EL-V1M2-Revised DRAFT-Rev 3.0: Summary of Changes

	Original Text	Suggested Change	Justification	Comments
				Summary of Changes made since the last DRAFT.
				Leading 1 (one) of numbering was removed from all sections for all Modules. For example, '1.2 Scope' became '2.0 Scope' and '1.3.1 Additional Terms and Definitions' became '3.1 Additional Terms and Definitions'.
				'Shall' was changed to 'must' in all Modules.
1	**1.2 Scope** The essential QC procedures applicable to microbiological analysis are included in this module. Additional QC or program requirements that are either specified by method, regulation or project shall be met by laboratories.	**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.	Current language repeatedly states these two requirements. It is clearer and more concise to state this in an overarching section of the module.	
2	**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.	**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.	The definition 'Test Reagent' has been added here as it will be used in the performance testing section. The definition clarifies that performance testing applies to more than media, and the definition is inclusive of non-organism analytes such as endotoxins.	

3	N/A	**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	This section was added to include any Technical Specialist requirements beyond what is required in Module 2, if any. The currently drafted language is not final and pending the completion of the Technical Specialist language in Module 2.	All subsequent section numbers were increased by one (1) due to the addition of this section.
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 (PT) 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	**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	• The updated header language is consistent with Module 4 DS language. Removed duplicative language to that added in Scope. • Language was updated to better reflect when verification is required versus validation and to improve clarity and consistency. • Updated language to 'reference methods' and 'non-reference methods' to improve clarity and consistency. • Language added to the accuracy section regarding positive controls is inclusive of non-organism analytes. • Updated language regarding concentration more clearly states the intent of the section.	

	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	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.	• Selectivity language updated to improve readability. • Accuracy, precision and selectivity requirements were placed directly under the applicable section on non-reference methods rather than having them placed elsewhere and citing them.	
5	**1.6.1.2** Thereafter, ongoing DOC (Section 1.6.3), must be performed and documented at least every twelve (12) months.	**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.	This language is consistent with 7.1.1, and use of the word 'annually' is consistent with glossary language.	
6	**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. **a)** 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	**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.	Changes were made in response to non-valid SIR 488, which pointed out a lack of clarity with regards to what matrix the Standard requires for initial DOCs (the Matrix definition, the Quality System Matrix definition, or the field of accreditation matrix on the laboratory's scope).	

	for plate methods or working range for most probable number (MPN) type methods.			
7	**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 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 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	**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,	• Updated and rearranged language to make it clearer that any of the listed options are acceptable, and that the list is not all-encompassing. • Updated language regarding matrix to be consistent with similar language in the Initial DOC section in response to non-valid SIR 488.	

	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 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)** 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.	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	**1.7 Technical Requirements**	**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.	Added language to clarify that laboratories do not need to be accredited for internal QC testing (aka support analyses).	
9	**1.7.1 Calibration** **1.7.1.1** The laboratory shall have documented procedures for calibration, verification, and QC 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.	**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.	Removed language redundant to Scope and Module 2.	

10	**1.7.2 Continuing Calibration** Reserved for specific procedures.		Removed language, as the Scope already states that method requirements must be followed.	
11	**1.7.3.1 Quality, Selectivity, and Sterility of Standards, Reagents, Materials, and Media** The laboratory performing the sample analysis, except where specified in Section 1.7.3.1.d.ii and Section 1.7.3.1.d.iii, shall perform and document the quality of the reagents and media used as appropriate for the analytical method.	**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.	Updated language to improve clarity and consistency.	The language regarding 'the laboratory performing the sample analysis' already existed in the Draft Standard and was not changed in the Revised Draft Standard. This language prevents future SIRs like 423 and 425, which inquire about quality control testing by the laboratory of use.
12	**1.7.3.1.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 laboratory 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. **a.** For chromo/fluorogenic media: add media to sterile deionized water and incubate at the appropriate temperature and time. **b.** For all other media, incubate un-inocculated 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-	**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.	• Updated and rearranged language to reduce redundancy and improve readability. Addressed non-valid SIR 484 regarding confusion over the language 'These checks shall include but are not limited to' by replacing with 'The materials to be checked must include, but are not limited to'.	

	sterilized single use funnels using nonselective growth media. The laboratory shall perform a sterility check on one (1) funnel/object per sterilization batch sterilized in the laboratory with 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 non-selective 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 non-selective growth media.	**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.		
13	**1.7.3.1.b) Media** – Culture media may be prepared from commercial dehydrated powders or may be purchased ready-to-use. **i.** All media shall be tested prior to or at minimum in conjunction with first use for sterility following Section 1.7.3.1. **ii.** All media shall be tested prior to or at minimum in conjunction with first use for selectivity to ensure the target organism(s) respond in an acceptable and predictable manner once per lot or batch. For selectivity the laboratory shall: **a)** Ensure that results are accurate, target organism identity shall be verified as specified in the method	**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	• Changed name of section to 'Performance Checks' to better reflect the information in the section, as it applies to more than just media. • Utilized the term 'Test Reagents' to reflect that the section applies to all substances used to identify, analyze or quantify the target organism(s)/analyte(s) of interest. • Removed redundant language.	

	(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). **b)** Ensure identity and traceability, by utilizing reference cultures used as positive and negative controls, 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. **8.** 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. **iii**. 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). Positive culture controls demonstrate that	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.	• Updated and rearranged language to improve readability. • Removed description of positive control as it was already described elsewhere. • Updated language regarding negative controls is inclusive of non-organism analytes. • Removed ‘or more’ and ‘at least’ language in response to SIR 494 and to make intent clearer. • Updated reference culture language to improve readability and consistency. Removed selectivity language, as earlier in the Standard it was made clear that selectivity testing requirements apply only to non-reference method validation. • Added requirement that a working stock subculture must not be used beyond 31 days, as this is standard good lab practice and integral to organism integrity. • Moved the requirement to verify and document final pH to this section. Moved expiration date and shelf-life requirements to a later section and combined with redundant language.	

	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). Each pre-prepared, ready-to-use lot of selective medium (including chromoflourogenic reagent) and each batch of selective medium prepared in the laboratory, shall be analyzed with one (1) or more known negative culture control(s) (i.e., non-target organisms) and at least one (1) or more known pure positive culture control(s) (i.e., target organism), as appropriate to the method and that produce typical results based on the method. **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.			
14	**1.7.3.1.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.	**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.	• Added additional examples of dilution water to improve clarity. • Specified that volume checks are only required for dilution water prepared in specific volumes. • Clarified that final pH will be used for pH verification testing. Specified that testing of dilution water must be done prior to first use.	

15	**1.7.3.1.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. The water shall be distilled water, deionized water, or reverse-osmosis-produced water. **ii.** The laboratory shall 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. 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	**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.	• Updated and rearranged language to improve readability. • Removed redundant language. • Added language to clarify that laboratories do not need to be accredited for internal QC testing (aka support analyses), as this was the intent of the 2016 Standard Module 5 language. • Outsourcing language updated to language consistent with Module 2. • Removed requirement for Bacteriological Water Quality Test. Silica testing presents a challenge to meet this requirement, and other quality control testing required in this Standard will show whether reagent water is of an acceptable quality. Specified that if no method or regulatory specification exists for results of this testing, the laboratory must define acceptability criteria. This ensures that results are evaluated for suitability.	

	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.	**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. **e)** Once opened, container(s) of purchased reagent water in use must be retested at the frequency outlined above.		
16	**1.7.3.1.b) Media** **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. **1.7.3.1.c)** The laboratory shall use reagents, media and commercial dehydrated powders within the shelf-life of the product, and shall maintain documentation as per Volume1, Module2 Quality Systems: General Requirements, Section 5.6.4.2.	**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.	Updated language based on changes being made to Module 2.	
17	**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. The filtration series may include single or multiple filtration units, which have been sterilized prior to beginning the series. At a minimum, the filtration series shall include a beginning and ending blank for each filtration unit. **b)** The filtration series is considered ended when more than thirty (30) minutes elapses between successive filtrations. During a filtration series, filter funnels	**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	Updated language to be consistent with implementation guidance documentation on filtration method blanks.	

	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.	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.		
18	**1.7.3.3 Test Variability/Reproducibility** For all methods that specify a quantitative result, duplicate counts must be performed monthly on 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, all analysts must count results on the same sample, when possible, with no more than ten percent (10%) difference between the counts. In a laboratory with only one (1) analyst, the same sample shall be counted twice by the analyst, with no more than a five percent (5%) difference between the counts.	**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.	• Updated language to better reflect the intent of the Standard as well as allow for creativity by the laboratory in how they meet this requirement. Added language requiring corrective action to ensure that this requirement is meaningful.	
19	**1.7.3.4 Sample Specific Controls (where applicable)** **a)** The laboratory shall perform matrix spikes per method requirements.		Removed language, as the Scope already states that method requirements must be followed.	

	b) The laboratory shall perform sample matrix duplicates per method requirements			
20	**1.7.3.6.b.ii.a.2** 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 laboratory shall, at least once during each month that the autoclave is used, demonstrate the effective sterilization with 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	**8.2.5.2.b.i.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.	Removed language specific to using biological indicators effective for lactose-based media, as the Standard already states that appropriate biological indicators must be used.	
21	**1.7.3.6.b.ii.a.4**. 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.	**8.2.5.2.b.i.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.	In response to SIR 471, removed the formula for pressure under the Ideal Gas Law, as steam does not act as an ideal gas and the inclusion of the formula as an option for performing pressure checks was causing confusion.	
22	**1.7.3.6.b.ii.a.5** The laboratory shall check the autoclave mechanical timing device quarterly against a stopwatch and document the actual time elapsed.	**8.2.5.2.b.i.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	• Removed requirement that a stopwatch must be used to check timing to allow for creativity by the laboratory in how they meet this requirement. Added language requires corrective action if discrepancies are identified to ensure that this check is meaningful.	
23	**1.7.3.6.b.ii.b. Ovens**	**8.2.5.2.b.ii Ovens**	Reworded language to improve readability	

	The laboratory shall check ovens used to sterilize 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.	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.		
24	**1.7.3.6.b.iii Volumetric Equipment** The laboratory shall verify equipment used for measuring volume as follows: **a.** Equipment with movable parts, such as automatic dispensers, dispensers/diluters, and mechanical hand pipettes, shall be verified for accuracy quarterly. **b.** 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. **c.** The volume of the disposable volumetric equipment, such as sample bottles and disposable pipettes, shall be checked once per lot. **d.** 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.	**8.2.5.2.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.	• Addressed non-valid SIR 478 by adding language allowing for alternate approaches for volumetric verifications. • Removed language redundant with Module 2. Updated language to improve readability.	
25	**1.7.3.6.b.v. Incubators, Water Baths** **a**. 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	**8.2.5.2.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	• Updated language to make intent clearer. • Added language requires corrective action if discrepancies are identified to ensure that this check is meaningful.	

	sample addition to re-establish equilibrium conditions under full capacity load appropriate for the intended use. **b.** 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 Volume 1, Module 2, Section 5.5.13.1 for Support Equipment. Records shall be maintained in accordance with Volume 1, Module 2, Section 4.13: Records Maintenance	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.	• Removed requirement for equilibrium testing, as incubation times are mandated by the method and not altered based on equilibrium test results. Removed language redundant with Module 2.	
26	**1.7.3.6.b.vi Labware (Glassware and Plasticware)** **a.** The laboratory shall have a documented procedure for washing labware, if applicable. Detergents designed for laboratory use shall be used.	**8.2.5.2.f Labware (Glassware and Plasticware)** **i.** The laboratory shall have a documented procedure for washing labware, if applicable. Detergents designed for laboratory use shall be used.	Updated language to improve readability.	
27	**1.7.3.6.b.vi.d.** 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.	**8.2.5.2.f.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.	Removed language redundant with Module 2.	
28	**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	**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.	Update this language to remove ambiguity, redundancy with Module 2 and language that is not auditable.	

	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.			