



ENVIRONMENTAL LABORATORY SECTOR

VOLUME 1

MANAGEMENT AND TECHNICAL REQUIREMENTS FOR LABORATORIES PERFORMING ENVIRONMENTAL ANALYSIS

Module 5: Microbiological Testing Requirements

TNI Standard

**This Document Presented for Review and Comment
Only**

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PREFACE

This Standard is the result of many hours of effort by those volunteers on The NELAC Institute (TNI) 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 supersedes and replaces preceding documents in whole or in part. It supplements Module 2, Quality Management 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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Microbiological Testing Requirements

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VOLUME 1, MODULE 5

Microbiological Testing Requirements

1.0 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 TNI Module 2. Adherence to those quality system requirements and all quality control (QC) procedures specified in this module will ensure that microbiological test results are fit for the intended use.

2.0 Scope

The essential QC procedures applicable to microbiological analysis are included in this module. If more stringent standards or requirements are included in a mandated test method or by regulation, the laboratory must demonstrate that such requirements are met. If it is not clear which requirements are more stringent, the method or regulation is to be followed.

3.0 Terms and Definitions

The relevant definitions from TNI Module 2, Section 3 apply. Definitions related to this document, which are used differently or do not exist in the above references, are defined below.

3.1 **Monthly:** Once per calendar month not to exceed 32 days.

3.2 **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.

3.3 **Test Reagent:** Substances used to identify, analyze or quantify the target organism(s)/analyte(s) of interest. Test reagents can include, but are not limited to: media, stains, dyes and biochemical identifiers.

4.0 Method Selection

Refer to TNI Module 2, Section 7.2.

5.0 Validation and/or Verification of Methods

5.1 Verification of reference methods must be completed by the laboratory prior to first use. For example, performance of a satisfactory initial demonstration of capability.

5.2 For non-reference methods, validation must comply with TNI Module 2, Section 7.2.2, and include the following:

5.2.1 Accuracy – Use at least one pure (single organism/analyte of interest) positive control at a concentration typical of the range for quantitative analyses. Compare the method results to that of a reference method and demonstrate evaluation for acceptability against an established accuracy criteria. In the absence of applicable method or regulatory requirements, the laboratory must define acceptance criteria. A positive control demonstrates that the medium can support the growth of the target organism(s)/analyte(s) of interest, and that the medium or test reagent produces the specified or expected reaction to the target organism(s)/analyte(s) of interest.

5.2.2 Precision – Perform at least ten replicate analyses with both the proposed and reference method, using a sample containing the target organism(s)/analyte(s) of interest. The results must show that

the precision of the proposed method is statistically equivalent or better than that of the reference method.

5.2.3 Selectivity (sensitivity) – Verify all responses, using microbial identification testing or equivalent processes, in at least ten samples of mixed cultures which include the target organism(s)/analyte(s) of interest at varying concentrations. Calculate the number of false positive and false negative results and demonstrate evaluation for acceptability against established criteria. In the absence of applicable method or regulatory requirements, the laboratory must define acceptance criteria.

5.3 The laboratory must maintain method development, validation, and verification records for as long as the method is in use, and for five years past the date of last use.

6.0 Demonstration of Capability (DOC)

6.1 General

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 6.2).

6.1.2 For each individual who performs any activity involved with preparation and/or analysis of samples, an ongoing DOC (see Section 6.3), must be performed and recorded annually.

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 where there have been no significant changes in instrument type or method, the ongoing DOC is acceptable as an initial DOC. The laboratory must have records on file to demonstrate that an initial DOC is not required.

6.1.4 The laboratory must maintain DOC records for as long as the method is in use, and for five years past the date of last use.

6.2 Initial DOC

An initial DOC must 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-month period.

6.2.1 The laboratory must record each initial DOC in a manner such that the following information is readily available for each affected employee:

- a) analyst(s) involved in preparation and/or analysis;
- b) matrix;
- c) target organism(s)/analyte(s) of interest
- d) identification of method(s) performed;
- e) identification of laboratory-specific Standard Operating Procedure (SOP) used for analysis, including revision number;
- f) date(s) of analysis; and
- g) summary of analyses, including information outlined in Section 6.2.2.c.

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 demonstrate that other approaches to initial DOC are adequate.

- a) The target organism(s)/analyte(s) of interest must be diluted in a volume of matrix appropriate for use and the method. Concurrently prepare at least four aliquots according to

- the method at a concentration in the countable range for plate methods or working range for most probable number (MPN) type methods.
- b) For qualitative tests, acceptable performance in a blind study, either internally or externally generated, may be used to meet this requirement, provided that the study consists of a minimum of a blank, a negative culture, and a positive culture for each target organism(s)/analyte(s) of interest.
 - c) Aliquots must be analyzed concurrently according to the method.
 - d) 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(s)/analyte(s) of interest. When it is not possible to determine mean and standard deviations, such as for presence/absence, the laboratory must assess performance against established criteria. In the absence of applicable method or regulatory requirements, the laboratory must define acceptance criteria.
 - e) Compare the information from d) above to the corresponding acceptance 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 any of the acceptance criteria, the analyst must proceed according to i) or ii) below.
 - i. Locate and correct the source of the problem and repeat the initial DOC for all organism(s)/analyte(s) of interest beginning with a) 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/analyte(s) of interest beginning with a) above.

6.3 Ongoing DOC

The laboratory must have a demonstrated procedure describing satisfactory ongoing DOC that includes how the laboratory will identify data associated with ongoing DOCs. The analyst(s) must demonstrate ongoing capability by routinely meeting the QC 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-month period, an initial DOC (Section 6.2) must be performed prior to performing analysis. This ongoing DOC must be one of the following:

- a) Performance of another initial DOC.
- b) Analysis of one sample of matrix appropriate for use and the method that is fortified with a known quantity of the target organism(s)/analyte(s) of interest, 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.
- c) Analysis of one positive control in duplicate for each target organism(s)/analyte(s) of interest and test, with results meeting acceptance criterion for precision. In the absence of applicable method or regulatory requirements, the laboratory must define acceptance criteria.
- d) Acceptable results for a blind proficiency test sample or sample set, as required by the program, for target organism(s)/analyte(s) of interest in each field of accreditation.
- e) A demonstrated procedure for reviewing QC samples performed by an analyst, or groups of analysts, relative to the QC 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.
- f) The analysis of positive real-world samples with results against established criteria. In the absence of applicable method or regulatory requirements, the laboratory must define acceptance criteria.

7.0 Technical Requirements

Unless otherwise specified, accreditation is not required for support analyses, such as those to ensure media, reagents, water, and supplies meet the applicable reference method and TNI requirements.

7.1 Calibration - For instruments that are continuous monitors, such as in-line specific conductance meters:

- a) the laboratory must demonstrate acceptable calibration verification monthly;
- b) an initial calibration must be performed if a calibration verification is unacceptable, or when the instrument is being returned to service after having been taken off-line.

7.2 Quality Control

7.2.1 Quality Control of Standards, Reagents, Materials, and Media

The laboratory performing the sample analysis must perform and record the quality control of standards, reagents, materials, and media used as appropriate for the analytical method except where noted.

7.2.1.1 Sterility Checks – Each lot of media, materials and supplies purchased, provided and used by the laboratory must be checked to demonstrate the absence of organism(s)/analytes of interest. Sterility checks meet the requirement to demonstrate the absence of organism(s)/analytes of interest.

When materials and supplies have been purchased by the laboratory pre-sterilized and a method blank utilizing the item(s) is analyzed prior to or in conjunction with first use, a Certificate of Analysis may be used to satisfy the sterility check. Materials and supplies which have been sterilized in the laboratory must be tested to verify sterility once per batch or lot. The materials and supplies to be checked include, but are not limited to: media, filter funnels, sample containers, dilution water, buffers, and membrane filters.

When the laboratory performs sterility checks:

- a) Chromo/fluorogenic media must be tested with sterile reagent water (as defined in 7.2.1.4.a).
- b) Each type of media must be incubated uninoculated using appropriate incubation time and temperature per each method.
- c) For each sterilization batch performed by the laboratory, one representative item of similar size, material type and use must be tested.
- d) Non-selective, single strength growth media must be used for sterility checks as appropriate for the item under test. The concentration of non-selective growth media must be single strength after the addition of a liquid item (e.g., dilution water, buffers, etc.).

7.2.1.2 Performance Checks- All test reagents (as defined in 3.0) must be checked by the laboratory for satisfactory performance once per purchased lot or prepared batch prior to or in conjunction with first use.

- a) Each test reagent must be analyzed with a positive control which verifies that the media/reagent produces the correct response. For selective media, the positive must be a known which is pure (single organism/analyte of interest) and produce typical results. Non-selective media is used to determine that growth is possible and therefore evidence of growth is deemed the correct response. As there is no specific target organism for non-selective media, any organism that produces evidence of growth, such as turbidity or colonies, would be deemed appropriate.
- b) Each selective test reagent must be analyzed with a known negative control. A negative control demonstrates that the medium or test reagent does not support the growth of non-

- target organism(s)/analyte(s) of interest or does not exhibit the typical positive reaction of the target organism(s)/analyte(s) of interest.
- c) When microorganisms are used for positive and negative controls for selective media and/or selective test reagents, the laboratory must use reference cultures traceable to national or international sources. Microorganisms may be single-use preparations or cultures maintained for their intended use by demonstrated procedures that demonstrate the continued purity and viability of the organism.
 - i. Reference cultures, once prepared, may be sub-cultured once to provide reference stocks. The reference stocks must be preserved by a technique that maintains the characteristics of the strains. Working stocks must be prepared from reference stocks. If reference stocks have been thawed, they must not be refrozen and re-used.
 - ii. Working stocks must not be sequentially cultured more than five times. Each sequential culture must not be used beyond 31 days. Working stocks must not be sub-cultured to replace reference stocks.
 - d) To ensure accurate results, target organism identity must be verified as specified in the method (e.g., by use of the completed test, secondary verification tests such as a catalase test, or a selective medium such as Brilliant Green Lactose Bile Broth (BGLB) or EC or EC + MUG broth).
 - e) When prepared in the lab, the laboratory must verify and record the final pH of all media. For media purchased by the laboratory, the laboratory must review the certificate of analysis to verify that the pH is suitable for the method.
- 7.2.1.3 Water types used in testing may include buffer water, peptone water, rinse water and/or reagent water.
- a) For all laboratory-prepared water types used in testing that have a regulation-, method-, or standard-defined acceptable pH, the laboratory must verify and record the final pH prior to first use.
 - b) When types of water used in testing are purchased by the laboratory, the laboratory must review the certificate of analysis to verify that the pH is suitable for the method prior to first use. If the certificate of analysis does not include pH, the laboratory must verify and record the pH prior to first use.
 - c) When types of water used in testing are also purchased or prepared in at-use volume for dilutions, the laboratory must verify and record each working volume once per lot or prepared batch prior to first use.
- 7.2.1.4 Reagent Water
- a) Laboratory reagent water must be distilled, deionized, or reverse-osmosis-produced water prepared in the laboratory or purchased from an outside source. The laboratory must monitor the quality of the reagent water used in testing.
 - i. The laboratory must monitor the quality of the water for disinfectant residual, conductivity, total organic carbon (TOC), 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.
 - ii. The laboratory must monitor the quality of the water for Cd, Cr, Cu, Ni, Pb, and Zn annually.
 - b) Because the supporting tests specified in 7.2.1.4 a) i. and ii. above are not for compliance testing, the laboratory does not need to be accredited for these tests provided these tests follow the relevant sections of Module 2, the reference method, and/or relevant state or federal regulatory requirements.
 - c) Each reagent water quality parameter which the laboratory subcontracts must be analyzed by a laboratory accredited to this Standard or by a laboratory that meets applicable statutory and regulatory requirements for performing the tests and submitting the results of tests performed, as applicable.

- d) Results of the above analyses must meet applicable method or regulatory requirements. In the absence of applicable method or regulatory requirements, the laboratory must define acceptance criteria.
 - e) Once opened, container(s) of purchased reagent water in use must be retested at the frequency outlined in Sections 7.2.1.4 a) i. and ii. above. The laboratory must ensure that each lot of purchased reagent water meets applicable method or regulatory requirements. In the absence of applicable method or regulatory requirements, the laboratory must define acceptance criteria.
- 7.2.1.5 With the exception of support measurements, the laboratory must not use standards, reagents, media or materials beyond the expiration date of the product, or as specified in the test method. This requirement is more stringent than that found in TNI Module 2, Section 6.6.4.4.
- 7.2.2 Method Blanks
- The laboratory must demonstrate that the filtration equipment and filters, sample containers, media, and reagents have not been contaminated through improper handling or preparation, or environmental exposure.
- 7.2.2.1 For filtration technique, the laboratory must conduct method blanks per the analytical method. The analysis may utilize a filter funnel manifold with single or multiple vacuum supply ports/positions. At a minimum, the filtration series must include a beginning and ending blank for each manifold port/position used. In addition, the laboratory must insert a method blank after every ten samples filtered per port/position unless the laboratory uses single-use funnel sets or sanitizes filtration units by UV light (254-nm) after sample filtration.
- All filtration units in a filtration series must have been sterilized prior to beginning the series. During a filtration series, filter funnels must be rinsed with three 20-30 mL portions of sterile rinse water after each sample filtration. The filtration series is considered ended when more than thirty minutes elapses between successive filtrations.
- 7.2.2.2 A method blank for pour plate media must be made by pouring one uninoculated plate for each lot of pre-prepared, ready-to-use media and for each batch of medium prepared in the laboratory.
- 7.2.3 Test Variability/Reproducibility
- Duplicate counts must be performed on all methods that result in a quantitative value. The count must be performed on a positive sample during each month that the test is performed. These counts may be performed on environmental samples or quality control samples.
- 7.2.3.1 When a laboratory has multiple analysts, all analysts who perform counting during a given month must perform a count on the same sample as another analyst. There must be no more than ten percent difference between any two analysts.
- 7.2.3.2 In a laboratory with one analyst, the sample must be counted twice by the analyst with no more than five percent difference.
- 7.2.3.3 If the difference exceeds the requirements of Sections 7.2.3.1 or 7.2.3.2, corrective measures must be identified and implemented.
- 7.2.3.4 The laboratory must document how acceptability is evaluated.

7.2.4 Constant and Consistent Test Conditions

7.2.4.1 Laboratory Facilities

Floors and work surfaces must be non-absorbent and easy to clean and disinfect. Work surfaces must be adequately sealed. Laboratories must provide sufficient storage space, and must be clean and free from dust accumulation.

7.2.4.2 Laboratory Equipment- laboratory equipment must be verified as per the appropriate section of Module 2 except where noted.

- a) Temperature Measuring Devices - The laboratory must use temperature measuring devices such as liquid-in-glass thermometers, thermocouples, or platinum-resistance thermometers to verify equipment temperatures. The temperature measuring devices must be of appropriate accuracy, range, graduation and/or resolution to meet specification(s) in the method.
- b) Sterilization Equipment
 - i. Autoclaves
 1. The laboratory must evaluate the performance of each autoclave initially by establishing its functional properties and performance, for example, heat distribution characteristics with respect to typical uses. Autoclaves must meet specified temperature tolerances. Pressure cookers must not be used for sterilization of growth media.
 2. The laboratory must demonstrate proper sterilization temperature by use of a continuous temperature recording device or by use of a maximum registering thermometer with every cycle.
 3. Monthly, when the autoclave is in use, the laboratory must demonstrate effective sterilization with use of appropriate biological indicators. The incubation time and temperature of the biological indicators must follow the manufacturer instructions.
 4. The laboratory must use an appropriate indicator, such as temperature-sensitive tape, with the contents of each autoclave sterilization cycle to indicate that the autoclave contents have been processed.
 5. The laboratory must maintain records of autoclave operations for every cycle. Records must include: date, contents, maximum temperature reached, pressure, sterilization cycle time elapsed, total run time (may be recorded as time in and time out), and analyst's initials.
 6. Autoclave maintenance, internally or by service contract, must be performed annually, and must include a pressure check and verification of temperature device. Records of the maintenance must be maintained. If the temperature is verified to be acceptable and it has been determined and recorded that the autoclave has no leaks, it is acceptable to state the pressure has been verified.
 7. When used to sterilize media, the laboratory must verify the autoclave timing device quarterly and record the actual sterilization cycle time elapsed. When discrepancies are identified, the laboratory must implement and record appropriate corrective actions.
 - ii. Ovens

Monthly, when the oven is used to sterilize, the laboratory must demonstrate effective sterilization with use of appropriate biological indicators. The incubation time and temperature of the biological indicators must follow the manufacturer instructions.

The laboratory must use an appropriate indicator, such as temperature sensitive tape, with the contents of each oven sterilization cycle to indicate that the contents have been processed.

Ovens must be preheated to temperature of use prior to loading. The laboratory must maintain records for each cycle that include date, sterilization cycle time elapsed, temperature, contents, and analyst's initials.

- c) Volumetric Equipment - The laboratory must verify the volumetric accuracy of equipment used for measuring volume. Verification must be either volumetric, as compared to Class A, or gravimetric. When neither of these methods are appropriate, it is the responsibility of the laboratory to demonstrate that other approaches to verification are at least equivalent. Verification of volume must be within 2.5% of expected volume to be considered acceptable for volumetric accuracy.
- d) UV Instruments - The laboratory must evaluate UV instruments used for sanitization quarterly for effectiveness with an appropriate UV light meter, by plate count, agar spread plates, or other methods providing equivalent results, such as UV-cide 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.
- e) Incubators and Water Baths
 - i. The laboratory must establish the uniformity of temperature distribution conditions in incubators and water baths prior to first use and after service to check for areas of temperature nonconformance. When such areas are identified, the laboratory must implement and record appropriate corrective actions.
 - ii. During periods when samples are under test, the laboratory must have a system in place to monitor and record the temperature of incubators and water baths twice daily, at least four hours apart. "Under test" is defined as the time period that the sample is in the incubation phase of the method. Data loggers, continuous temperature monitoring devices, or other temperature monitoring equipment can be used as long as they are calibrated in accordance with TNI Module 2, Section 6.4.6.1.

NOTE: There is no intent to take the temperature of incubation units during periods when there are no samples under test.

- f) Labware
 - i. The laboratory must have a demonstrated procedure for washing labware, if applicable. When washing labware, the detergent used must be designed for laboratory use.
 - ii. Glassware must be made of borosilicate or other non-corrosive material, free of chips and cracks, and must have readable measurement marks.
 - iii. Labware that is washed and reused must 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 laboratory changes the detergent formulation or washing procedures.
 - iv. One representative piece of labware from each batch that is washed in the laboratory must be tested for possible acid or alkaline residue using a suitable pH indicator, such as bromothymol blue.

7.3 Sample Handling

7.3.1 Samples which require thermal preservation but do not meet the maximum temperature requirement may be considered acceptable if appropriate evidence of sample cooling is present.

7.3.2 Microbiological samples from known chlorinated sources (such as wastewater effluent), unknown sources where disinfectant (e.g. chlorine) usage is suspected (such as a new client or a new source), and all potable water supplies (including source water) must be checked for absence of disinfectant residual in the laboratory. Alternatively, the laboratory does not need to test as above if all of the following are met:

- a) The laboratory can show that the received sample containers are from its laboratory or have been appropriately verified to have contained sodium thiosulfate;

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- b) 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;
 - c) 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 recorded;
 - d) Disinfectant residual is checked in the field and actual concentration is recorded with sample submission.

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