A2LA FOOD MICROBIOLOGY PROGRAM REQUIREMENTS

June 2001

(Based upon the FLAWG document:
“AOAC INTERNATIONAL Accreditation Criteria for Laboratories Performing Food Microbiological Testing”)

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1 This revision updates the references to ISO/IEC 17025:1999, including renumbering and re-ordering of the requirements. No changes have been made to the technical requirements.
FOOD MICROBIOLOGY PROGRAM REQUIREMENTS

June 2001

TABLE OF CONTENTS

Cover Page 1
Table of Contents 2
Introduction 3

1. Scope 3
2. References 4
3. Definitions 5
4. Management Requirements 7
  4.1 Organization 7
  4.2 Quality System 8
  4.3 Document Control 8
  4.4 Review of Requests, Tenders and Contracts 8
  4.5 Subcontracting of Tests 8
  4.6 Purchasing Services and Supplies 8
  4.7 Service to the Client 8
  4.8 Complaints 8
  4.9 Control of Nonconforming Testing 8
  4.10 Corrective Action 8
  4.11 Preventive Action 8
  4.12 Control of Records 8
  4.13 Internal Audits 9
  4.14 Management Reviews 9

5. Technical Requirements 9
  5.1 General 9
  5.2 Personnel 9
  5.3 Accommodation and Environmental Conditions 10
  5.4 Test Methods and Method Validation 11
  5.5 Equipment 13
  5.6 Measurement Traceability 16
  5.7 Sampling 19
  5.8 Handling of Test Items 20
  5.9 Assuring the Quality of Test Results 20
  5.10 Reporting the Results 21

6. Proficiency Testing Requirements 21
Introduction

The general criteria for accreditation of laboratories are as found in ISO/IEC 17025-1999, “General Requirements for the Competence of Testing and Calibration Laboratories”. Laboratories that perform analyses or calibrations in the field or in mobile facilities must also meet the A2LA Specific Criteria for the Accreditation of Site Testing and Site Calibration Laboratories.

This document describes additional, specific accreditation requirements for laboratories performing microbiological analyses in the examination of food products, ingredients in the production of food, in-process food samples, environmental samples pertinent to foods (swabs, debris, scrapings, air, condensate, etc.) and final products. These specific criteria were developed to meet the needs of the United States Department of Agriculture (USDA) and are applicable to those testing laboratories seeking to meet USDA requirements for food microbiology testing under the A2LA Biological field of testing.

For the purposes of this document, it is assumed that food microbiology will be involved in the testing of foods and related substances for bacteria, yeasts, molds, protozoa and viruses, although principally for bacteria, yeasts and molds. Typically, these microbiological tests will include:

- Pathogens – Salmonella, Campylobacter jejuni/coli, Listeria monocytogenes, enteropathogenic Escherichia coli, Staphylococcus aureus, Bacillus cereus, Clostridium botulinum, Shigella, Vibrio cholera, Vibrio parahaemolyticus, Yersinia enterocolitica, Clostridium perfringens, etc.;

- Sanitation indices – E coli 0157:H7, coliforms, fecal coliforms, streptococci, etc.;

- General tests for process control, quality and spoilage, lactogacilli, yeasts, molds, aerobic plate counts, standard plate counts, anaerobic, lipolytic, proteolytic, saccharolytic, amolytic, osmophilic, thermophilic, psychrophilic plate counts, etc.

Included in these lists will be diagnostic steps including toxin testing, serological tests, animal tests, biochemical tests and metabolic products analysis.

4. Scope

This document applies to testing by methods that are nationally recognized – such as AOAC International, American Public Heath Association (APHA), Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM), United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), USDA Agricultural Marketing Service (AMS) and United States Pharmacopeia (USP) – as well as newly developed methods and laboratory developed methods. It is also applicable to all types of laboratories, whether they are in the private sector (independent or in-house) or in the government sector.

In addition, this document specifically addresses testing proficiency and is not generally intended for research and/or product development laboratories, unless specified by a client and/or proficiency to a test method is critical to these functions.
4. References

GENERAL:

ISO/IEC Guide 2; 1986, General Terms and Their Definitions Concerning Standardization and Related Activities.

International Vocabulary of Basic and General Terms in Metrology (VIM); 1984, issued by BIPM, IEC, ISO and OIML.

ISO 8402; 1986, Quality – Vocabulary.


FOOD MICROBIOLOGY QUALITY AND SAFETY:


USPHS. CDC Laboratory Manual, Quality Control in Microbiology. 1987. Atlanta, GA.


National Environmental Laboratory Accreditation Conference (NELAC), Proficiency Testing Program, Revision 4, Jan. 8, 1997, pg. 11-12.

SCC Laboratory Accreditation Program for Pesticide Residue Laboratories Guideline.
Proficiency Testing in Food Microbiology, Sue Scotter, Food Science & Technology Today, 10 (4), 1996.


**REFERENCE CULTURES:**

**Bacteria**


**Yeasts**


Molds, Protozoa, Viruses

Refer to type culture collections description.

4. **Definitions**

**Audit**: A systematic and independent examination to determine whether quality activities and related results comply with planned arrangements and whether these arrangements are implemented effectively and are suitable to achieve objectives.

**Calibration**: Comparison and adjustment to a standard of known accuracy.

**Certified Reference Culture (CRC)**: Microbiological; a reference culture certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body; e.g., cultures used to verify test systems, validate methods, perform quality control of test media, etc. must be traceable to a type culture collection. Synonymous with Standard Reference Materials (SRM)

**Check Samples**: Sets of samples tested by laboratories to determine if their processes are in control. A test sample with known properties of microorganisms examined on a routine basis to evaluate laboratory performance.
Client: An entity (e.g., customer, agency, company, person, etc.) that receives a test result done according to specified requirements.

Conformance: Compliance with specified requirements.

Control: To exercise authority over and regulate.

Controlled Document: A policy or procedure related to the documented quality system that is subjected to controls to ensure that the same version of the document and any revisions are held by or available to all personnel to whom the document is applicable.

Corrective Action: Measures taken to rectify conditions adverse to quality and to eliminate recurrence.

Culture: An isolated microorganism grown on laboratory medium.

Documentation: Recorded information.

Food Testing Laboratory: Laboratory that performs tests on finished food product, ingredients, in-process samples and associated environmental samples for microorganisms.

In-process Samples: Samples in the laboratory that are in the process of being tested (not to be confused with in-process product samples from a manufacturing standpoint).

Inspection: Activities such as measuring, testing and examining one or more characteristics of a product or service and comparing these with specified requirements to determine conformity.

Internal Audit: A formal review of the performance of a quality system conducted by laboratory personnel from outside of the laboratory or department under review.

Laboratory Information Management System (LIMS): The computer and software used to identify, schedule, prioritize, perform calculations, generate reports, store results and perform any other function necessary to control the flow of a sample through the laboratory.

Method: A document that provides detailed “how to” instructions to accomplish a task.

Monitor: A substance, device or system for observing, recording or detecting the operation, condition or performance of a microbiological test procedure.

Nonconformity: The non-fulfillment of a specified requirement.

Proficiency Test Samples: Test materials (split samples) with microorganisms (antibiotics and toxins) that are tested periodically by a number of locations to determine the proficiency of recovery, using statistical analysis where appropriate.

Quality: Conformance to specified requirements.

Quality Assurance: All those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality.
**Quality Control:** The operational techniques and activities that are used to fulfill requirements for quality.

**Quality System:** The organizational structure, responsibilities, procedures, processes and resources for implementing quality management.

**Raw Material:** A material used in food processing whose properties may impact the quality of the final result.

**Reference Culture (RC):** A culture with cultural characteristics sufficiently well established to be used to calibrate/verify test systems and test media and validate methods.

**Replicate tests:** Samples of RMs or CRMs which are tested by the same analyst in duplicate or by two different analysts. In each case, the results are compared for precision.

**Report:** Final presentation of results sent to a customer.

**Sample:** Any material brought into the laboratory for analysis.

**Self-Audit:** A review of the performance of the quality system within a limited area conducted by the personnel with responsibility for the area.

**Split Samples:** Unknown test samples of adequate homogeneity, sub-sampled and sent to laboratories for proficiency testing.

**Standard Operating Procedure:** A document that specifies or describes how an activity is to be performed. It may include methods to be used and a sequence of operations.

**Traceability:** The property of a result of a measurement whereby it can be related to appropriate standards, generally international or national standards, through an unbroken chain of comparisons. (e.g., all media, reagents and kits must be traceable to a result and to the appropriate Certified Reference Material, Certified Reference Culture, Reference Culture or Reference Material.)

**Validated method:** A method whose performance characteristics (selectivity and specificity, range, linearity, sensitivity, ruggedness, accuracy and precision and quantitation and detection limits) meet the specifications related to its intended use.

**Verification:** Confirmation by examination and provisions of evidence that specified requirements have been met.

Example: Verification of a microbiological test process would be the adequate recovery of Salmonella from a known inoculated sample run concurrently with other samples; the adequate recovery of selective/differential versus non-selective (TGY) S. aureus on media incubated concurrently with other samples. For quantitative tests such as S. aureus, these must be a quantitative assessment of recovery such as < 50% difference or <1 log # of differences.

4. **Management Requirements**

4.1 **Organization** (No Additions)
4.2 Quality System

4M.2.1 For multi-functional laboratories, those sections of the quality manual pertaining specifically to the food microbiology laboratory shall be easily identifiable.

4.3 Document Control (No Additions)

4.4 Review of Requests, Tenders and Contracts (No Additions)

4.5 Subcontracting of Tests (No Additions)

4.6 Purchasing Services and Supplies (No Additions)

4.7 Service to the Client (No Additions)

4.8 Complaints (No Additions)

4.9 Control of Nonconforming Testing (No Additions)

4.10 Corrective Action (No Additions)

4.11 Preventive Action (No Additions)

4.12 Control of Records

4M.12.1 All records shall be traceable to the sample and testing performed including the equipment, materials, calibration, maintenance, performance verifications, environmental data and analyst(s). All records shall allow traceability through the following progression of events:

Sampling (where applicable)
↓
Sample Receipt and Check-In
↓
Sample Preparation
↓
Sample Handling, Storage and Disposal
↓
Sample Analysis
↓
Equipment Performance (CRC, proficiency checks, daily checks)
↓
Calibration Records (CRC)
↓
Analyst Training (RM, proficiency checks)
↓
Media Performance (CRC, RC)
4.13 Internal Audits

4M.13.1 The laboratory shall conduct an internal audit on an annual basis. All audit records shall be kept in the laboratory.

4.14 Management Reviews (No Additions)

5. Technical Requirements

5.1 General (No Additions)

5.2 Personnel

5M.2.1 The laboratory shall have a selection procedure and training system to ensure technical competence of all staff members. Microbiological testing shall be done by or supervised by a competent microbiologist or technician. For the purposes of this document, there are two types of laboratories: full service laboratories and limited service laboratories.

5M.2.1.1 In full service laboratories (where most organisms – if not all – of significance to foods are tested) there shall be a trained, competent supervisory microbiologist on staff having at least a Bachelors Degree in microbiology, food science or a related discipline with at least two years of laboratory experience. The person filling this position shall have successfully completed at least 20 credit hours in microbiology, public health, food safety or other related topics.

5M.2.1.2 In small, limited service laboratories (usually of no more than 5 people; where 1-3 tests are performed) the technician performing the tests or supervising the tests shall be trained with demonstrated competence in the limited number of tests performed by the laboratory. The person filling this position shall have at least two years of laboratory experience with the testing concerned.

5M.2.2 Training shall include all methods or portions of methods and techniques that each person is responsible for performing. At a minimum, each analyst shall demonstrate competency through observation by management and verification using replicate and/or check samples. For technicians performing only portions of a specific method, competency may be confirmed/verified by observation only.

5M.2.3 The continued competence of staff must be monitored/appraised using appropriate means (e.g., by objective measurements or visual observation as appropriate.

5M.2.4 Training records shall include documentation of all relevant internal and external education and method performance verifications.
5.3 Accommodation and Environmental Conditions

5M.3.1 The laboratory shall be arranged to minimize cross contamination and shall be segregated from other activities in the laboratory with limited access. Suggested means of accomplishing this are:

- Carry out procedures in a sequential manner using appropriate precautions to ensure test and sample integrity (e.g., use of sealed containers) (Eurachem);
- Segregate activities in time and space (Eurachem);
- Use biological containment hoods;
- Restrict highly contaminated samples to separate areas;
- Restrict operations to selected areas when high levels of pathogens may be encountered (e.g., pre-enrichments, selective enrichment transfers).

5M.3.2 Laboratories located in facilities where products or ingredients are manufactured shall not test for infectious pathogens (such as Listeria monocytogenes, Salmonella, Escherichia coli 0157:H7, Shigella, Campylobacter, Vibrio cholera), unless the laboratory is physically separated with limited access, negative air flow and supervision by a qualified microbiologist.

5M.3.3 The laboratory shall be ventilated to reduce the levels of contamination. The laboratory test area should be air-conditioned to control humidity and temperature.

5M.3.3.1 Work space temperature and test area humidity shall be monitored. The recommended relative humidity in the test area is 45-50% RH and the temperature in the test area should be 20-25°C.

5M.3.4 Excessive numbers of environmental bacteria, yeasts and molds shall be controlled by air systems with filters. Verification and monitoring of control shall be performed using air sampling devices, air settling plates, surface swabs or other appropriate means. These checks are critical to aerobic plate count procedures and yeast and mold tests.

5M.3.5 Bench tops (work surfaces) and floors shall be made of impervious, smooth, easily cleaned materials. There shall be at least six linear feet of bench or surface workspace for each analyst while working. Walls and ceilings should be made of materials that are smooth and easily cleaned.

5M.3.6 Pathogen testing shall be strictly controlled so as to prevent cross contamination. Critical work surfaces shall be monitored for pathogens pertinent to the laboratory’s scope of testing (Salmonella, Listeria monocytogenes and E. coli 0157:H7) - after sanitation but before testing operations begin.

5M.3.7 Handling of microorganisms, laboratory hygiene and housekeeping shall be consistent with the essential elements described in “Centers for Disease Control, 1988, Biosafety in Microbiological and Biomedical Laboratories, 2nd Edition, U.S. Government Printing Office, Washington, D.C.”
5M.3.7.1 Full service laboratories handling infectious agents shall implement the essential elements of “Biosafety Level 2”.

5M.3.7.2 Small, limited service laboratories, not generally testing for infectious agents, shall implement the essential elements of “Biosafety Level 1”.

5M.3.8 The laboratory shall have a pest control program.

5M.3.9 There shall be at least 50 (preferably 100) foot-candles intensity at working surfaces.

5M.3.10 Where applicable, the laboratory shall comply with the Public Health Services Policy of Human Care and Use of Animals, Public Law 99-158, November 20, 1985, “Animals in Research”. (A supporting document for animal care is “The NIST Guide for the Care and Use of Laboratory Animals”, Pub. No. 86-23, Rev. 1985.) In some circumstances, it may be desirable to obtain American Association of Laboratory Animal Clinicians (AALAC) certification.

5M.3.11 Reference cultures and certified reference cultures shall be kept separated from samples at check-in and during storage.

5M.3.12 Sample check-in and storage shall be segregated (ideally, in a separate area from the testing laboratory) and shall include proper sanitation to exclude the possibility of cross contamination.

5M.3.13 The pre-enrichment set-up area shall be segregated or separated from the main testing area. (It is also recommended that the media preparation and sterilization area be separated from the testing areas.)

5M.3.14 The laboratory shall have a Chemical Hygiene Plan (CFR 29.1910.1450) and documented, regular safety meetings.

5.4 Test Methods and Method Validation

5M.4.1 The laboratory shall have documented QA/QC procedures, including, but not limited to, media QC, incubation times and temperatures, equipment calibration and maintenance process control QC and standards for approving/rejecting results.

5M.4.2 Test methods and/or procedures shall include:

- **Scope**
- Description of the food types to be tested
- Quantities to be tested
- Material, equipment and tolerances required
- Description of procedure
- Physical and environmental conditions required
- Sample identification
- Method of recording observations and results
- Safety measures
- Data required for reporting
- Sensitivity of method
- Method of data analysis and reporting
5M.4.3 The laboratory shall use test methods that meet the needs of the client. Where possible, these methods shall comply with the essential/critical elements of international, national and/or regional standards. Where no method is specified, the laboratory shall use an appropriate method that is traceable to a recognized, validated method.

(NOTE: Most food microbiological methods are found in AOAC, FDA Bacteriological Analytical Manual, USDA, FSIS and AMS sources, APHA SMEDP, APHA Compendium of Methods for the Microbiological Examination of Foods, ISO and ICMSF. Many trade associations publish their own methods and may be useful resources – such as, National Food Processors, American Association of Cereal Chemists, Association of Dressings and Sauces, etc. New methods, especially involving emerging pathogens, may be obtained from scientific journals, texts or key researchers.)

5M.4.4 All methods not taken from authoritative, validated sources shall be agreed upon by the laboratory and client, with clearly defined expectations and requirements. Validation of the appropriateness of these methods (including nonstandard, commercialized tests systems (kits) and new test methods) shall be performed and documented and shall be subject to review by and agreement with the client to ensure that the range and accuracy of values obtainable from the method (e.g., detection limit, selectivity, matrix effects, repeatability/reproducibility, ease of use, etc.) are relevant to the client’s needs. Where methods exist that are superior to officially recognized methods, these methods may be used if agreed upon with the client and validated as meeting their intended purpose. (For U.S. purposes, official methods – including commercialized test systems (kits) from the AOAC International Official Methods of Analysis, FDA Bacteriological Analytical Manual and the USDA Laboratory Manuals – are considered validated.)

5M.4.4.1 The laboratory shall validate standard methods applied to food matrices not specified in the standard procedures.

5M.4.4.2 Qualitative test methods shall be validated to demonstrate estimated sensitivity and specificity, relative accuracy to official methods (where appropriate), positive and negative deviation, limit of detection, matrix effect, repeatability and reproducibility.

5M.4.4.3 Quantitative test methods shall be validated to demonstrate specificity, sensitivity, relative accuracy, positive and negative deviation, repeatability, reproducibility and limit of determination.

5M.4.4.4 In cases where new methods are developing rapidly (e.g., for emerging pathogens), normal validation procedures may be circumvented. In these cases, the laboratory shall – at a minimum - demonstrate qualitative recovery of a microorganism (CRC) for enrichment procedures carried out in replicate and demonstrate quantitative recovery of a microorganism (CRC) for an enumeration procedure carried out in replicate.

5M.4.4.5 The suitability of the method shall be checked and confirmed by comparing with specified requirements typical for the intended use of the method. For example, a filtration method for a non-filterable food, a five day test where three days are required, a 1 gm test where 100 gm are required, surface area specific tests for CFU/sq. area where CFU/gm is required.
5M.4.5 When sampling is performed by the laboratory, there shall be procedures ensuring that representative samples are obtained.

5M.4.6 Electronic records, electronic signatures and handwritten signatures executed to electronic records shall be demonstrated as being equivalent to records and handwritten signatures executed to paper per 21 CFR Part II (Docket No. 92NO251) RIN 0910-AA29; Federal Register: March 20, 1997, 1 Volume 62, Number 54, Rules and Regulations, pages 13429-13466.

5M.4.7 The laboratory shall have procedures (for both hardware and software) for safeguarding adjustments that would invalidate test results.

5.5 Equipment

5M.5.1 The laboratory shall be furnished with all items of sampling, measurement and test equipment required for the correct performance of the tests, including sampling, preparation of test items, processing and analysis of test data.

5M.5.2 All equipment (especially those items having an impact on the uncertainty of the results) listed in the methods shall meet the specifications relevant to the method and shall be calibrated and/or verified to those specifications.

5M.5.3 The laboratory shall have documented procedures for the handling, transport, storage and use of measuring equipment to prevent contamination or deterioration.

5M.5.4 The laboratory shall document maintenance schedules and procedures. Maintenance records shall be maintained. The following equipment shall undergo maintenance and servicing as specified:

5M.5.4.1 Incubators shall be cleaned and sanitized at a frequency specified by the laboratory.

5M.5.4.2 Refrigerators shall be cleaned and sanitized at a frequency specified by the laboratory.

5M.5.4.3 Freezers shall be cleaned and sanitized at a frequency specified by the laboratory.

5M.5.4.4 Ovens shall be cleaned and sanitized at a frequency specified by the laboratory.

5M.5.4.5 Water baths shall be cleaned and sanitized at a frequency specified by the laboratory.

5M.5.4.6 Centrifuges shall be serviced annually and cleaned and sanitized monthly.

5M.5.4.7 Safety cabinets shall be serviced annually and cleaned and sanitized with each use.
5M.5.4.8 Laminar flow hoods shall be serviced annually and cleaned and sanitized with each use.

5M.5.4.9 Microscopes shall be serviced annually. The eyepiece and objective lens shall be checked and, if necessary, cleaned with each use.

5M.5.4.10 pH meters shall be serviced annually. The electrodes shall be cleaned with each use.

5M.5.4.11 Balances shall be serviced annually and cleaned with each use.

5M.5.4.12 Vial fillers shall be cleaned at a frequency specified by the laboratory.

5M.5.4.13 Autoclaves shall be serviced semi-annually. Visual checks shall be performed at a frequency recommended by the manufacturer.

5M.5.4.14 The deionized water/reverse osmosis system cartridges shall be replaced at a frequency recommended by the manufacturer.

5M.5.4.15 Stills shall be cleaned per manufacturer’s instructions and at a frequency specified by the manufacturer.

5M.5.4.16 Glassware and plasticware shall be cleaned and sterilized with each use.

5M.5.4.17 Spiral platers shall be cleaned and decontaminated with each use.

5M.5.4.18 Spectrophotometers shall be cleaned per manufacturer’s instructions and at a frequency specified by the manufacturer.

5M.5.4.19 Other special incubation equipment shall be cleaned and sanitized with each use.

5M.5.4.20 The laboratory itself shall be cleaned and sanitized at a frequency specified by the laboratory.

5M.5.5 All media shall be labeled utilizing an appropriate identification scheme (preferably a number or alphanumeric system) and dated.

5M.5.6 There shall be a lot acceptance procedure for evaluating media lots for suitability. (This can be satisfied by manufacturer QC data following the CDC Lab Manual, Quality Control: Microbiology, 1987, U.S. Dept. of Health and Human Services, United States Public Heath Service (USPHS), CDC, Atlanta, GA for all lots of prepared dehydrated media or prepared media.)

5M.5.6.1 Laboratories that compound their own media shall follow the protocol specified parenthetically in 5M.5.6.

5M.5.7 Upon receipt of dehydrated media, records shall be kept including media name or description, manufacturer’s lot number, laboratory-assigned number, date received, date opened, date prepared for QC, manufacturer-assigned expiration date, discard date and initials of responsible person.
5M.5.7.1 All dehydrated media shall be labeled with laboratory number, identification and date approved.

5M.5.8 The laboratory shall have policies/procedures for the disposition of expired media.

5M.5.9 Each batch of media prepared internally or purchased must be examined for suitability. Records of these checks shall include:

(a) laboratory media identification number;
(b) batch number;
(c) date of media preparation;
(d) date results were read/taken;
(e) productivity (+ culture);
(f) selectivity (optional);
(g) sterile control; and
(h) date approved or rejected with initials of person approving/rejecting.

5M.5.10 All media shall be identified and traceable to QC results and each test. This includes preparation, traceability to media, pH (before and after sterilization), appearance, sterilization batch (with related records), fill volumes (if appropriate), batch size and quantity.

5M.5.11 For reagents, kits and identification systems, each lot shall be labeled upon receipt. Each lot shall be approved following a specified procedure. Each lot shall be identified with a laboratory number or alphanumeric system that includes the date approved.

5M.5.11.1 Records shall include:

(a) a description of the material;
(b) manufacturer lot number;
(c) date received;
(d) date opened;
(e) appearance; and
(f) date of approval/disapproval with initials of responsible person.

5M.5.11.2 Serological tests shall include a positive control and a saline negative control.

5M.5.12 All batches of media and reagents shall be traceable to autoclave records which shall document:

(a) date;
(b) run number;
(c) autoclave number (where appropriate);
(d) media and reagents/load;
(e) time into autoclave;
(f) time at desired temperature;
(g) time out of autoclave; and
(h) initials of responsible person(s).
5M.5.13 Sterilization equipment and sterilization processing cycles shall be validated and documented.

5M.5.14 All batches of media and reagents, sterilized by means other than an autoclave, shall be traceable to sterilization or decontamination processing records which shall document:

(a) date;
(b) media or reagent;
(c) confirmation of heating conditions (or filtration); and
(d) initials of responsible person(s).

5M.5.15 The laboratory’s water source shall be tested to ensure that it is inhibitor free (i.e., “microbiologically suitable water”). Documentation of performance of the following tests shall be maintained:

5M.5.15.1 Daily Testing resistance shall be > 1.0 megohms/cm at 25°C;

5M.5.15.2 Monthly Testing: Total residual chlorine shall be <0.01mg/l; aerobic plate count shall be < 1000 colony forming units (cfu)/mL.

5M.5.15.3 Annual Testing: Heavy metals (Cd, Cr, Cu, Ni, Pb, Zn - individually) shall be < 0.05 mg/L; Heavy metals (total) shall be < 10 mg/L; Bacteriological Suitability shall be “passing”.

5.6 Measurement Traceability

5M.6.1 The laboratory shall have a program for calibrating/verifying the performance of all critical equipment and media, traceable to national standards or cultures. For the purpose of food microbiology, media are critical materials that shall be calibrated/verified against national standards – reference cultures and/or certified reference cultures. The following equipment shall be calibrated/verified at the specified frequency:

**Calibration**

5M.6.1.1 The NIST reference thermometers shall be recertified every five years.

5M.6.1.2 Reference thermocouples shall be calibrated to the boiling point and ice point annually.

5M.6.1.3 Working thermometers shall be calibrated against the NIST reference thermometer annually.

5M.6.1.4 Working thermocouples shall be calibrated against the reference thermocouple or by other NIST traceable means annually.

5M.6.1.5 Reference weights shall be recertified (traceable to NIST) every five years.

5M.6.1.6 Balances shall be calibrated (traceable to NIST) annually.

5M.6.1.7 Timers shall be calibrated to a national time standard annually.
5M.6.1.8  Volumetric glassware (non Class A) shall be calibrated gravimetrically (traceable to NIST weights) annually.

5M.6.1.9  Microscope stage micrometers shall be calibrated at installation.

5M.6.1.10 Hydrometers shall be calibrated to a reference chemical compound annually.

5M.6.1.11 Autoclaves shall be calibrated to NIST traceable thermometers or thermocouples annually.

**Calibration for Use**

5M.6.1.12 The stability and uniformity of temperature for all incubators shall be verified at installation.

5M.6.1.13 The stability and uniformity of temperature for all refrigerators shall be verified at installation.

5M.6.1.14 The stability and uniformity of temperature for all freezers shall be verified at installation.

5M.6.1.15 The stability and uniformity of temperature for all ovens shall be verified at installation.

5M.6.1.16 The stability and uniformity of temperature for all waterbaths shall be verified at installation.

5M.6.1.17 The uniformity of temperature for all autoclaves shall be verified at installation and annually thereafter.

5M.6.1.18 Spectrophotometer wavelengths shall be calibrated/verified at installation.

5M.6.1.19 The mass/volume delivery of vial fillers shall be verified at installation and daily thereafter for each volume.

5M.6.1.20 Safety cabinets and laminar airflow cabinets shall be inspected for particulates at installation and annually thereafter.

5M.6.1.21 Spiral platers shall be verified against conventional testing at installation.

**Performance Verification**
(Note: “Daily” refers to each day of use)

5M.6.1.22 Incubator temperatures shall be monitored daily – once in the morning and once in the evening/afternoon.

5M.6.1.23 Refrigerator temperatures shall be monitored daily – once in the morning or once in the evening/afternoon.

5M.6.1.24 Freezer temperatures shall be monitored daily – once in the morning or once in the evening/afternoon.
5M.6.1.25 Oven temperatures shall be monitored daily – once in the morning or once in the evening/afternoon. (Note: If an oven is used at different temperatures on different days, temperature shall be monitored and recorded each time samples are entered into and removed from the oven.)

5M.6.1.26 Waterbath temperatures shall be monitored with each use.

5M.6.1.27 Autoclave time and temperature readings shall be monitored daily and with each load. All media shall be sterility controlled and spore vials or strips shall be used to verify sterility weekly.

5M.6.1.28 A blank spectrophotometer reading shall be taken daily.

5M.6.1.29 The mass/volume delivery of micropipettors shall be verified daily.

5M.6.1.30 Each lot of pipettes shall be cleaned and sterilized. The mass/volume delivery of each lot of pipettes shall be verified. (NOTE: Certification of sterility and/or cleanliness from the manufacturer fulfills this requirement.)

5M.6.1.31 Glassware cleanliness shall be monitored via residue tests; glassware sterility shall be verified with each lot or load.

5M.6.1.32 The sterility of each lot of petri dishes shall be verified. (NOTE: Certification of sterility and/or cleanliness from the manufacturer fulfills this requirement.)

5M.6.1.33 Balances shall be verified daily using at least 1 weight within the expected range of use.

5M.6.1.34 Prepared media shall be checked for sterility, productivity (and + culture), pH and appearance with each batch.

5M.6.1.35 Rapid test kits shall be checked for +/- reaction with each lot of +/- culture reference.

5M.6.1.36 The ID system shall be checked for +/- reaction with each lot of +/- culture reference.

5M.6.1.37 Test reagents shall be checked for +/- reaction with each lot.

5M.6.1.38 pH meters shall be calibrated daily using standard buffers covering the expected range of use.

5M.6.1.39 The deionized water/reverse osmosis system and stills shall be checked for conductivity (weekly) and microbial density (monthly).

5M.6.1.40 Open medium control sterility checks shall be performed on safety cabinets and laminar airflow cabinets with each use. The airflow of these cabinets shall be monitored monthly.

5M.6.1.41 Colony counters shall be verified against a manual count annually.
5M.6.1.42 Spiral platers shall be compared to standard plating procedures annually. Siphon condition shall be checked daily and volume dispersal shall be checked monthly.

5M.6.2 When national or international standards are not available for the calibration/verification of the performance of microbiology procedures, there shall be a documented procedure for demonstrating appropriate recovery each time a method is performed. This may be accomplished through:

5M.6.2.1 Participation in a suitable program of interlaboratory comparison or proficiency testing;

5M.6.2.2 The use of RCs and CRCs to quantify recovery on every occasion that a test is performed;

5M.6.2.3 Mutually agreed-upon expectations of the laboratory and client.

5M.6.3 The laboratory shall have procedures for the safe handling, transport, storage and use of reference standards, reference materials, reference cultures and certified reference cultures to prevent contamination or deterioration.

5M.6.4 Reference standards (e.g., reference thermometers, weights, etc.) shall be used for calibration or verification purposes only.

5M.6.5 Certified reference cultures shall be traceable to a nationally or internationally recognized type culture collection.

5M.6.5.1 Reference cultures from laboratory sources shall be identified and traceable to standard reference sources.

5M.6.5.2 Reference cultures shall be handled to maintain their biochemical reaction and physiological characteristic integrity.

5M.6.5.3 RCs and CRCs shall not be transferred more than five times from the original sources. After the fifth transfer, the laboratory may purchase another culture from a type culture collection or re-identify the culture for key biochemical and physiological characteristics using nationally or internationally recognized reference sources. Alternatively, the type culture may be grown then freeze dried, kept in frozen storage, etc. and used periodically thereby extending the length of time after which they must be repurchased or reidentified.

5M.6.6 The laboratory shall ensure that their suppliers of CRCs comply with the requirements of ISO/IEC Guide 25 relevant to calibration laboratories.

5.7 Sampling (No Additions)
5.8 Handling of Test Items

5M.8.1 The laboratory’s system for the identification of samples shall include the following information, records of which must be retained throughout the testing life of the sample:

5M.8.1.1 unique and unambiguous sample identification (usually a number or alpha numeric identification);

5M.8.1.2 name of the person(s) to whom the final report will be sent;

5M.8.1.3 manufacturer’s name or sample source and date of sampling (if available);

5M.8.1.4 identification number or description from client (if any);

5M.8.1.5 product description;

5M.8.1.6 tests desired and/or methods requested;

5M.8.1.7 date of receipt;

5M.8.1.8 delivery carrier;

5M.8.1.9 sample condition and physical appearance (including temperature);

5M.8.1.10 sample identification (if different from 11M.1.4).

5M.8.2 If any deviations from specifications are noted, the continued suitability of the sample for testing shall be discussed with the client. Cross contamination is the most critical issue – resulting from broken or leaking samples – for both qualitative and quantitative tests. However, it is much more important to the qualitative (infectious pathogens) tests.

5M.8.3 The laboratory shall have documented procedures for the handling, sampling, transport, storage, preparation, retention and disposal of test items.

5M.8.3.1 Sample handling and storage procedures shall include precautions for preventing cross contamination and deterioration (death or growth of microorganisms).

5.9 Assuring the Quality of Test Results

5M.9.1 All laboratories shall have a daily (every test day) control using CRCs (+ culture), where available, or RCs (+ culture) for running concurrently with all tests. There shall be procedures and policies for interpreting these results as related to the validity of the tests. In cases where there is adequate control and documentation, this procedure can be used to verify the acceptability of media (concurrent with the test).

5M.9.2 CRC, CRM, RM and RC shall be used to evaluate performance on a daily basis to include media and daily process control checks. These data shall be used to evaluate the validity of the test results following written procedures available before testing.
5.10 Reporting the Results

5M.10.1 For microbiological testing of infectious agents where diagnostic (qualitative) tests can differentiate serological types or biotypes, there shall be policies and procedures for interpreting, evaluating and reporting unequivocal results, since environmental factors/data may invalidate or cast doubt on the integrity of the results. (This is especially pertinent to Salmonella analyses where serological tests are well developed.) The client shall be notified of any factors that have affected or may potentially affect the integrity of reported results and shall be informed of any data interpretations or evaluations that are made.

(NOTE: On a case-by-case basis, exceptions will be made for the report content requirements outlined in ISO/IEC Guide 25, Section 13.2 for laboratories reporting results for regulatory purposes and not utilizing the A2LA logo on these reports.)

6. Proficiency Testing Requirements

Please refer to the A2LA Proficiency Testing Requirements for Accredited Testing and Calibration Laboratories.

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Document Revision History

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<tr>
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<td>Specific Criteria numbering and order changed to match ISO/IEC 17025.</td>
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