



ENVIRONMENTAL LABORATORY SECTOR

WORKING DRAFT STANDARD (WDS)

This WDS is a proposed revision of the 2012 standard (EL-V1M5-2012). It has been prepared by the Microbiology Expert Committee. It will be presented to the membership and the public for discussion and input at the **TNI Semiannual Meeting: Forum on Laboratory Accreditation in Washington, DC** on August 5, 2014 and through a Webinar on that same date.

Note: A clean copy is presented below to improve readability. Contact Ilona Taunton (Ilona.taunton@nelac-institute.org) if you want to view a copy where track changes shows the proposed changes from the 2012 Standard (EL-V1M5-2012).

VOLUME 1

MANAGEMENT AND TECHNICAL REQUIREMENTS FOR LABORATORIES PERFORMING ENVIRONMENTAL ANALYSIS

Module 5: Quality Systems for Microbiological Testing

TNI Standard

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PREFACE

This Standard is the result of many hours of effort by those volunteers on The NELAC Institute (TNI) Quality Systems Committee and Microbiology Expert Committee. The TNI Board of Directors wishes to thank these committee members for their efforts in preparing this Standard as well as those TNI members who offered comments during the voting process.

This Standard supplements Module 2, Quality Systems General Requirements, and may be used by any organization that wishes to implement a program for the accreditation of environmental laboratories.

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VOLUME 1, MODULE 5

Quality Systems for Microbiological Testing

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VOLUME 1, MODULE 5

Quality Systems for Microbiological Testing

1.0 MICROBIOLOGICAL TESTING

1.1 Introduction

This Standard applies to laboratories undertaking microbiological analysis of environmental samples. Microbiological testing refers to and includes the detection, isolation, enumeration, or identification of microorganisms and/or their metabolites, or determination of the presence or absence of growth in materials and media. The evaluation of laboratories for this discipline is in conjunction with a quality system as specified in general requirements module. Adherence to quality systems requirements will ensure that all quality control procedures specified in this module are being followed.

1.2 Scope

The essential quality control procedures applicable to microbiological analysis are included in this module. Additional quality control or program requirements that are either specified by method, regulation or project shall be met by laboratories.

1.3 Terms and Definitions

The relevant definitions from TNI, Volume 1, Module 2, Section 3.0 apply. Definitions related to this document, which are used differently or do not exist in the above references are defined below.

1.3.1 Additional Terms and Definitions

Source Water – When sampled for drinking water compliance, untreated water from streams, rivers, lakes, or underground aquifers, which is used to supply private and public drinking water supplies.

1.3.2 Exclusions and Exceptions

Reserved

1.4 Method Selection

Refer to Volume 1, Module 2, Sections 5.4.2, 5.4.3, and 5.4.4.

1.5 Method Validation

1.5.1 For methods, other than reference methods, validation must comply with Volume 1, Module 2. This validation must include the minimum requirements outlined in Sections 1.5.4, 1.5.5, and 1.5.6 of this module.

1.5.2 For both reference and non-standard methods, laboratories shall participate in proficiency testing programs.

1.5.3 The laboratory shall maintain documentation of the validation procedure for as long as the method is in use and for at least five (5) years past the date of last use.

1.5.4 Accuracy – Use at least one (1) known pure reference culture at the anticipated environmental conditions, and compare the method results to that of a reference method.

- 1.5.5 Precision – Perform at least ten (10) replicate analyses with both the proposed and reference method, using the target microorganisms of choice. The results shall show that the methods are not statistically different.
- 1.5.6 Selectivity (sensitivity) – Verify all responses in at least ten (10) samples using mixed cultures that include the target organism(s), and at varying concentrations (microbial identification testing or equivalent processes may be used). Calculate the number of false positive and false negative results.

1.6 Demonstration of Capability (DOC)

1.6.1 General

- 1.6.1.1 An individual who performs any activity involved with preparation and/or analysis of samples must have constant, close supervision (as defined in the laboratory's training procedure) until a satisfactory initial DOC is completed (see Section 1.6.2).
- 1.6.1.2 Thereafter, ongoing DOC (Section 1.6.3), as per the quality control requirements in Section 1.7.3, is required.
- 1.6.1.3 In cases where an individual has prepared and/or analyzed samples using a method that has been in use by the laboratory for at least one year prior to applying for accreditation, and there have been no significant changes in instrument type or method, the ongoing DOC shall be acceptable as an initial DOC. The laboratory shall have records on file to demonstrate that an initial DOC is not required.

All demonstrations shall be documented. All data applicable to the demonstration shall be retained and readily available at the laboratory.

1.6.2 Initial DOC

An initial DOC shall be made prior to using any method, and at any time there is a change in instrument type, personnel or method or any time that a method has not been performed by the laboratory or analyst in a twelve (12) month period.

- 1.6.2.1 The laboratory shall document each initial DOC in a manner such that the following information is readily available for each affected employee:
- analyst(s) involved in preparation and/or analysis;
 - matrix;
 - organism(s);
 - identification of method(s) performed;
 - identification of laboratory-specific SOP used for analysis, including revision number;
 - date(s) of analysis;
 - summary of analyses, including information outlined in Section 1.6.2.2.c.
- 1.6.2.2 If the method or regulation does not specify an initial DOC, the following procedure is acceptable. It is the responsibility of the laboratory to document that other approaches to initial DOC are adequate.
- The target organism(s) shall be diluted in a volume of sterile, quality system matrix (a sample in which no target organisms or interferences are present at concentrations that will impact the results of a specific method). When required by method, the diluent shall be sterile buffered water and/or sterile peptone water unless specified by the manufacturer. Prepare at least four (4) aliquots at the concentration specified, or if unspecified, to the countable range for plate methods or working range for most probable number (MPN) type methods.

- b. At least four (4) aliquots shall be prepared and analyzed according to the method either concurrently or over a period of days.
- c. Using all of the results, convert these results to logarithmic values, then calculate the mean recovery and standard deviation of the log converted results in the appropriate reporting units for each organism of interest. When it is not possible to determine mean and standard deviations, such as for presence/absence, the laboratory shall assess performance against established and documented criteria.
- d. For qualitative tests, acceptable performance in a blind study, either internally or externally generated, may be used to meet this Standard, provided that the study consists of a minimum of a blank, a negative culture, and a positive culture for each target organism or metabolite (e.g. b-glucuronidase in *E. coli*).
- e. Compare the information from c) above to the corresponding acceptance criteria for precision and accuracy in the method (if applicable) or in laboratory-generated acceptance criteria such as relative standard deviation (if there are not established mandatory criteria). If all parameters meet the acceptance criteria, the analysis of actual samples may begin. If any one of the parameters does not meet the acceptance criteria, the performance is unacceptable for that parameter.
- f. When one or more of the tested parameters fail at least one of the acceptance criteria, the analyst shall proceed according to i) or ii) below.
 - i. Locate and correct the source of the problem and repeat the initial DOC for all parameters of interest beginning with b) above.
 - ii. Repeat the initial DOC for all parameters that failed to meet criteria.
- g. Repeated failure, however, confirms a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all organisms of interest beginning with b).

1.6.3 Ongoing DOC

- 1.6.3.1 The laboratory shall have a documented procedure describing ongoing DOC that includes how the laboratory will identify data associated with ongoing DOCs. The analyst(s) shall demonstrate ongoing capability by routinely meeting the quality control requirements of the method, laboratory SOP, client specifications, and/or this Standard. If the method has not been performed by the analyst in a twelve (12) month period, an Initial DOC (1.6.2) shall be performed. It is the responsibility of the laboratory to document that other approaches to ongoing DOC are adequate.
- 1.6.3.2 This ongoing demonstration may include one of the following or by performing another initial DOC.
 - a. Analysis of one sample or clean matrix that is fortified with a known quantity of the target organism, with results meeting the laboratory acceptance criteria for accuracy and, where applicable to the testing technique, also meeting the observational details expected for the presumptive, confirmed and completed phases defined in the method.
 - b. Analysis of one sample in duplicate for each target organism and test, with results meeting the laboratory acceptance criterion for precision.
 - c. Acceptable results for one-single-blind proficiency test sample for target organisms in each field of accreditation.
 - d. Performance of an alternate adequate procedure for the field of accreditation, the procedure and acceptance criteria being documented in the laboratory's quality system.

- e. A documented process of reviewing QC samples performed by an analyst or groups of analysts relative to the quality control requirements of the method, laboratory SOP, client specifications, and/or this Standard. This review can be used to identify patterns for individuals or groups of analysts and determine if corrective action or retraining is necessary; or
- f. If a) through e) are not technically feasible, then analysis of real-world samples with results within predefined acceptance criteria (as defined by the laboratory or method) shall be performed.

1.7 Technical Requirements

1.7.1 Calibration

- a. The laboratory shall have documented procedures for calibration, verification, and quality control of support equipment including conductivity meters, oxygen meters, pH meters, hygrometers, and other similar measurement instruments. These procedures shall refer to applicable reference methods.
- b. For instruments that are continuous monitors, such as in-line specific conductance meters:
 - i. The laboratory shall document acceptable calibration verification at least once a month.
 - ii. An initial calibration shall be performed if a continuing calibration is unacceptable, or when the instrument is being returned to service after having been taken off line.

1.7.2 Continuing Calibration

Reserved for specific procedures.

1.7.3 Quality Control

1.7.3.1 Quality and Sterility of Standards, Reagents, Materials and Media

The laboratory shall ensure that the quality of the reagents and media used is appropriate for the test concerned.

- a. **Sterility Checks:** All materials and supplies that are needed to process the sample and are required to be sterile prior to use (whether sterilized in the lab or purchased as sterilized) must be checked once per purchase or prepared lot using non-selective growth media as appropriate. Certificates of analysis provided by vendors shall be verified by the laboratory. These sterility checks may be performed by a contracted laboratory if the laboratory does not have the requisite equipment to perform them. These checks shall include but are not limited to:
 - i. A sterility check shall be analyzed for each lot of prepared ready to use media and for each batch of media prepared in the laboratory.
 1. For chromo/fluorogenic media, add media to sterile DI water and incubate at the appropriate temperature and time.
 2. For all other media incubate uninoculated at the appropriate temperature and time.

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- ii. For pre-sterilized single use funnels, a sterility check shall be performed on one funnel per lot. For laboratory-sterilized funnels, a sterility check shall be performed on one funnel per sterilization batch.
 - iii. Sterility checks on sample containers shall be performed on at least one (1) container for each lot of purchased, pre-sterilized containers. For containers prepared and sterilized in the laboratory, a sterility check shall be performed on one (1) container per sterilized batch with nonselective growth media.
 - iv. A sterility check shall be performed on each batch of dilution water prepared in the laboratory and on each lot of pre-prepared, ready-to-use dilution water with non-selective growth media. The concentration of the non-selective growth media shall be single strength after the addition of the dilution water.
 - v. At least one (1) filter from each new lot of membrane filters shall be checked for sterility with nonselective growth media.
- b. Media – Culture media may be prepared from commercial dehydrated powders or may be purchased ready-to-use.
- i. Laboratory-prepared media
 - 1. Media prepared by the laboratory from basic ingredients and/or commercial dehydrated powder shall be tested for performance (e.g., for selectivity, sensitivity, sterility, growth promotion, and growth inhibition). These tests shall be performed at a minimum with first use.
 - 2. Media shall be used within the holding time limits specified in the accredited method.
 - 3. Detailed testing criteria information shall be defined in the laboratory's methods, SOPs, or similar documentation.
 - ii. Ready-to-use media
 - 1. See 1.7.3.1(b)(i)(1)
 - 2. Any ready-to-use media shall be used within the expiration date provided by the manufacturer.
- c. Reagents and commercial dehydrated powders shall be used within the shelf life of the product, and shall be documented as per TNI Volume 1, Module 2 Quality Systems General Requirements.
- d. Reagent Water
- i. The quality of the reagent water used in the laboratory, which will come into contact with test organisms, and used in preparation of media, solutions, and buffers shall be monitored for bactericidal and inhibitory substances. This water shall be distilled water, de-ionized water, or reverse-osmosis produced water.
 - ii. The quality of the water shall be monitored for chlorine residual, specific conductance, total organic carbon, and heterotrophic bacteria plate count monthly (when in use), when maintenance is performed on the water treatment system, or at startup after a period of disuse longer than one month.

- iii. Analysis for metals (Cd, Cr, Cu, Ni, Pb, and Zn) and the Bacteriological Water Quality Test (to determine presence of toxic agents or growth promoting substances) shall be performed annually. An exception to performing the Bacteriological Water Quality Test shall be given to laboratories that can supply documentation to show that their water source meets the criteria, as specified by the method, for High Quality (Type I) or Medium Quality (Type II) reagent water.
 - iv. Results of the above analyses shall meet the specifications of the required method and records of analyses shall be maintained for five (5) years.
 - v. Reagent water purchased from an outside source and used for the preparations of media, solutions and buffers shall meet the criteria specified in items ii) and iii) above. The laboratory shall have documented records of this information. Purchased reagent water that has been in use for longer than the testing intervals specified in items i) through iv) or in the accredited method shall either be re-tested or discarded.
- e. Documentation for media prepared in the laboratory shall include date of preparation, preparer's initials, type, manufacturer, lot number, final pH, expiration date, and the amount of reagents used. Documentation for media purchased pre-prepared, ready-to-use (including reagent water purchased from outside sources) shall include manufacturer, lot number, type of media received, date of receipt, expiration date of the media, and pH of the media.

1.7.3.2 Method Blanks

The laboratory shall demonstrate that the filtration equipment and filters, sample containers, media and reagents have not been contaminated through improper handling or preparation, inadequate sterilization, or environmental exposure.

- a. For filtration technique, the laboratory shall conduct method blanks per the analytical method. At a minimum, the filtration series shall include a beginning and ending blank. The filtration series may include single or multiple filtration units, which have been sterilized prior to beginning the series.
- b. The filtration series is considered ended when more than thirty (30) minutes elapses between successive filtrations. During a filtration series, filter funnels shall be rinsed with three (3) 20-30 ml portions of sterile rinse water after each sample filtration. In addition, laboratories shall insert a method blank after every ten (10) samples or sanitize filtration units by UV light (254-nm) after each sample filtration.
- c. For pour plate technique, method blanks of the medium shall be made by pouring, at a minimum, one uninoculated plate for each lot of pre-prepared, ready-to-use media and for each batch of medium prepared in the laboratory.

1.7.3.3 Test Variability/Reproducibility

For methods that specify colony counts such as membrane filter or plated media, duplicate counts shall be performed monthly on one positive sample, for each month that the test is performed. If the laboratory has two or more analysts, each analyst shall count typical colonies on the same plate. Counts shall be within 10% difference to be acceptable. In a laboratory with only one microbiology analyst, the same plate shall be counted twice by the analyst, with no more than 5% difference between the counts.

1.7.3.4 Sample Specific Controls (where applicable)

- a. Matrix spikes shall be performed per method requirements.
- b. Sample matrix duplicates shall be performed per method requirements.

1.7.3.5 Data Reduction

The calculations, data reduction and statistical interpretations specified by each method shall be identified and followed.

1.7.3.6 Selectivity

- a. All growth and recovery media shall be checked to assure that the target organism(s) respond in an acceptable and predictable manner.
- b. To ensure that analysis results are accurate, target organism identity shall be verified as specified in the method (e.g., by use of the completed test, or by use of secondary verification tests such as a catalase test or by the use of a selective media such as brilliant green (BG) or E. coli (EC or EC + MUG) broth.
- c. In order to ensure identity and traceability, reference cultures used for positive and negative controls shall be obtained from a recognized national collection, organization, or manufacturer recognized by the accreditation body. Microorganisms may be single use preparations or cultures maintained for their intended use by documented procedures that demonstrate the continued purity and viability of the organism.
 - i. Reference cultures may be revived (if freeze-dried) or transferred from slants and sub-cultured once to provide reference stocks. The reference stocks shall be preserved by a technique that maintains the characteristics of the strains. Reference stocks shall be used to prepare working stocks for routine work. If reference stocks have been thawed, they shall not be refrozen and re-used.
 - ii. Working stocks shall not be sequentially cultured more than five (5) times and shall not be sub-cultured to replace reference stocks.
- d. Culture Controls
 - i. Negative Culture Controls
 1. Negative culture controls demonstrate that the medium does not support the growth of non-target organisms or does not exhibit the typical positive reaction of the target organism(s).
 2. Each pre-prepared, ready-to-use lot of selective medium (including chromofluorogenic reagent) and each batch of selective medium prepared in the laboratory shall be analyzed with one or more known negative culture controls (i.e. non-target organisms), as appropriate to the method. This shall be done prior to first use of the medium.
 - ii. Positive Culture Controls
 1. Positive culture controls demonstrate that the medium can support the growth of the target organism(s), and that the medium produces the specified or expected reaction to the target organism(s).
 2. Each pre-prepared, ready-to-use lot of medium (including chromofluorogenic reagent) and each batch of medium prepared in the laboratory shall be tested with at least one pure culture of a known positive reaction as appropriate to the method. This shall be done prior to first use of the medium.

1.7.3.7 Constant and Consistent Test Conditions

a. Laboratory Facilities

Floors and work surfaces shall be non-absorbent and easy to clean and disinfect. Work surfaces shall be adequately sealed. Laboratories shall provide sufficient storage space, and shall be clean and free from dust accumulation. Plants, food, and drink shall be prohibited from the laboratory work area.

b. Laboratory Equipment

i. Temperature Measuring Devices

Temperature measuring devices such as liquid-in-glass thermometers, thermocouples, or platinum resistance thermometers used to assess and document equipment temperatures shall be the appropriate quality to meet specification(s) in the method. The graduation and range of the temperature measuring devices shall be appropriate for the required accuracy of the measurement. Temperature measuring devices shall be verified to national or international standards for temperature. Verification shall be performed at least annually (see TNI Volume 1, Module 2, Section 5.5.13.1). This verification may be accomplished by a single point provided that it represents the method mandated temperature and use conditions.

ii. Sterilization Equipment

1. Autoclaves

The performance of each autoclave shall be initially evaluated by establishing its functional properties and performance, for example heat distribution characteristics with respect to typical uses. Autoclaves shall meet specified temperature tolerances. Pressure cookers shall not be used for sterilization of growth media.

Demonstration of sterilization temperature shall be provided by use of a continuous temperature recording device or by use of a maximum registering thermometer with every cycle. At least once during each month that the autoclave is used, appropriate biological indicators shall be used to determine effective sterilization. The selected biological indicator shall be effective at the sterilization temperature and time needed to sterilize lactose-based media. Temperature sensitive tape shall be used with the contents of each autoclave run to indicate that the autoclave contents have been processed.

Records of autoclave operations shall be maintained for every cycle. Records shall include: date, contents, maximum temperature reached, pressure, time in sterilization mode, total run time (may be recorded as time in and time out) and analyst's initials.

Autoclave maintenance (either internally or by service contract) shall be performed annually, and shall include a pressure check and verification of temperature device. Records of the maintenance shall be maintained in equipment logs. When it has been determined that the autoclave has no leaks, pressure checks can be documented using the formula $PV = nRT$.

The autoclave mechanical timing device shall be checked quarterly against a stopwatch and the actual time elapsed documented.

2. Ovens

Ovens used for sterilization shall be checked for sterilization effectiveness monthly with appropriate biological indicators. Records shall be maintained for each cycle that include date, cycle time, temperature, contents and analyst's initials.

iii. Volumetric Equipment

Volumetric equipment shall be verified as follows:

1. Equipment with movable parts such as automatic dispensers, dispensers/diluters, and mechanical hand pipettes shall be verified for accuracy quarterly.
2. Equipment such as filter funnels, bottles, non-Class glassware, and other containers with volumetric markings (including sample analysis vessels) shall be verified once per lot prior to first use.
3. The volume of the disposable volumetric equipment such as sample bottles, and disposable pipettes shall be checked once per lot.
4. Verification of volume shall be considered acceptable if the accuracy is within 2.5% of expected volume. This verification can be volumetric as compared to class A or gravimetric.

iv. UV Instruments

UV instruments, used for sanitization, shall be tested quarterly for effectiveness with an appropriate UV light meter, by plate count agar spread plates or other methods providing equivalent results such as uvicide strips. Replace bulbs if output is less than 70% of original for light tests or if count reduction is less than 99% for a plate containing 200 to 300 organisms.

v. Incubators, Water Baths,

The uniformity of temperature distribution in incubators and water baths shall be established prior to first use. Temperature of incubators and water baths shall be documented twice daily, at least four hours apart, on each day of use when samples are under test. Under test is defined as the time period that the sample is in the incubation phase of the method.

For tests where samples are under test during weekends or holidays, the laboratory must have a system in place to ensure that the temperature monitoring requirements are met for the entire incubation period. Data loggers, continuous temperature monitoring devices, or other temperature monitoring equipment can be used as long as they can be calibrated in accordance with TNI Volume 1, Module 2, section 5.5.13.1 for support equipment.

An exception to the twice-daily temperature measurement documentation is permitted for the last day of the incubation period when all samples are removed from the incubator or waterbath, the initial temperature(s) is measured and documented, and no other samples are or will be present in the incubator or waterbath that day.

vi. Labware (Glassware and Plasticware)

1. The laboratory shall have a documented procedure for washing labware, if applicable. Detergents designed for laboratory use shall be used.
2. Glassware shall be made of borosilicate or other non-corrosive material, free of chips and cracks, and shall have readable measurement marks.
3. Labware that is washed and reused shall be tested for possible presence of residues that may inhibit or promote growth of microorganisms by performing the Inhibitory Residue Test initially, and each time the lab changes the detergent formulation or washing procedures.
4. Washed labware shall be tested at least once daily, each day of washing, for possible acid or alkaline residue by testing at least one piece of labware with a suitable pH indicator such as bromothymol blue. Records of tests shall be maintained.

1.7.4 Data Acceptance/Rejection Criteria

Methods criteria and evaluation methods shall be used.

1.7.5 Sample Handling

- a. Samples that require thermal preservation shall be considered acceptable if the arrival temperature of a representative sample container meets the method or mandated temperature requirement.
 - i. Samples that are delivered to the laboratory on the same day they are collected may not meet the requirements of Section 1.7.5.a) or the method, if the time frame between collection and delivery is too short for the cooling process to complete. In these cases, the samples shall be considered acceptable if the samples were received nestled in ice with evidence that the cooling process has begun and the temperature of the sample(s) (or representative sample) is recorded upon receipt and is less than the temperature recorded at the time of sampling.
 - ii. If sample analysis is begun within fifteen (15) minutes of collection, thermal preservation is not required.
 - iii. Thermal preservation is not required in the field if the laboratory receives the sample and either begins the analysis or refrigerates the sample within fifteen (15) minutes of collection.
- b. Microbiological samples from known chlorinated sources (such as wastewater effluent), unknown sources where chlorine usage is suspected (such a new client or a new source) and all potable water supplies (including source water) shall be checked for absence of chlorine residual in the laboratory unless all of the following conditions are met:
 - i. the laboratory can show that the received sample containers are from their laboratory or have been appropriately tested and documented;
 - ii. sufficient sodium thiosulfate was in each container before sample collection to neutralize at minimum 5 mg/l of chlorine for drinking water and 15 mg/l of chlorine for wastewater samples;

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- iii. one container from each batch of laboratory prepared containers or lot of purchased ready-to-use containers is checked to ensure efficacy of the sodium thiosulfate to 5 mg/l chlorine or 15 mg/l chlorine as appropriate and the check is documented;
 - iv. chlorine residual is checked in the field and actual concentration is documented with sample submission.