combinations are adequate (e.g., 6 replicates or greater at each spike level for quantitative methods and 9 replicates or greater for qualitative methods).

** Number of spike levels is recommended for at least one source of matrix. Other similar sources of matrix (*e.g.*, within the same category; see Appendix 4) may be studied at one or two spike levels (*e.g.*, at an action/guidance or tolerance level or close to the lower limit of quantitation/detection).

3.0 ADDITIONAL PROCEDURAL GUIDANCE

In addition to the criteria described above in Table 1 for standard quantitative and qualitative methods, additional guidance is provided in this section for specific types of methods or validation situations.

3.1 Platform/Instrumentation Extension

Expanding the use of a validated method to include another significantly different instrument or platform requires further validation. 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 have major differences in configuration, or detection scheme.

Platform extension validation should generally be performed using Table 1, Level 2 as a guide and should compare the proposed new platform to the platform used in the reference method. In planning platform extension validation, one must determine what degree of cross-correlation between the results obtained on the two platforms will be acceptable.

Examples:
Method A is a validated method for the screening of pesticides on a gas chromatograph coupled to a single quadrupole mass spectrometer (GC-MSD). Gas chromatography coupled to a triple quadrupole mass spectrometer (GC-QQQ), offers certain advantages over the GC-MSD platform in terms of sensitivity, selectivity and scope. In this instance, a comparative method extension validation is indicated to ensure equivalent results. However, if new analytes are added to the scope of the method via the use of the new platform, a new method validation is indicated for the GC-QQQ method.

Method Z is a validated method for the screening of polycyclic aromatic hydrocarbons in seafood using liquid chromatography with a fluorescence detector (LC-FLD). A laboratory would like to transfer this method to a liquid chromatography system that utilizes only a diode-array detector (LC-DAD). In this instance, a comparative method extension validation would be indicated to ensure that the new detection system produces equivalent results to the originally validated method.

3.2 Analyte Extension

Multi-residue, multi-class methods are becoming more common. Many of these methods are semi-quantitative (limits tests) or qualitative broad band screens. Performance requirements for these types of procedures are described below. However, if a multi-residue method is meant to be used for quantitation, the same performance characteristics as required for single analyte methods should be evaluated for each analyte (accuracy, precision, selectivity, limit of detection, limit of quantitation, linearity range, uncertainty, and ruggedness). It is understood that with a large multi-residue method , not all analytes will meet the recommended acceptability ranges listed in Appendix 2, but the performance for each compound should be tested and reported so that the accuracy and precision are known for any given analyte and are sufficient for the intended purpose of the method.

When new analytes are added to a quantitative multi-residue method, tests should be performed to ensure that the addition of new compounds do not affect the performance of the instrumental conditions, e.g. duty cycle or scan rates for other eluting analytes, and that

the analytes do not present a chemical or physical interaction with the stabilities of the other tested analytes.

3.3 Food Matrix Extension

The validation of method performance with a new matrix is intended to assure that the method will continue to produce accurate and reliable results. Emergency matrix extensions (Level 1 in Table1) are intended for those instances in which a validated method is used with a matrix not previously validated in response to a real or perceived threat to food safety or public health, and in this type of urgent situation it is not expected that the MVS would be consulted. Matrix extensions of validated methods that are intended to increase the regulatory scope and applicability on a recurring basis would minimally fall under Level 2 validation in Table 1. This section provides guidance to extend validated methods to matrices in anticipation that these food commodities will be included in Agency-wide testing. Method developers may wish to consult with the appropriate Technical Advisory Group or MVS before initiating any Level 2 validation work on matrix extension.

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. It is also usually the case that the regulatory chemical methods employed by FDA are used to analyze a diversity of products representing a large spectrum of matrices. It becomes unfeasible to carry out a matrix extension validation for each single matrix in order to expand the scope of the method. A more reasonable approach to demonstrate the applicability of a method to a set of product matrices is to validate the method for different "categories" of products. For instance, a multi-residue pesticide method can be validated for "high-sugar", "high-fat", "high-water", "dry" and "high-protein" matrices. Appendix 4 provides guidance on commodity categories and gives examples of representative matrices in each category.

The number of different food categories to be validated depends on the applicability and intended use of the method. If the method is specific to only one category, only one type of food need be included. If the applicability is wider (e.g., detection of phthalates in processed foods), then an appropriate number of food categories should be included to represent all anticipated matrices. Depending on how many categories will be validated, a minimum of 1 – 3 representative matrices from each category should be selected.

3.4 Limit Tests (common semi-quantitative screening method)

One specific category of qualitative methods includes limit tests (binary or pass/fail tests) for analytes that have a defined level of concern. The purpose of these screening methods is to determine if analyte is present with a concentration near or above the level of concern. This is in contrast to screening methods whose intended purpose is to determine the presence or absence of an analyte at any level. Limit test method validations must include determination of the precision of the method for an analyte(s) at the level(s) of concern.

Limit test screening methods, in general, should avoid false negatives with false negative rates representing less than 5% of the analytical results. The occurrence of false positives is less critical since presumptive positives are further analyzed by quantitative or confirmatory methods. However, false positive rates should typically be less than 10-15% to avoid unnecessary confirmatory testing. Ideally, limit tests are capable of rapidly screening a large number of samples to minimize the need for additional analysis. A common approach used in limit test screening methods is to use a confidence interval to set a laboratory threshold or cut-off value whereby only responses above that value require further testing. For a limit

test based on an instrument response, a threshold or cut-off value can be determined by a confidence limit, based on an estimate of the standard deviation of the response or concentration of an analyte in samples fortified with the analyte at the level of concern.

Example:
Milk samples (n=21) were fortified with sulfamethazine at the level of concern (10 ng/mL). A LC-MS/MS limit test screening method was used to measure this drug in the extracted milk samples. The mean concentration found was to be 10.99 ng/mL with a standard deviation of 2.19. A threshold or cut-off value was calculated so that 95% of samples containing sulfamethazine at or above 10 ng/mL would have a response above the threshold value:

Threshold value = [mean concentration – (t * standard deviation)]
= [10.99 – (1.725 * 2.19)] = 7.21 ng/mL

Where t = one-tailed Student's t value for n-1 degrees of freedom at the 95% confidence level

This approach can also be used for immunosorbent assays such as enzyme linked immunosorbent assay (ELISA) or optical biosensor assays. These tests may be non-competitive (direct measurement of analyte response) or competitive (indirect measurement). Analysis of data from a competitive immunosorbent test should account for the fact that the observed response decreases with increasing analyte concentration; therefore, a response lower than the threshold or cut-off would be considered a presumptive positive response. For immunosorbent assays, it is also important to measure the response observed for blank matrix samples and to verify that the blank response is distinguishably (statistically) different from that of the threshold.

Performance characteristics of limit tests:
Validation of new limit tests should include, at a minimum, evaluation of the following performance characteristics: sensitivity, specificity, precision, threshold or cut-off value, false positive rate, false negative rate, minimum detectable concentration (should be lower than the threshold/cut-off value), and ruggedness/robustness.

3.5 Qualitative Broad-band Analyte Screening

Broad-band methods that can detect many compounds are being utilized more frequently as an initial screening step as part of chemical contaminant testing in FDA laboratories. These methods usually involve mass spectrometric analyses and provide qualitative information. For example, the data obtained may be compared to an established reference such as a database of compounds with exact mass and molecular formula information or spectra in a compiled library. For regulatory action, any positive findings from this screen should be confirmed by a targeted method (for example using a LC-MS/MS or GC-MS/MS platform).

Typically, initial validation of these methods is performed using a limited set of representative analytes and representative matrices. For example, sets of analytes that contain compounds from a variety of chemical classes from the area of interest (e.g. pesticides, veterinary drug residues, or common chemical toxins) are tested with the method using representative matrices. The performance characteristics that may be evaluated include: sensitivity, selectivity, false positive rate, false negative rate, minimum detectable concentration, ruggedness, and confirmation of identity. It is understood that the method

performance may vary with the different classes of compounds, but it is important to have an initial evaluation of the method's capabilities.

Laboratories continuously expand the scope of these broad-band methods by adding new analytes that come to their attention through various sources of intelligence. In addition, a new compound might be found in a sample after acquired data are compared to the reference databases. In these cases, some verification that the analyte can be detected reliably by the screening method is required. When a new compound is added to the scope of a qualitative method, it should first be determined whether this compound belongs to a class of compounds that has already been validated for the broad-band method. If the new compound shares chemical characteristics with an existing class of compounds in the scope of the method, then it may suffice to select a few representative matrices, perform a single level spike in these representative matrices in duplicate and determine that reproducible recovery is obtained in order to assess whether the analyte can be detected effectively by the method. Scenarios that may require a full validation would include a new analyte being added to the scope of the broad-band method that was not represented by any of the compound classes already in the scope. Also, if the new analyte requires modifications in the extraction protocol due to its chemical characteristics, then its inclusion in the scope should be fully validated as recommended by this guidance.

Although positive findings by the broad-band method are subjected to confirmatory testing using a targeted method, it is still important to determine, through proper validation and verification protocols, that the broad-band method does not give rise to a high number of false negative findings. False negative in this context means the method fails to detect a residue in its scope when the residue is present in the matrix at or above the level of concern or minimum detectable concentration. While the positive finding by the broad-band method is subjected to further analysis and scrutiny, negative findings are upheld as such and a regulatory decision is made based on these results, *e.g.*, to release the products into commerce.

4.0 REFERENCES AND SUPPORTING DOCUMENTS

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APPENDIX 1 - Glossary of Terms

Generally, references 13-17 were utilized in preparation of this glossary.

Accuracy: The closeness of agreement between a test result and an accepted reference value. When applied to test results, accuracy includes a combination of random and systematic error. When applied to test method, accuracy refers to a combination of trueness and precision.

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

Analyte: The chemical substance measured and/or identified in a test sample by the method of analysis.

Analytical batch: An analytical batch consists of samples, standards, and blanks 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 (usually within one day) or in continuous sequential time periods.

Bias: The difference between the expectation of the test result and the true value or accepted reference value. Bias is the total systematic error, and there may be one or more systematic error components contributing to the bias.

Blank: A substance that does not contain the analytes of interest and is subjected to the usual measurement process. Blanks can be further classified as method blanks, matrix blanks, reagent blanks, instrument blanks, and field blanks.

Calibration: Determination of the relationship between the observed analyte signal generated by the measuring/detection system and the quantity of analyte present in the sample measured. Typically, this is accomplished through the use of calibration standards containing known amounts of analyte.

Calibration Standard: A known amount or concentration of analyte used to calibrate the measuring/detection system. May be matrix matched for specific sample matrices.

Carryover: Residual analyte from a previous sample or standard which is retained in the analytical system and measured in subsequent samples. Also called *memory*.

Certified Reference Material (CRM): Reference material accompanied by documentation (certificate) issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceability, using valid procedures. Note: Standard Reference Material (SRM) is the trademark name of CRMs produced and distributed by the National Institute of Standards and Technology (NIST).

Check Analysis: Result from a second independent analysis which is compared with the result from the initial analysis. Typically, check analyses are performed by a different analyst using the same method.

Confirmation of Identity: Unambiguous identification of an analyte(s) by a highly specific technique such as mass spectrometry or by demonstration of results from two or more independent analyses in agreement.

Confirmatory Analysis/Method: Independent analysis/method used to confirm the result from an initial or screening analysis. A different method is often used in confirmation of screening results.

Cut-off Concentration: In qualitative analysis, the concentration of the analyte that is either statistically lower than the level of concern (for limit tests) or at which positive identification ceases (for confirmation of identity methods). See also *Threshold Value*.

False Negative Rate: In qualitative analysis, a measure of how often a test result indicates that an analyte is not present, when, in fact, it is present or, is present in an amount greater than a threshold or designated cut-off concentration.

False Positive Rate: In qualitative analysis, a measure of how often a test result indicates that an analyte is present, when, in fact, it is not present or, is present in an amount less than a threshold or designated cut-off concentration.

Fitness for Purpose: Degree to which data produced by a measurement process enables a user to make technically and administratively correct decisions for a stated purpose.

Guidance Level: Level of concern or action level issued under good guidance practices that must be reliably identified or quantified in a sample.

Incurred Samples: Samples that contain the analyte(s) of interest, which were not derived from laboratory fortification but from sources such as exogenous exposure or endogenous origin. Exogenous exposure includes, for example, pesticide use, consumption by an animal, or environmental exposure.

Interference: A positive or negative response or effect on response produced by a substance other than the analyte. Includes spectral, physical, and chemical interferences which result in a less certain or accurate measurement of the analyte.

Intermediate Precision: Within-laboratory precision obtained under variable conditions, e.g., different days, different analysts, and/or different instrumentation.

Internal Standard: A chemical added to the sample, in known quantity, at a specified stage in the analysis to facilitate quantitation of the analyte. Internal standards are used to correct for matrix effects, incomplete spike recoveries, etc. Analyte concentration is deduced from its response relative to that produced by the internal standard. The internal standard should have similar physico-chemical properties to those of the analyte.

Laboratory Fortified Matrix: See *Matrix Spike*.

Level of Concern: Level of concern is the concentration of an analyte in a sample that has to be exceeded before the sample can be considered violative. This concentration can be a regulatory tolerance, safe level, action level, guidance level or a laboratory performance level.

Limit of Detection (LOD): The minimum amount or concentration of analyte that can be reliably distinguished from zero. The term is usually restricted to the response of the detection system and is often referred to as the *Detection Limit*. When applied to the complete analytical method it is often referred to as the *Method Detection Limit* (MDL).

Limit of Quantitation (LOQ): The minimum amount or concentration of analyte in the test sample that can be quantified with acceptable precision. Limit of quantitation (or quantification) is variously defined but must be a value greater than the MDL and should apply to the complete analytical method.

Limit Test: A type of semi-quantitative screening method in which analyte(s) has a defined level of concern. Also referred to as binary or pass/fail tests.

Linearity: The ability of a method, within a certain range, to provide an instrumental response or test results proportional to the quantity of analyte to be determined in the test sample.

Matrix: All the constituents of the test sample with the exception of the analyte.

Matrix Blank: A substance that closely matches the samples being analyzed with regard to matrix components. Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples. The matrix blank is used to determine the absence of significant interference due to matrix, reagents and equipment used in the analysis.

Matrix Effect: An influence of one or more components from the sample matrix on the measurement of the analyte concentration or mass. Matrix effects may be observed as increased or decreased detector responses, compared with those produced by simple solvent solutions of the analyte.

Matrix Source: The origin of a test matrix used in method validation. A sample matrix may have variability due to its source. Different food matrix sources can be defined as different commercial brands, matrices from different suppliers, or in some cases different matrices altogether. 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.

Matrix spike: An aliquot of a sample prepared by adding a known amount of analyte(s) to a specified amount of matrix. A matrix spike is subjected to the entire analytical procedure to establish if the method is appropriate for the analysis of a specific analyte(s) in a particular matrix. Also referred to as a *Laboratory Fortified Matrix*.

Method blank: A substance that does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples. An aliquot of reagent water is often used as a method blank in the absence of a suitable analyte-free matrix blank.

Method Detection Limit (MDL): The minimum amount or concentration of analyte in the test sample that can be reliably distinguished from zero. MDL is dependent on sensitivity, instrumental noise, blank variability, sample matrix variability, and dilution factor.

Method Development: The process of design, optimization and preliminary assessment of the performance characteristics of a method.

Method Validation: The process of demonstrating or confirming that a method is suitable for its intended purpose. Validation includes demonstrating performance characteristics such as accuracy, precision, specificity, limit of detection, limit of quantitation, linearity, range, ruggedness and robustness.

Method Verification: The process of demonstrating that a laboratory is capable of replicating a validated method with an acceptable level of performance.

Minimum Detectable Concentration (MDC): In qualitative analysis, an estimate of the minimum concentration of analyte that must be present in a sample to ensure at a specified high probability (typically 95% or greater) that the measured response will exceed the detection threshold, leading one to correctly conclude that an analyte is present in the sample.

Precision: The closeness of agreement between independent test results obtained under specified conditions. The precision is described by statistical methods such as a standard deviation or confidence limit of test results. See also *Random Error*. Precision can be further classified as *Repeatability, Intermediate Precision*, and *Reproducibility*.

Qualitative Analysis/Method: Analysis/method in which substances are identified or classified on the basis of their chemical, biological or physical properties. The test result is either the presence or absence of the analyte(s) in question.

Quantitative Analysis/Method: Analysis/method in which the amount or concentration of an analyte may be determined (or estimated) and expressed as a numerical value in appropriate units with acceptable accuracy and precision.

Random error: Component of measurement error that in replicate measurements varies in an unpredictable manner. See also *Precision*.

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

Reagent Blank: Reagents used in the procedure taken through the entire method. Reagent Blanks are used to determine the absence of significant interference due to reagents or equipment used in the analysis.

Recovery: The proportion of analyte (incurred or added) remaining at the point of the final determination from the analytical portion of the sample measured. Usually recovery is expressed as a percentage.

Reference material: A material, sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process or in examination of nominal properties.

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: Precision obtained under observation conditions where independent test results are obtained with the same method on identical test items in the same test facility by the same operator using the same equipment within short intervals of time.

Representative Analyte: An analyte used to assess probable analytical performance with respect to other analytes having similar physical and/or chemical characteristics. Acceptable data for a representative analyte are assumed to show that performance is satisfactory for the represented analytes. Representative analytes should include those for which the worst performance is expected. Representative analytes are used mostly for non-targeted analysis and unknown screening procedures.

Representative Matrix: Matrix used to assess probable analytical performance with respect to other matrices, or for matrix-matched calibration, in the analysis of broadly similar commodities. For food matrices, similarity is usually based on the amount of water, fats, protein, and carbohydrates. Sample pH and salt content can also have a significant effect on some analytes.

Reproducibility: Precision obtained under observation conditions where independent test results are obtained with the same method on identical test items in different test facilities with different operators using different equipment.

Ruggedness/Robustness: A measure of the capacity of an analytical procedure to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Screening Analysis/Method: An analysis/method intended to detect the presence of analyte in a sample at or above some specified concentration (action or target level). Screening methods typically attempt to use simplified methodology for decreased analysis time and increased sample throughput.

Selectivity: The extent to which a method can determine particular analyte(s) in a mixture(s) or matrix(ces) without interferences from other components of similar behavior. Selectivity is generally preferred in analytical chemistry over the term *Specificity*.

Sensitivity: The change in instrument response which corresponds to a change in the measured quantity (*e.g.*, analyte concentration). Sensitivity is commonly defined as the gradient of the response curve or slope of the calibration curve at a level near the LOQ.

Specificity: In quantitative analysis, specificity is the ability of a method to measure analyte in the presence of components which may be expected to be present. The term *Selectivity* is generally preferred over Specificity.

Spike Recovery: The fraction of analyte remaining at the point of final determination after it is added to a specified amount of matrix and subjected to the entire analytical procedure. Spike Recovery is typically expressed as a percentage. Spike recovery should be calculated for the method as written. For example, if the method prescribes using deuterated internal standards or matrix-matched calibration standards, then the reported analyte recoveries should be calculated according to those procedures.

Standard: A substance of known identity and purity and/or concentration.

Standard Reference Material (SRM): A certified reference material issued by the National Institutes of Standards and Technology (NIST) in the United States. (www.nist.gov/SRM).

Systematic error: Component of measurement error that in replicate measurements remains constant or varies in a predictable manner. This may also be referred to as *Bias*.

Threshold Value: In qualitative analysis, the concentration of the analyte that is either statistically lower than the level of concern (for limit tests) or at which positive identification ceases (for confirmation of identity methods). See also *Cut-off Concentration*.

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

Uncertainty: Non-negative parameter characterizing the dispersion of the values being attributed to the measured value.

APPENDIX 2 – Examples of Acceptability Criteria for Certain Performance Characteristics

Examples of acceptability criteria are found in references 7,9,10,14 and 18. No single set of acceptability is going to be truly applicable to all methodology covered in the FVM program. However a good starting point for many methods is found in the Codex Alimentarius Commission, Procedural Manual, Twenty-second ed., 2014 [10]

A. Quantitative Method Acceptability Criteria

Table A2.1. Method Criteria for Method Levels at Increasing Orders of Magnitude
(reproduced in part from reference 10, Table 4, p. 72 and reference 7)

ML* unit	0.001 mg/kg	0.01 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	100 mg/kg	1 g/kg	10 g/kg
Alternative ML* unit	1 ppb	10 ppb	100 ppb	1 ppm	10 ppm	100 ppm	0.1%	1 %
Concentration ratio of ML* (C_{ML})	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Minimum applicable range	From 0.0006 to 0.0014 mg/kg	From 0.006 to 0.014 mg/kg	From 0.03 to 0.17 mg/kg	From 0.52 to 1.48 mg/kg	From 6.6 to 13.3 mg/kg	From 76 to 124 mg/kg	From 0.83 to 1.2 g/kg	From 8.8 to 11 g/kg
LOD (≤ mg/kg)	0.0002	0.002	0.01	0.1	1	10	100	1000
LOQ (≤ mg/kg)	0.0004	0.004	0.02	0.2	2	20	200	2000
RSD_r**	22%	22%	11%	8%	6%	4%	3%	2%
$PRSD_R$#	22%	22%	22%	16%	11%	8%	6%	4%
RSD_R##	≤ 44%	≤ 44%	≤ 44%	≤ 32%	≤ 22%	≤ 16%	≤ 12%	≤ 8%
Recovery	40%-120%	60%-115%	80%-110%	80%-110%	80% - 110%	90% - 107%	95% – 105%	97%-103%

*ML is a method level and can be defined for the analyte(s)/sample matrice(s) combination as a maximum level, minimum level, normative level or concentration range depending on the intended use of the method.

"The RSD_r or Repeatability Precision refers to the degree of agreement of results when conditions are maintained as constant as possible within a short period of time (e.g., relative standard deviation of replicates or best precision exhibited by a single laboratory). Typically, acceptable values for RSD_r are between ½ and 2 times the value shown ($HorRat_r = RSD_r$ (found, %)/ RSD_r (calculated, %)). For concentration ratios ≥ 10-7 Horwitz theory is applied. For concentration ratios < 10^{-7}, Thompson theory is applied.

#The $PRSD_R$ or Predicted Relative Reproducibility Standard Deviation is based on the Horwitz/Thompson equation. For concentration ratios < 10^{-7}, Thompson theory is applied.

##The RSD_R or Reproducibility Precision refers to the degree of agreement of results when operating conditions are as different as possible (*e.g.*, same test samples in different laboratories) and should be calculated from the Horwitz/Thompson equation. When the Horwitz/Thompson equation is not applicable (for an analytical purpose or according to a regulation) or when "converting" methods into criteria then it should be based on the RSD_R from an appropriate method performance study. The ratio between the found and predicted value should be ≤ 2. ($HorRat_R = RSD_R / PRSD_R \leq 2$)

B. Qualitative Method Acceptability Criteria

There are significantly fewer examples of acceptability criteria for qualitative methods available. AOAC is using a relatively new Probability of Detection (POD) model as a way to characterize the performance of qualitative methods [9].

As discussed above, limit test screening methods, in general, should minimize false negatives particularly at the level of concern or reporting level. The occurrence of false positives is less critical since presumptive positives are further analyzed by quantitative or confirmatory methods. However, false positive rates should typically be less than 10-15% in order to avoid unnecessary confirmatory testing (14, 18).

Table A2.2. General Method Criteria for Limit Tests/Screening Methods

False Negative Rate	≤ 5% at the level of concern[1]
False Positive Rate	≤ 10-15%

[1] Acceptable false negative rate depends significantly on the intended purpose of the method.

APPENDIX 3 - Examples of Validation Plans

A. Extension to other matrices with the same analyte(s) at Level One Validation

This scheme represents an emergency use method extension plan for Matrix Y and Analyte Z. This plan utilizes two different sources of matrix. *In cases where a representative matrix is being used to characterize a whole family of commodities, it is recommended that additional, different commodities from that family are used as "sources".* Note that this plan is for emergency use only – the new matrix (or matrices) cannot be officially included in the scope of the method until at the minimum a Level Two Validation is performed.

Table A3.1. Plan for Matrix Extension (Level One Validation, Example)

	Matrix	Samples 1 & 2	Analyte Z Fortified Samples 3 & 4	Analyte Z Fortified Samples 5 & 6	Analyte Z Fortified Samples 7 & 8
Day 1	Matrix Y (Source 1)	Blank	½X Spike Level	X Spike Level	2X Spike Level
Day 1	Matrix Y (Source 2)	Blank	½X Spike Level	X Spike Level	2X Spike Level

Notes:

i. Test portion matrices listed as Matrix Y represent 2 different commercial brands.
ii. Fortification levels: fortification will be at the level of concern or action level (X) as stated in the method and at levels corresponding to 1/2X and 2X.
iii. Fortification of each matrix can be done on the same day.
iv. Other fortification plans meeting requirements specified in Table 1 may be used.

B. Extension to similar analytes in the same matrix at Level Two Validation

A validated method can be extended to other potential analyte(s) belonging to the same chemical group. For example, a toxin method can be extended to other toxins. An example of the composition of a set of validation studies for method extension is shown in the following table for new analytes Y and Z in canned corn from 3 different sources where the method is validated originally for analyte A in corn.

Table A3.2. Plan for Extension to Similar Analytes (Level Two Validation, Example)

	Matrix	Analyte Y fortification levels	Analyte Z fortification levels
Day 1	Corn 1,2,3	0, 1/2X, X, 2X	0, 1/2X, X, 2X
Day 2	Corn 1,2,3	0, 1/2X, X, 2X	0, 1/2X, X, 2X

Day 3	Corn 1,2,3	0, 1/2X, X, 2X	0, 1/2X, X, 2X

Notes:
i. Three different commercial brands of same product will be analyzed.
ii. Fortification levels: fortification will be at the level of concern or action level (X) as stated in the method and at levels corresponding to 1/2X and 2X.
iii. Each analyte will be analyzed in blank matrix and at 1/2X, X and 2X fortification levels.
iv. Simultaneous analysis of the analytes can be undertaken if warranted.
v. Other fortification plans meeting requirements specified in Table 1 may be used.

C. Validation at Level Two for single matrix and single analyte

This plan utilizes 3 different commercial brands of one matrix. The single matrix is being validated for a single analyte.

Table A3.3. Plan for Single Matrix and Single Analyte Level Two Validation (Example)

	Matrix 1 Source 1	Matrix 1 Source 2	Matrix 1 Source 3
Day 1	Blank Fortified (X)	Fortified (X) Fortified (2X)	Blank Fortified (1/2X)
Day 2	Fortified (2X) Fortified (1/2X)	Blank Fortified (1/2X)	Blank Fortified (2X)
Day 3	Fortified (1/2X) Fortified (X)	Fortified (2X) Blank	Fortified (X) Fortified (X)
Day 4	Fortified (2X) Blank	Fortified (X) Fortified (1/2X)	Fortified (2X) Fortified (1/2X)

Notes:
i Sample matrix, represents one matrix from 3 different sources of matrix.
ii Fortification levels: fortification will be at the level of concern or action level (X) as stated in the method and at levels corresponding to 1/2X and 2X.
iii Each of 3 different sources of matrix will be analyzed 8 times (replicate analyses) over the course of experiment, two times unfortified, two times fortified at each level.
iv. The validation will take place over a period of 4 days.
v. Other fortification plans meeting requirements specified in Table 1 may be used.

APPENDIX 4 – Selection of Representative Matrices

Two tools that can aid in selection of representative matrices and CRMs when designing a validation protocol for a method intended to have applicability to a broad scope of products are shown below. Food composition varies greatly making the validation of methods intended for a wide variety of foods a difficult balance between available resources and sufficient validation with a variety of food types.

A. Commodity groups and representative commodities

Table A4.1. Vegetable and Fruits, Cereals and Food of Animal Origin (reproduced in part from reference 14)

Commodity groups	Typical commodity categories	Typical representative commodities
1. High water content	Pome fruit	Apples, pears
	Stone fruit	Apricots, cherries, peaches
	Other fruit	Bananas
	Alliums	Onions, leeks
	Fruiting vegetables/cucurbits	Tomatoes, peppers, cucumber, melon
	Brassica vegetables	Cauliflower, Brussels sprouts, cabbage, broccoli
	Leafy vegetables and fresh herbs	Lettuce, spinach, basil
	Stem and stalk vegetables	Celery, asparagus
	Forage/fodder crops	Fresh alfalfa, fodder vetch, fresh sugar beets
	Fresh legume vegetables	Fresh peas with pods, peas, mange tout, broad beans, runner beans, French beans
	Leaves of root and tuber vegetables	Sugar beet and fodder beet tops
	Fresh Fungi	Champignons, canterelles
	Root and tuber vegetables or feed	Sugar beet and fodder beet roots, carrots, potatoes, sweet potatoes
2. High acid content and high water content	Citrus fruit	Lemons, mandarins, tangerines, oranges
	Small fruit and berries	Strawberry, blueberry, raspberry, black currant, red currant, white currant, grapes
	Other	Kiwifruit, pineapple, rhubarb

Table A4.1. Vegetable and Fruits, Cereals and Food of Animal Origin (continued)

Commodity groups	Typical commodity categories	Typical representative commodities
3. High sugar and low water content	Honey, dried fruit	Honey, raisins, dried apricots, dried plums, fruit jams
4a. High oil content and very low water content	Tree nuts	Walnuts, hazelnuts
	Oil seeds	Oilseed rape, sunflower, cotton-seed, soybeans, peanuts, sesame, etc.
	Pastes of tree nuts and oil seeds	Peanut butter, tahini, hazelnut paste
	Oils from tree nuts, oil seeds and oily fruits	Olive oil, rapeseed oil, sunflower oil, pumpkin seed oil
4b. High oil content and intermediate water content	Oily fruits and products	Olives, avocados and pastes thereof
5. High starch and/or protein content and low water and fat content	Dry legume vegetables/pulses	Field bean, dried broad bean, dried haricot bean (yellow, white/navy, brown, speckled), lentils
	Cereal grain and products thereof	Wheat, rye, barley and oat grain; maize, rice, whole meal bread, white bread, crackers, breakfast cereals, pasta
6. "Difficult or unique commodities"		Hops, cocoa beans and products thereof, Coffee, tea, spices
7. Meat (muscle) and Seafood	Red muscle	Beef, pork, lamb, game, horse
	White muscle	Chicken, duck, turkey
	Offal	Liver, kidney
	Fish	Cod, haddock, salmon, trout
	Crustaceans	Shrimp, scallop, crab
8. Milk and milk products	Milk	Cow, goat and buffalo milk
	Cheese	Cow and goat cheese
	Dairy products	Yogurt, cream
9. Eggs	Eggs	Chicken, duck, quail, and goose eggs
10. Fat from food of animal origin	Fat from meat	Kidney fat, lard
	Milk fat	Butter
	Fish oil	Cod liver oil

B. AOAC Food Matrix Triangle

The AOAC Food Matrix Triangle (Figure A4.1) can be used to categorize foods and food matrix reference materials into nine sectors based on relative fat, protein and carbohydrate content [9, 19, 20]. This tool can be useful in the validation of methods intended for a wide variety of food matrices and to help in categorizing similar food matrices for methods intended for more limited applicability.

Figure A4.1. Foods Partitioned into Sectors Based on Their Protein, Fat, and Carbohydrate Content