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- FOR COMMENT

ENVIRONMENTAL LABORATORY SECTOR

VOLUME 1

MANAGEMENT AND TECHNICAL REQUIREMENTS FOR LABORATORIES PERFORMING ENVIRONMENTAL ANALYSIS

Module 5: Quality Management 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) Microbiology and Quality Systems Expert Committees. 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 Systems General Requirements, and may be used by any organization that wishes to implement a program for the accreditation of environmental laboratories.

Standard Revision History

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VOLUME 1, MODULE 5
Quality Management Systems for Microbiological Testing

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VOLUME 1, MODULE 5
Quality Management Systems for Microbiological Testing

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 the 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 shall demonstrate that such requirements are met. If it is not clear which requirements are more stringent, the standard from the method or regulation is to be followed. Records must be retained by the laboratory in accordance with TNI Module 2, Section 5.4.6.2.

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 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.

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.

3.2 Exclusions and Exceptions

Reserved

4.0 Technical Specialist Requirements

4.1 Any technical specialist responsible for microbiological testing must meet the requirements of TNI Module 2, Section 6.2.2.2. The following requirements must also be met:

a) an earned bachelor's degree in microbiological sciences, biological sciences, chemistry, environmental sciences, physical sciences, biochemical engineering, molecular biology engineering, or equivalent scientific discipline;

b) successful completion of one (1) college-level microbiology course that includes a laboratory component

5.0 Method Selection

Refer to TNI Module 2, Sections 5.4.2, 5.4.3, and 5.4.4.

6.0 Validation and/or Verification of Methods

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

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

6.2.1 Accuracy – Use at least one (1) 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. 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.

6.2.2 Precision – Perform at least ten (10) replicate analyses with both the proposed and reference method, using a sample containing the target microorganisms of choice. The results must show that the precision of the proposed method is statistically equivalent or better than that of the reference method.

6.2.3 Selectivity (sensitivity) – Verify all responses, using microbial identification testing or equivalent processes, in at least ten (10) samples of mixed cultures which include the target organism(s) at varying concentrations. Calculate the number of false positive and false negative results.

6.3 For both reference and non-reference methods, laboratories must participate in proficiency testing (PT) programs, where available.

6.4 The laboratory must maintain documentation for as long as the method is in use, and for at least five (5) years past the date of last use.

7.0 Demonstration of Capability (DOC)

7.1 General

7.1.1 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 7.2).

7.1.2 For each individual who performs any activity involved with preparation and/or analysis of samples, an ongoing DOC (see Section 7.3), must be performed and documented annually.

7.1.3 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 (1) 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.

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

7.2 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 (12) month period.

7.2.1 The laboratory must document 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) organism(s);

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 7.2.2.c.

7.2.2 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.

a) The target organism(s) must be diluted in a volume of matrix appropriate for use. Prepare at least four (4) aliquots at a concentration of a countable range for plate methods or working range for most probable number (MPN) type methods.

b) At least four (4) aliquots must be prepared and analyzed concurrently according to the method.

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 must 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.

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 must 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) above.

7.3 Ongoing DOC

7.3.1 The laboratory must have a documented 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 (12) month period, an initial DOC (Section 7.2) must be performed prior to performing analysis.

NOTE: This ongoing demonstration may include, but is not limited to, any one of the following:

a) Performance of another initial DOC

b) Analysis of one (1) sample of matrix appropriate for use 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.

c) Analysis of one (1) positive sample in duplicate for each target organism and test, with results meeting the laboratory acceptance criterion for precision.

d) Acceptable results for a blind proficiency test sample or sample set, as required by the program, for target organisms in each field of accreditation.

e) A documented 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 real-world samples with results within predefined acceptance criteria (as defined by the laboratory or method).

g) If the laboratory uses an alternate procedure for an ongoing demonstration of capability, the procedure and acceptance criteria must be documented.

8.0 Technical Requirements

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

8.1 Calibration

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

a) the laboratory must document acceptable calibration verification at least once a month;

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

8.2 Quality Control

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

The laboratory performing the sample analysis, except where specified in Section 8.2.1.4 b) and Section 8.2.1.4 c), must perform and document the quality control of standards, reagents, materials, and media used as appropriate for the analytical method.

8.2.1.1 Sterility Checks – All materials and supplies that are needed to process the sample and are required to be sterile must be checked by the laboratory once per purchased lot or prepared batch prior to or in conjunction with first use. The materials to be checked must include, but are not limited to: media, filter funnels, sample containers, dilution water, buffers, and membrane filters.

a) One item from each purchased pre-sterilized lot, or one item or object representative in size and use per sterilization batch sterilized by the laboratory, must be tested.

b) Non-selective, single strength growth media must be used 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).

c) Chromo/fluorogenic media must be tested with sterile deionized water.

d) Where media are made as concentrates (e.g., double strength), the media must be diluted to working strength with sterile deionized water before testing.

e) All media must be incubated uninoculated using appropriate incubation time and temperature.

f) Certificates of analysis provided by vendors must be verified by the laboratory and retained in accordance with TNI Module 2 Section 5.6.4.2.a.

8.2.1.2 Performance Checks- All test reagents 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 one (1) known negative and one (1) pure (single organism/analyte of interest) positive control as appropriate to the method and produce typical results. The laboratory must have a procedure for this testing. 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.

b) When microorganisms are used for positive and negative controls, the laboratory must use reference cultures that have been obtained from a recognized national collection, organization, or a 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, 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 (5) times. Each sequential culture must not be used beyond 31 days. Working stocks must not be sub-cultured to replace reference stocks.

c) 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).

d) The laboratory must verify and document the final pH of all media.

8.2.1.3 Dilution water, however used, includes buffer water, peptone water, rinse water and/or reagent-free water. The laboratory must verify the volume once per lot or prepared batch of dilution water prepared in specific volumes. The laboratory must verify the final pH of all dilution water. These verifications must take place prior to first use.

8.2.1.4 Reagent Water

a) The laboratory must monitor the quality of the reagent water used in the laboratory, including reagent water purchased from an outside source, which will come into contact with test organisms and is used in preparation of media, solutions, and buffers, for bactericidal and inhibitory substances. The water must be distilled water, deionized water, or reverse-osmosis-produced water.

b) The laboratory must monitor the quality of the water for disinfectant residual, conductivity, 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. If the laboratory performs these tests internally for the purpose of reagent water quality monitoring, the laboratory does not need to be accredited for these tests. When the laboratory subcontracts work, this work must be placed with a laboratory accredited to this Standard for the tests to be performed or with a laboratory that meets applicable statutory and regulatory requirements for performing the tests and submitting the results of tests performed.

c) The laboratory must monitor the quality of the water for Cd, Cr, Cu, Ni, Pb, and Zn annually. When the laboratory subcontracts work, this work must be placed with a laboratory accredited to this Standard for the tests to be performed or with a laboratory that meets applicable statutory and regulatory requirements for performing the tests and submitting the results of tests performed.

d) Results of the above analyses must meet method or regulatory specifications. In the absence of method or regulatory specifications, the laboratory must define acceptance criteria.

f) Once opened, container(s) of purchased reagent water in use must be retested at the frequency outlined above.

8.2.1.5 The laboratory must not use standards, reagents, materials, and media whether prepared by the laboratory or purchased from a vendor, beyond the expiration date of the product, or as specified in the accredited method. This language is more stringent than that found in TNI Module 2, Section 6.6.4.4.

8.2.2 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.

8.2.2.1 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 (10) 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.

8.2.2.2 A filtration series must include filtration units that have been sterilized prior to beginning the series. During a filtration series, filter funnels must be rinsed with three (3) 20-30 mL portions of sterile rinse water after each sample filtration. The filtration series is considered ended when more than thirty (30) minutes elapses between successive filtrations.

8.2.2.3 For pour plate technique, method blanks of the medium must be made by pouring, at a minimum, one (1) uninoculated plate for each lot of pre-prepared, ready-to-use media and for each batch of medium prepared in the laboratory.

8.2.3 Test Variability/Reproducibility

For all methods that specify a quantitative result, a duplicate count must be performed monthly on at least one (1) positive sample for each month that the test is performed. These counts may be performed on environmental samples or quality control samples. If the laboratory has multiple analysts, each analyst must perform a count a sample that has also been counted by another analyst. The difference between the counts must be no more than ten percent (10%) or corrective action must be taken. In a laboratory with only one (1) analyst, the same sample must be counted twice by the analyst, with no more than a five percent (5%) difference between the counts or corrective action must be taken.

8.2.4 Data Reduction

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

8.2.5 Constant and Consistent Test Conditions

8.2.5.1 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.

8.2.5.2 Laboratory Equipment

a) Temperature Measuring Devices

The laboratory must use temperature measuring devices such as liquid-in-glass thermometers, thermocouples, or platinum-resistance thermometers to assess and document equipment temperatures. The temperature measuring devices must be appropriate quality to meet specification(s) in the method. The graduation and range of the temperature measuring devices must be appropriate for the required accuracy of the measurement. Verification must be performed as per TNI Module 2, Section 5.5.13.1.

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. The laboratory must, at least once during each month that the autoclave is used, demonstrate the effective sterilization with use of appropriate biological indicators. The laboratory must use temperature-sensitive tape with the contents of each autoclave run to indicate that the autoclave contents have been processed.

3. The laboratory must maintain records of autoclave operations for every cycle. Records must 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.

4. 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 in equipment logs. If the temperature is verified to be acceptable and it has been determined and documented that the autoclave has no leaks, it is acceptable to state the pressure has been verified.

5. The laboratory must verify the autoclave timing device quarterly and document the actual time elapsed. When discrepancies are identified, the laboratory must implement and document appropriate corrective actions.

ii. Ovens

At least once during each month that an oven is used to sterilize, the laboratory must demonstrate the effective sterilization with use of appropriate biological indicators. The laboratory must maintain records for each cycle that include date, cycle time, temperature, contents, and analyst's initials. The laboratory must use temperature sensitive tape with the contents of each run to indicate that the contents have been processed.

c) Volumetric Equipment

The laboratory must verify equipment used for measuring volume. Class A glassware are exempt from any verification requirements. 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 document that other approaches to verification are at least equivalent. In addition to the requirements in Module 2, the below requirements must be met:

i. Reusable volumetric equipment, such as filter funnels, bottles, and non-Class A glassware must be verified prior to first use.

ii. Disposable volumetric equipment, such as filter funnels, sample bottles, sample analysis vessels, and disposable pipettes must be checked once per lot prior to first use.

iii. Verification of volume must be considered acceptable if the accuracy is within 2.5% of expected volume.

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, Water Baths

i. The laboratory must establish the uniformity of temperature distribution conditions in incubators and water baths prior to first use after installation or service to check for areas of temperature nonconformance. When such areas are identified, the laboratory must implement and document appropriate corrective actions.

ii. During periods when samples are under test, the laboratory must have a system in place to monitor and document the temperature of incubators and water baths twice daily, at least four (4) 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 can be calibrated in accordance with TNI Module 2, Section 5.5.13.1 for Support Equipment.

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

f) Labware (Glassware and Plasticware)

i. The laboratory must have a documented procedure for washing labware, if applicable. If used, these detergents 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. Washed labware must be tested at least once daily, each day of washing, for possible acid or alkaline residue by testing at least one (1) piece of labware with a suitable pH indicator such as bromothymol blue.

8.3 Data Acceptance/Rejection Criteria

Methods criteria and evaluation methods must be used.

8.4 Sample Handling

Receipt of samples must comply with TNI Module 2, Sections 5.8.6 and 5.8.7, as well as:

8.4.1 If the arrival temperature of a representative sample container meets the method or regulatory temperature requirement, the sample shall be considered acceptable.

8.4.2 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 the below exemptions are met:

a) The laboratory can show that the received sample containers are from its laboratory or have been appropriately tested and documented;

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 (1) 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;

d) Disinfectant residual is checked in the field and actual concentration is documented with sample submission.