



ENVIRONMENTAL LABORATORY SECTOR

This VDS is a proposed revision of the 2012 Standard (EL-V1M5-2012). It has been prepared by the Microbiology Expert Committee. A version with track changes turned on is available from Ilona Taunton by request (ilona.taunton@nelac-institute.org).

VOLUME 1

MANAGEMENT AND TECHNICAL REQUIREMENTS FOR LABORATORIES PERFORMING ENVIRONMENTAL ANALYSIS

Module 5: Quality Systems for Microbiological Testing

TNI Standard

**P.O. Box 2439
Weatherford, TX 76086
817-598-1624
www.nelac-institute.org**

This page intentionally left blank.

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.

Standard Revision History

Action	Date
Working Draft Standard Published	January 14, 2007
Voting Draft Standard Published	June 15, 2007
Draft Interim Standard Published	December 15, 2007
Approved by Quality Systems Committee	December 22, 2007
Modified by Editorial Changes	March 12, 2009
Adopted by NELAP Board	September 8, 2009
Scheduled for Implementation by NELAP	July 1, 2011
Working Draft Standard Published	July 3, 2014
Modified Working Draft Standard Published	November 21, 2014
Voting Draft Standard Published	May 20, 2015

This page intentionally left blank.

VOLUME 1, MODULE 5
Quality Systems for Microbiological Testing

Table of Contents

1.0	MICROBIOLOGICAL TESTING	1
1.1	Introduction	1
1.2	Scope	1
1.3	Terms and Definitions	1
1.3.1	Additional Terms and Definitions	1
1.3.2	Exclusions and Exceptions	1
1.4	Method Selection	1
1.5	Method Validation	1
1.5.1	Accuracy	1
1.5.2	Precision	2
1.5.3	Selectivity (sensitivity)	2
1.6	Demonstration of Capability (DOC).....	2
1.6.1	General	2
1.6.2	Initial DOC.....	2
1.6.3	Ongoing DOC	3
1.7	Technical Requirements.....	4
1.7.1	Calibration	4
1.7.2	Continuing Calibration.....	4
1.7.3	Quality Control	4
1.7.4	Data Acceptance/Rejection Criteria	10
1.7.5	Sample Handling.....	10

This page intentionally left blank.

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 the general requirements module. Adherence to those quality system requirements and all quality control procedures specified in this module will ensure that microbiological test results are fit for the intended use.

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

- a. For methods other than reference methods, validation must comply with Volume 1, Module 2. This validation must include the minimum requirements outlined in Sections 1.5.1, 1.5.2, and 1.5.3 of this module.
- b. For both reference and non-standard methods, laboratories shall participate in proficiency testing programs, where available.
- c. 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.

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

- 1.5.2 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 shall show that the precision of the proposed method is statistically equivalent or better than that of the reference method.
- 1.5.3 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), must be performed and documented at least every twelve (12) months.
- 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 (1) year prior to applying for accreditation and where 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.
- 1.6.1.4 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 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 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.
- 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) above.

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 (Section 1.6.2) shall be performed prior to performing analysis. 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 (1) sample of 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 (1) positive sample in duplicate for each target organism and test, with results meeting the laboratory acceptance criterion for precision.
 - c. Acceptable results for a blind proficiency test sample or sample set, as required by the program, 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.
- 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

1.7.1.1 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.

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

- a. The laboratory shall document acceptable calibration verification at least once a month.
- b. 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 demonstrate and document that the quality of the reagents and media used is appropriate for the test concerned including, but not limited to, test conditions and incubation times.

- 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 by the laboratory once per purchased or prepared lot using non-selective growth media as appropriate. Certificates of analysis provided by vendors shall be verified by the laboratory and retained in accordance with V1M2 5.6.4.2.a. These checks shall include, but are not limited to:
 - i. The laboratory shall perform a sterility check for each lot of prepared ready to use media and on 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. Where media are made as concentrates (e.g., double strength), then the medium shall be diluted to working strength with sterile deionized water before testing.

-
- ii. The laboratory shall perform a sterility check on one (1) funnel per lot of pre-sterilized single use funnels using non-selective growth media. The laboratory shall perform a sterility check on one (1) funnel per batch of laboratory-sterilized funnels, using non-selective growth media.
 - iii. The laboratory shall perform a sterility check on at least one (1) container for each lot of purchased, pre-sterilized sample containers with non-selective growth media. The laboratory shall perform a sterility check on one (1) container/object per sterilization batch sterilized in the laboratory with nonselective growth media.
 - iv. The laboratory shall perform a sterility check 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 dilution water.
 - v. The laboratory shall perform a sterility check on at least one (1) filter from each new lot of membrane filters with nonselective growth media.
- b. Media – Culture media may be prepared from commercial dehydrated powders or may be purchased ready-to-use.
- i. All media 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.
 - ii. The laboratory shall use all media within the expiration date or shelf life provided by the manufacturer.
 - iii. The laboratory shall use all laboratory-prepared media within the holding time limits specified in the accredited method.
 - iv. The laboratory shall have detailed testing criteria information defined in the laboratory's methods, SOPs, or similar documentation.
- c. The laboratory shall use reagents, media and commercial dehydrated powders within the shelf life of the product, and shall maintain documentation as per V1M2 Quality Systems: General Requirements. 5.6.4.2
- d. Reagent Water
- i. The laboratory shall monitor the quality of the reagent water used in the laboratory, which will come into contact with test organisms and is used in preparation of media, solutions, and buffers, for bactericidal and inhibitory substances. This water shall be distilled water, de-ionized water, or reverse-osmosis-produced water.
 - ii. The laboratory shall monitor the quality of the water 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. Analysis may be performed by another certified laboratory.
 - iii. The laboratory shall monitor the quality of the water 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) 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. Analysis may be performed by another certified laboratory.
- iv. Results of the above analyses shall meet the specifications of the required method. 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.
 - vi. Reagent water that has been opened 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. Dilution water, however used, includes buffer water and/or peptone water. The laboratory shall monitor the quality of the dilution water for sterility, pH and volume once per lot or batch whether purchased or lab prepared.
 - f. Documentation for media and reagents 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. Records shall be retained by the laboratory in accordance with V1M2 5.4.6.2.

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, 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 sample filtration.
- c. For pour plate technique, method blanks of the medium shall 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.

1.7.3.3 Test Variability/Reproducibility

For methods that specify counts (i.e. cfu/100mL or MPN/100mL) such as membrane filter, plated media or other methods which specify a quantitative result, duplicate counts shall be performed monthly on one (1) positive sample for each month that the test is performed. If the laboratory has two (2) or more analysts, each analyst shall count typical results on the same sample. Counts shall be within ten percent (10%) difference to be acceptable. In a laboratory with only one (1) microbiology analyst, the same sample shall be counted twice by the analyst, with no more than a five percent (5%) difference between the counts.

1.7.3.4 Sample Specific Controls (where applicable)

- a. The laboratory shall perform matrix spikes per method requirements.
- b. The laboratory shall perform sample matrix duplicates 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 once per lot or batch.
- 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 medium such as *Brilliant Green Lactose Bile Broth* (BGLB) or *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.e. working cultures)
 - 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 (1) 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 chromo/fluorogenic reagent) and each batch of medium prepared in the laboratory shall be tested with at least one (1) or more known pure positive culture controls (i.e. target organism) as appropriate to the method and that produce typical results based on 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.

b. Laboratory Equipment

i. Temperature Measuring Devices

The laboratory shall 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 shall be 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 laboratory shall 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 shall meet specified temperature tolerances. Pressure cookers shall not be used for sterilization of growth media.
- The laboratory shall demonstrate proper sterilization temperature by use of a continuous temperature recording device or by use of a maximum registering thermometer with every cycle. The lab shall at least once during each month that the autoclave is used, demonstrate the effective sterilization through the use of appropriate biological indicators. The selected biological indicator shall be effective at the sterilization temperature and time needed to sterilize lactose-based media. The laboratory shall use temperature sensitive tape with the contents of each autoclave run to indicate that the autoclave contents have been processed.
- The laboratory shall maintain records of autoclave operations 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, 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 laboratory shall check the autoclave mechanical timing device quarterly against a stopwatch and document the actual time elapsed.

2. Ovens

The laboratory shall check ovens used for sterilization for sterilization effectiveness monthly with appropriate biological indicators. The laboratory shall maintain records for each cycle that include date, cycle time, temperature, contents, and analyst's initials. The laboratory shall use temperature sensitive tape with the contents of each run to indicate that the contents have been processed.

iii. Volumetric Equipment

The laboratory shall verify equipment used for measuring volume 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 A 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

The laboratory shall 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.

v. Incubators, Water Baths

1. The laboratory shall establish the uniformity of temperature distribution and equilibrium conditions in incubators and water baths prior to first use after installation or service. The equilibrium check shall include time required after test sample addition to re-establish equilibrium conditions under full capacity load appropriate for the intended use.

2. During periods when samples are under test, the laboratory shall 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 V1M2 Section 5.5.13.1 for Support Equipment. Records shall be maintained in accordance with V1M2 4.13 Records Maintenance.

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

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 (1) 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

Receipt of samples must comply with V1M2 Sections 5.8.6 and 5.8.7, as well as:

- 1.7.5.1 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. Samples that are delivered to the laboratory on the same day they are collected may not meet the requirements of this section or the method or the regulatory requirement. In these cases, the samples **may** be considered acceptable if the samples are received on ice with evidence that the cooling process has begun.

NOTE: The intent is for the samples to be preserved immediately and analyzed as soon as possible.

- 1.7.5.2 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:

- 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 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. Chlorine residual is checked in the field and actual concentration is documented with sample submission.