NYSDOH ELAP MICROBIOLOGY CHECKLIST

This checklist incorporates references to both TNI 2016 Standards, where applicable, and specific method, state and / or federal regulatory requirements.

Directions: Place a mark (e.g., /, √ or X) in the appropriate column (Yes (Y), No (N), or Not Applicable (NA)). If it is an observation on areas for possible improvement, place a mark under the Suggestion (S) column. In database, use code "SGST."

Lab ID: _____ Assessment ID: _____

Lab Name: _____

Personnel Interviewed: Reports Reviewed:

_____ _____ _____

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At the time of the assessment, a question marked 'yes' indicates that no evidence of a deficiency was observed.

Assessment Date(s):_____ Assessor (Signature): _____

If this was a team assessment, indicate the Lead Assessor's name. _____

Microbiological Testing Detailed Method Review	Data Records observed	Comments
Method Number: **SOP Number:** **Rev.:** **SOP date:** **Personnel records observed:**		
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Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
The laboratory is in adherence to the Quality Control procedures and program requirements specified in the NELAC standard, method, regulation, and project.	M5,1.2					000d30	
SM9215A, 5 & 7-8: **Heterotrophic Plate Count** SM9215B: **Pour Plate Method**							
1__ All dilution plates analyzed in duplicate.						0d31a1	
2__ Incubated at 35.0 ± 0.5 ºC for 48 ± 3 hours.						0d31a2	
3__ Colonies counted with a dark-field colony counter, or one with equivalent magnification & illumination. (SM9215A, 8.a. & b.& ANSI/AAMI RD52:2004, 7.2.3)						0d31a3	
4__ Incubated at 35.0 +/- 0.5 degrees Celsius for 72 ± 4 hours for finished bottled water. (EPA 600/8-78-017, Part III, Sec. 5.5.2)						0d31a4	
5__ Incubated at 35-37 ºC for 48 hours (for dialysis product water – ANSI/AAMI RD52:2004, 7.2.3 and RD62:2006, 5.1)						0d31a10	
6__Sample volume chosen yields between 30 and 300 colonies. (SM9215A, 8.a. & b. & ANSI/AAMI RD52:2004, 7.2.3)						0d31a101	
SimPlate							
7__ Inverted and incubated at 35.0 ± 0.5 ºC for 48 hours.						0d31a5	
8__ When doing unit dose, 10 ± 0.2 mL sample is added to media tube.						0d31a6	
9__ When doing multi dose, 1 mL of sample and 9 mL of rehydrated media is pipetted onto center of the plate.						0d31a7	
10__ 6 W, 365 nm, light held 6-12 inches above plate used to count fluorescent wells.						0d31a	
SM9215C & ANSI/AAMI RD52:2004 & RD62:2006: **Spread Plate**							
11__ An inoculum of at least 0.5 mL of sample spread equally over the surface of the agar. (ANSI/AAMI RD52:2004, 7.2.3)						0d31a8	
12__ Inoculated agar plate with glass rod or pipette. Calibrated loop is not allowed. (ANSI/AAMI RD52:2004, 7.2. & RD62:2006, 5.1.1)						0d31a9	
13__ Incubated at 35-37 ºC for 48 hours. (ANSI/AAMI RD52:2004, 7.2.3 and RD62:2006, 5.1)						0d31a10	
14__ Colonies counted with a dark-field colony counter, or one with equivalent magnification & illumination. (SM9215A, 8.a. & b.&						0d31a33	

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ANSI/AAMI RD52:2004, 7.2.3)							
15__ Sample volume chosen yields between 30 and 300 colonies. (SM9215A, 8.a. & b. & ANSI/AAMI RD52:2004, 7.2.3)						0d31a101	
Note: If colony yield is not met, lab can use smaller or larger volumes. Smaller volumes can be reached by doing 1:10 serial dilutions using sterile phosphate buffer. If larger volumes are required, the MF method should be used							
SM9215D & ANSI/AAMI RD52:2004 & RD62:2006: **Membrane Filter Method**							
16__ Dispensed 5-mL portion of sterile agar into 50- x 9- mm petri dishes **Note:** m-HPC agar may not be sterile.						0d31a12	
17__ Incubated at 35-37 ºC for 48 hours. (ANSI/AAMI RD52:2004, 7.2.3 and RD62:2006, 5.1)						0d31a10	
18__Colonies counted with a stereoscopic microscope at 10 to 15 x magnification. (SM9215A, 8. b.& ANSI/AAMI RD52:2004, 7.2.3)						0d31a13	
19__ Sample volume chosen yields between 20 and 200 cfu. (SM9215A, 8b. & ANSI/AAMI RD52:2004, 7.2.3)						0d31a11	
Note: If colony yield is not met, lab can use smaller or larger volumes. Smaller volumes can be reached by doing 1:10 serial dilutions using sterile phosphate buffer. If larger volumes are required, the MF method should be used.							
b. SM9221A&B, 1.b.: **Total Coliform Multiple Tube Fermentation with Lauryl Tryptose Medium**							
1__ SDWA: 100 mL sample analyzed. (five 20 mL tubes, ten 10 mL tubes, or one 100 mL bottle)	40 CFR 141.21(f)(1)					0d31b1	
2__ CWA: 5-tube per dilution for each sample.						0d31b2	
3__ Incubated at 35.0 ± 0.5 ºC for 24 +/- 2 hours.						0d31b3	
4__ SDWA: If no gas detected after 24 hours, incubate for another 24 hours.						0d31b4	
Note: For other waters (NW), pull positives after 24 +/- 2 hours, transfer them, and still check the ones that are negative after 24 hours at 48 +/- 3 hours.							
5__ All samples producing turbid cultures with no gas production invalidated, with another sample requested.						0d31b5	

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6_ Commercially available Lactose Broth (LB) may be used in lieu of LTB if system conducts at least 25 parallel tests using water normally tested and false positive and false negative results using LB is <10%. Note: No requirement to run completed phase	40 CFR 141.21(f)(3) & 40 CFR 136.3(a) Table IA 40 CFR 141.21(f)(3)					0d31c	
c. SM9221D, 1.a. & b.: **Total Coliform with Presence/Absence Medium**							
1__ 100 mL sample analyzed						0d31c1	
2__ Incubated at 35.0 ± 0.5 ºC for 24 hours						0d31c2	
3__ If purple color indicator does not turn yellow, incubate for another 24 hours						0d31c3	
4__ All samples producing turbid cultures with no color change invalidated, with another sample requested						0d31c4	
Note: Media 6x formulation strength may be used if media is filter sterilized instead of autoclaved.	40 CFR 141.21(f)(3)						
d. SM9221E, 1.a. & b:Thermotolerant (**Fecal) Coliform Most Probable Number with EC Medium**							
1__ 3-dilution (sample volumes), 5-tube (per sample volume) technique for each sample						0d31d1	
2__ Each tube inoculated from positive culture grown on m-Endo or LTB medium						0d31d2	
3__ Incubated at 44.5 ± 0.2 ºC for 24 ± 2 hours						0d31d3	
4__ Gas formation indicates Fecal Coliform, no further verification needed						0d31d4	
5__ Must be incubated in a water bath	SM and 40 CFR 141 Subpart C(f)(5)					0d31d5	
e. SM9221E, 2.a. & b: Thermotolerant (**Fecal) Coliform Most Probable Number with A-1 Medium**							
1__ 3-dilution (sample volumes), 5-tube (per sample volume) technique for each sample						0d31e1	
2__ Direct inoculation with sample possible						0d31e2	
3__ Incubated at 35.0 ± 0.5 ºC for 3 hours, then at 44.5 ± 0.2 ºC for 21 ± 2 hours						0d31e3	
4__ Gas formation indicates Thermotolerant (Fecal) Coliform; no further verification needed						0d31e4	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
f. SM9221F **PW/NW E. coli enumeration & NW Thermotolerant coliform with EC-MUG**							
1__Tube contains inverted Durham tube	40 CFR 136					0d32a	
2__ Enrich in presumptive medium for Total Coliform using 9221 B.2-2014 prior to EC-MUG	40 CFR 136					0d32f	
3__Presumptive tubes or bottles showing any amount of gas, growth, or acidity within 48 ± 3 h transferred to EC-MUG using sterile 3- or 3.5 mm diameter sterile loop or sterile wooden applicator inserted at least 2.5 cm to transfer growth from fermentation tube to culture tube.						0d32b	
Note: Wooden applicator must be plunged to bottom of EC-MUG tube.							
4__Incubate 44.5 ± 0.2° C for 24 ± 2 hours.						0d32c	
5__Growth and gas indicates thermotolerant coliform						0d32d	
6__Blue fluorescence under 6 W 365-366 nm UV light indicates E. coli						0d32e	
g. SM9222B, 5.a.-d.: **Total Coliform by Membrane Filtration**							
1__ SDWA: 100 mL sample filtered						0d31f1	
2__ CWA: Filter 3 different sample volumes so that at least one dilution will give 20-80 colonies, but not more than 200 colonies.						0d31f2	
3__ Enhancement recovery required for stressed organisms in chlorinated samples (e.g., spas and swimming pools).						0d31f3	
4__ Incubated at 35.0 ± 0.5 ºC for 22-24 hours						0d31f4	
h. SM9222D, 2.a.-d.: **Thermotolerant (Fecal) Coliform by Membrane Filtration**							
1__ Filter volumes or dilutions that will give 20-60 fecal coliform colonies per membrane filter						0d31g1	
2__ Incubated at 44.5 ± 0.2 ºC for 24 ± 2 hours						0d31g2	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
i. SM9223B, 2: **Total Coliform by MMO-MUG** **1**__ SDWA: 100 mL sample analyzed (for drinking waters) **2**__ Colilert: Incubated at 35.0 ± 0.5 ºC for 24 hours. **3**__ Colilert: When indeterminate after 24 hours, incubate for another 4 hours. **4**__ Colisure: Incubated at 35.0 ± 0.5 ºC for ≥ hours, but ≤ 48 hours. **5**__ Colilert-18: Incubated at 35.0 ± 0.5ºC for 18 hours (up to 22 hours if indeterminate after 18 hours); first 20 minutes MUST be in 35 ºC water bath or 7-10 minutes in 44.5 ºC water bath. **6**__ Readycult: Incubated at 35.0 ± 0.5 ºC for 24 hours ±1 hour. **7**__ Fluorocult LMX: Incubated at 35.0 ± 0.5 ºC for 24 hours ± 1 hour. **8**__ Colitag: Incubated at 35.0 ± 0.5 ºC for 16-48 hours. If results are Read before 22 hours, sample is prewarmed for 7-10 min in 44.5 ±0.2°C water bath. **9**__ E*Colite: Incubated at 35.0 ± 0.5 ºC for 28 hours. **10**__ Color change indicates Total Coliform; 365-366 nm, 6 W UV light used to determine fluorescence to indicate E. coli; and no further verification needed. **11**__ When enumerating coliforms using Colilert, the lab uses a Quanti-Tray for each sample dilution tested. **12**__ The lab checks the Quanti-Tray sealer monthly by adding a dye to the water. The lab reports quantitative (aka estimate of bacterial Density or enumeration) data for E. coli for source water under the SDWA Surface Treatment Rule.	40 CFR 141.21(f)(1)					0d31h1 0d31h2 0d31h3 0d31h4 0d31h5 0d31h6 0d31h7 0d31h8 0d31h9 0d31h10 0d31h11 0d31h12	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
. **j. Fecal Coliform by Colilert-18** **1**_ Incubated 44.5 ºC ± 0.2 ºC for 18 hours (up to 22 hours if indeterminant after 18 hours) **2**_ Water bath incubator must be used	40 CFR 136.3 Table 1A					0d31z1 0d31z2	
k. **Enterococci by Enterolert** **1**__ 100 mL sample analyzed (for drinking waters) **2**__ Incubated at 41.0 ± 0.5 ºC for 24 hours (up to 28 hours if indeterminate after 24 hours) **l**. **EPA 1600**, 9.5.2, 11.5 & 11.8: **Enterococci by Membrane Filtration with mEI Medium** **1**__ Filter volumes or dilutions that will give 20-60 enterococci colonies per membrane filter **2**__ Incubated at 41.0 ± 0.5 ºC for 24 hours +/- 2 hours **m**. **SM 9230C: Enterococci by Membrane Filtration with mE → EIA Medium** **1**__ If mE agar is used, incubated inverted plate for 48 hours at 41°±0.5°C, ± 3 hours and then transfer filter to EIA medium. Incubated at 41°± 0.5°C for 20 minutes.						0d31i1 0d31i2 0d31j1 0d31j2 0d31j3	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
Method Validation:							
1 For methods other than reference methods, validation must comply with V1M2 and include the following minimum requirements from M5:	M5,1.5 (a)						
a__ Determine **accuracy** by comparison of at least one positive reference culture result to that of a reference method;	M5,1.5.1					0d364ar	
b__ Determine **precision** by analyzing a minimum of ten replicate analyses spiked with the target microorganism with both the proposed and reference method and determine that the proposed method is statistically equivalent or better than the reference method;	M5,1.5.2					0d364br	
c__ Determine **selectivity** by analyzing a minimum of ten spiked samples using mixed cultures that include the target microorganisms and at various concentrations. Calculate the number of false positive and false negative results.	M5,1.5.3					0d364bb	
2 For both reference and non-standard methods, the laboratory participates in proficiency testing programs, where available.	M5,1.5 (b)					00d320a	
3 The laboratory maintains documentation of the validation procedure for as long as the method is in use and for at least five years past the last date of use.	M5,1.5 (c)					00d319r	
The quality control protocols specified by the laboratory's method manual are followed by all analysts.	M2,5.9.3 (c)					000d12	
All essential quality control measures are incorporated in the lab's method manual.	M2,5.9.3(c)					000d13	
All quality control measures are assessed and evaluated on an on-going basis and quality control acceptance criteria are used to determine the validity of the data.	M2,5.9.3(b)					000d14	
The laboratory has procedures for developing acceptance/rejection criteria for each test where no method or regulatory criteria exist.	M2,5.9.3(c)					000d15	
Samples are stored, handled, and prepared in accordance with written procedures and in a manner to avoid deterioration, loss, or damage. See also Quality Systems Checklist deficiency 51126. Examples of deterioration, loss, or damage include, but are not limited to, "pouring off" sample collection container to reduce volume to allow analysis in collection container and dipping a test strip into the sample.	M2,5.8.4					000d20	

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Microbiological samples from **known chlorinated water sources, unknown sources where disinfectant usage is suspected, and all potable water supplies** are checked in the laboratory for absence of residual chlorine, unless all of the following conditions are met: **a**__ sufficient sodium thiosulfate is added to each container to neutralize at minimum 5 mg/L of chlorine for drinking water and 15 mg/L chlorine for wastewater. **b**__ 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, **c**__ chlorine residual is checked in the field and actual concentration is documented with sample submission. **d**__ the laboratory can show that the received sample containers are from its laboratory or have been appropriately tested and documented.	M5,1.7.5.2(a-d)					55818 55818ar 55818br 55818cr 55818dr	
Samples requiring thermal preservation are acceptable if the arrival temperature of a representative sample container meets the method or mandated temperature requirement. Samples delivered to the laboratory on the same day as collection that do not meet the requirement **may** be considered acceptable if the samples are received on ice with evidence that the cooling process has begun.	M5,1.7.5.1					00d370	
The **maximum hold time** has not been exceeded for the bacteriological samples analyzed by the laboratory. **Note:** Refer to ELAP Certification Manual Item 245.	SWTR, BWR, TCR, GWR, NPDES, AAMI/ANSI					00d335z	
The laboratory has documented procedures, which refer to applicable reference methods, for the calibration, verification, and QC of support equipment including conductivity meters, oxygen meters, pH meters, hygrometers, and other similar measurements instruments.	M5,1.7.1.1					00d3677	
The following support equipment associated with microbiological testing is checked with NIST traceable materials (where possible): **a**__ pH meter **b**__ Balance(s) **c**__ Conductivity meter **d**__ Refrigerator(s) for sample storage and/or media storage	M2,5.5.13.1; ELAP Certification Manual Item 231					5916 or 00d34ar 00d34br 00d34cr 00d34dr	

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e__ Incubators **f**__ Water baths **g**__ Freezers						00d34er 00d34fr 00d34gr	
Quality Control							
The lab demonstrates and documents the quality of reagents and media used is appropriate for the test concerned including, but not limited to, test conditions and incubation times.	M5,1.7.3.1					000d37d	
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) are checked by the laboratory once per purchased or prepared lot using non-selective growth media.	M5,1.7.3.1(a)					000d37ar	
Certificates of Analysis (COA) provided by vendors documenting sterility are **verified by the laboratory** and retained in accordance with V1M2 5.6.4.2.a.	M5,1.7.3.1(a)					000d37br	
Sterility Checks: **1**__ The laboratory performs a sterility check for each lot of pre-prepared, ready-to-use media (including chromofluorogenic reagent) and for each batch of media prepared in the laboratory, prior to use: **a** __ For chromo/fluorogenic media, the media is added to sterile deionized water and incubated at the appropriate temperature and time for the method used and documented. **b**__ For all other media, the media is incubated uninoculated at working strength (single strength). **2**__The laboratory performs a sterility check on one (1) funnel per lot of pre-sterilized single use funnels and 1 funnel per batch of laboratory-sterilized funnels with non-selective growth media. **3**__ **a**__The laboratory performs a sterility check on at least one (1) container for each lot of purchased, pre-sterilized sample containers with non-selective growth media. **b**__The laboratory performs a sterility check on one (1) container/object per sterilization batch sterilized in the laboratory with non-selective growth media. **4**__ The laboratory performs 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.	M5,1.7.3.1(a)(i) M5,1.7.3.1(a)(i)(a) M5,1.7.3.1(a)(i)(b) M5,1.7.3.1.(a)(ii) M5,1.7.3.1(a)(iii) M5,1.7.3.1(a)(iv)					00d381r 00d382r 0d382a1r 00d388r 00d390 00d391 00d385r	

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The concentration of the non-selective growth media shall be single strength after addition of the dilution water.							
5__ The laboratory performs a sterility check on at least one filter from each new lot of membrane filters with non-selective growth media.	M5,1.7.3.1.(a)(v)					00d387r	
(When using a non-selective broth, incubate at 35 +/- 0.5 ° C for 24 and 48 hours and check for growth)						00d387a	
Media:							
1__ Media is tested for performance (e.g., selectivity, sensitivity, sterility, growth promotion, and growth inhibition) at a minimum with first use.	M5,1.7.3.1(b)(i)					00d377a	
2__ Media is used within the expiration date or shelf-life provided by the manufacturer.	M5,1.7.3.1(b)(ii)					00d377b	
3__ Laboratory prepared media is used within the holding time specified in the accredited method.	M5,1.7.3.1(b)(iii)					00d377c	
For SM Methods:							
a_ Broth in screw-cap flask is used within 96 h and stored at 2-8°C.						00d336a	
b_ Agar plates with tight-fitting covers used within 2 weeks and stored at 2-8°C						00d336b	
c_ Agar or broth loose-capped tubes is used within 2 weeks and stored at 2-8°C						00d336c	
d_ Agar or broth tightly closed screw-cap tubes is used within 3 months. Note: Hold at <30° C.	SM 9020B, 4.i.4 and, Table 9020:V					00d336d	
e_ Poured agar plates with loose-fitting covers, sealed in plastic bags are used within 2 weeks and stored at 2-8°C.						00d336e	
f_ Large volume of agar in tightly closed screw cap flask or bottle is used within 3 months and stored at 2-8°C.						00d336f	
g_ Tubes or plates with growth and/or bubbles are discarded.						00d336g	
h_ Media in tubes and plates stored >2 weeks with evaporation exceeding 10% original volume or weight is discarded.						00d336h	
i_ Refrigerated medium is warmed to room temperature before use.						00d336i	
j_Prepared, ready to use media with expiration dates later than noted above is verified weekly by testing recoveries with known densities of control cultures that also meet QC check requirements.						00d336j	
4__ The laboratory has detailed testing criteria information defined in the laboratory's methods, SOPs, or similar documentation.	M5,1.7.3.1(b)(iv)					00d337a	

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Reagents, media, and commercial dehydrated products are used within the expiration date or shelf-life provided by the manufacturer of the product and documentation is retained per V1M2 5.6.4.2.	M5,1.7.3.1(c)					00d337b	
Reagent Water:							
1 The quality of the reagent water which will come in contact with test organisms and is used in the preparation of media, solutions, and buffers is monitored for bactericidal and inhibitory substances.	M5,1.7.3.1(d)(i)					00d332b	
2 The reagent water which will come in contact with test organisms and is used in the preparation of media, solutions, and buffers is distilled water, deionized water, or reverse-osmosis-produced water.	M5,1.7.3.1(d)(i)					00d332a	
3 The quality of the water is monitored for the following on a monthly basis (when in use), when maintenance is performed on the water system, or startup after a period of disuse greater than one month: **a**___ Disinfectant residual **b**___ Specific conductance **c**___ Total organic carbon **d**___ Heterotrophic plate count	M5,1.7.3.1(d)(ii)					00d333r	
4 The quality of the water is monitored for metals (Cd, Cr, Cu, Ni, Pb, and Zn and the Bacteriological Water Quality Test (to determine the presence of toxic agents or growth promoting substances) annually. **Note:** Documentation demonstrating water source meets the criteria, as specified by the method, for High Quality (Type I) or Medium Quality (Type II) are not required to monitor for the Bacteriological Water Quality Test.	M5,1.7.3.1(d)(iii)					00d333ar	
5 Reagent water analyses results meet method specifications and are retained five years. (See below or method specific requirements.) From SM 23 Table 9020:II:	M5,1.7.3.1(d)(iv)					0d334fr	
a_ Conductivity: continuously or usage day <2 µmhos/cm at 25 °C						0d334br	
b_ Total organic carbon <1.0 mg/L						0d334gr	
c_ Heavy metals: each < 0.05 mg/L						0d334er	
total < 0.10 mg/L							
d_ Total residual chlorine: each use < 0.1 mg/L						0d334ar	

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e_ Heterotrophic plate count < 500 CFU/mL or MPN <500/mL **f**_ Use Test (new water source) Student's t ≤ 2.78 **g**_ Water Quality Test 0.8 – 3.0 ratio						0d334cr 0d334ir 0d334dr	
6 Records of reagent water purchased from an outside source and used in the preparation of media, solutions, and buffers meet the criteria specified in 3 and 4 above are documented.	M5,1.7.3.1(d)(v)					000d334	
7 Reagent water that has been opened for longer than the testing intervals specified above or in the accredited method is re-tested or discarded.	M5,1.7.3.1(d)(vi)					000d335	
The quality of dilution water, including buffer water and/or peptone water, is monitored for sterility, pH, and volume once per batch whether lab prepared or purchased.	M5,1.7.3.1(e)					0d334jr	
Documentation for **media and reagents prepared in the laboratory** includes the following: **a**___ Date of preparation, **b**___ Preparer's initials, **c**___ Type and amount of media prepared, **d**___ Manufacturer and Lot #, **e**___ Final pH of the media, and **f**___ Expiration date	M5,1.7.3.1(f)					0d337ar 0d337br 0d337cr 0d337dr 0d337er 0d337fr	
Documentation for media purchased pre-prepared, ready-to-use (including reagent water purchased from outside sources) includes the following: **a**___ Manufacturer, **b**___ Lot #, **c**___ Type and amount of media received, **d**___ Date of receipt **e**___ Expiration date of the media, and **f**___ pH of the media	M5,1.7.3.1(f)					0d338ar 0d338br 0d338cr 0d338dr 0d338er 0d338fr	
Method Blanks: **1** For filtration techniques, method blanks are conducted per the analytical method and at a minimum of beginning and ending blank for each filtration series.	M5,1.7.3.2(a)					0d382e	
2 Filtration series is ended when more than 30 minutes elapses between successive filtrations.	M5,1.7.3.2(b)					0d382f	
3 Filter funnels are rinsed with three 20-30 mL portions of sterile rinse water	M5,1.7.3.2(b)					0d382cr	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
after each filtration. **4** After every ten samples, a method blank is inserted, or the filtration unit is sanitized with UV light (254 nm) after every sample filtration. **5** For pour plate methods, at least one uninoculated plate is poured for each lot of pre-prepared, ready to-to-use medium and for each batch of laboratory prepared medium.	M5,1.7.3.2.(b) M5,1.7.3.2.(c)					0d382d 00d383r	
Test Variability/Reproducibility: For test methods that specify counts (i.e. cfu/100ml or MPN/100 ml), such as membrane filter, plated media, or other methods which specify a quantitative result, **duplicate counts** are performed **monthly** on one positive sample for each month that the test is performed: **a** If the lab has two or more analysts, each analyst counts typical results on the same sample for each month the test is performed. **i)** __ Counts shall be within **10% difference** to be acceptable. **b** In a lab with one microbiology analyst, the same sample is counted twice by the analyst for **each month** the test is performed. **ii)** __ Counts with no more than **5% difference** are between the counts are acceptable.	M5,1.7.3.3					0d3161r 0d3162r 0d3162ar 0d3163r 0d1363ar	
Sample Specific Controls: **1** Matrix spikes are performed per the method requirements. **2** Matrix duplicates are performed per the method requirements.	M5,1.7.3.4(a) M5,1.7.3.4(b)					0d390a 0d390b	
Data Reduction: The calculations, data reduction and statistical interpretations specified by each method are followed. a. 9221D - Reported result as presence-absence test positive or negative for total coliforms in 100 mL of sample. b. 9222B - Compute the count, using membrane filters with 20 to 80 coliform colonies and not more than 200 colonies of all types per membrane, by the following equation: (Total) coliforms/100 mL = (coliform colonies counted x 100)/mL sample filtered c. 9215B - To compute the heterotrophic plate count, CFU/mL, divide total number of colonies or average number (if duplicate plates of the same dilution) per plate by the sample volume. d. 9223B - If performing an MPN procedure, calculate the MPN value for	M5,1.7.3.5					00d326r	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
total coliforms and E. coli from the number of positive tubes as described in Section 9221C. If using the presence-absence procedure, report results as total coliform and E. coli present or absent in 100-mL sample. e. EPA 1600 – Compute the count per 100 mL of sample by dividing the # of enterococci colonies by the volume of sample filtered and then multiplying by 100. Refer to rules in Appendix B of method. For example, if there is > 1 dilution, calculate the arithmetic mean for those results in the acceptable counting range. f. ISO 11731:2017 (E) – For enumeration, select the plate or set of plates from the same culture showing the maximum number of confirmed colonies per water volume and taking any dilutions into account. Do not average the counts from different methods, treatments, or culture media as these are not replicates. Calculate the # of colonies in original water per liter using the equations in section 9 for direct plating, MF, indirect filtration, and plating after dilution.							
Selectivity:							
1. All growth and recovery media are checked to assure that the target organisms respond in an acceptable and predictable manner once per lot or batch.	M5,1.7.3.6(a)					00d325r	
2. In order to ensure identity and traceability, reference cultures used for positive and negative controls are obtained from a recognized national collection, organization, or manufacturer recognized by the accreditation body.	M5,1.7.3.6(c)					00d341r	
3. Reference cultures are single use preparations or cultures maintained for their use by documented procedures that demonstrate continued purity and viability of the organisms.	M5,1.7.3.6(c)					00d343r	
a. Reference cultures are revived (if freeze dried) or transferred from slants and sub-cultured once to provide reference stocks.	M5,1.7.3.6(c)(i)					00d342r	
b. The reference stocks are preserved by a technique that maintains the desired characteristics of the strains.	M5,1.7.3.6(c)(i)					00d344r	
c. Reference stocks are used to prepare working stocks for routine work.	M5,1.7.3.6(c)(i)					00d345r	
d. When reference stocks are thawed, they are not re-frozen and re-used.	M5,1.7.3.6(c)(i)					00d346r	
e. Working stocks are not sequentially cultured more than 5 times.	M5,1.7.3.6(c)(ii)					00d348r	
f. Working stocks are not sub-cultured to replace reference stocks.	M5,1.7.3.6(c)(ii)					00d349r	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
4. Culture Controls: a. Each batch of pre-prepared, ready-to-use lot of selective medium (including chromofluorogenic reagent) and each batch of selective medium prepared in the laboratory is analyzed with at least one known **negative culture control** as appropriate to the method prior to first use of the medium.	M5,1.7.3.6(d)(i)(b)					00d311r	
b. Each batch of pre-prepared, ready-to-use lot of selective medium (including chromofluorogenic reagent) and each batch of selective medium prepared in the laboratory is analyzed with at least one known **positive culture control** as appropriate to the method prior to first use of the medium.	M5,1.7.3.6(d)(ii)(b)					00d312r	
5. To ensure that analysis results are accurate, a target organisms' identity is 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 Bile Broth (BGLG) or EC or EC+MUG broth). **Note**: See below for selected methods. Additional method verifications can be found in the promulgated method.	M5,1.7.3.6(b)					0d325ar	
a. SM9221B, 2b; SM9221D, 2b: Total Coliform by Fermentation Broth method							
i__ Each positive culture from LTB (gas formation or color change) is inoculated onto BGLB (Note: If all 5 tubes produced gas in 2 or more sample dilutions, only the 5 tubes with gas from the highest dilution need be confirmed)						d340a11	
ii__ Incubated at 35.0 ± 0.5 ºC for 24 ± 2 hours						d340a2r	
iii__ If no gas formation, re-incubate for additional 24 hours (total of 48 ± 3 hours)						d340a3r	
iv__ Gas formation in BGLB confirms Total Coliform for purposes of MPN calculation or Presence-Absence reporting						d340a4r	
v_ SDWA samples also tested according to SM9221E or EPA 1104 Note: No requirement to run completed phase						0d340a5r	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
b. SM9222B, 5f: Total Coliform by Membrane Filter method							
i__ Inoculate at least 10 colonies from filter into LTB & BGLB						d340b1	
ii__ SDWA: Inoculate all colonies (can swab entire filter) into one LTB tube & one BGLB tube						d340b2	
iii__ Incubate at 35.0 ± 0.5 ºC for 48 hours						d340b3	
iv__ Gas production in LTB & BGLB confirms Total Coliform						d340b4	
v __ SM9222B: May use rapid-test or commercial multi-test verification systems that utilize test reactions for cytochrome oxidase & b-galactosidase; negative reaction for cytochrome oxidase & positive reaction for b-galactosidase confirms Total Coliform						d340b5	
vi__ SDWA: Positive cultures from LTB or membrane filter colonies also tested according to SM9221E, EPA 1104, or EPA 1105. (Note: May inoculate m-Endo colonies directly into BGLB medium. However, if gas is observed in LTB, but not in the corresponding BGLB tube, another BGLB tube must be inoculated & tested with the positive culture from the LTB tube						d340b6	
vii__ SM 9020B, 9.b.1 Membrane Filter Method Confirmation:							
a)__ For drinking water, all colonies from positive samples on m-Endo medium are verified.	40 CFR 136 Table IA					d340b7a	
b)__ If there are no positives, at least one known positive source water is tested quarterly.						d340b7b	
c)__ For other waters, at least 10 sheen colonies are verified monthly using LTB and BGLB, followed by count adjustment based on these results.						d340b7c	
d)__ For other waters, non-sheen colonies are verified monthly using LTB.						d340b7d	
c. SM9221E, 1b: Fecal Coliform with EC Medium (A-1 is not allowed for SDWA)	40 CFR 136 Table IA						
i__ Incubated at 44.5 ± 0.2 ºC for 24 ± 2 hours						d340c1	
ii__ Gas formation confirms that the Total Coliform is a Fecal Coliform						d340c2	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
d. SM 9222G/EPA 1104, 11: E. coli by EC + MUG Tube Procedure							
i__ Incubated at 44.5 ± 0.2 ºC for 24 ± 2 hours						d340d1	
ii__ 366-nm blue-light fluorescence confirms that the Total Coliform is E. coli						d340d2	
iii__ Membrane filter is transferred in its entirety to EC + MUG medium.						d340d3	
e. SM 9222G/EPA 1105, 11: E. coli by Nutrient Agar + MUG Membrane Filter Procedure							
i__ Membrane filter transferred in its entirety to NA + MUG medium (Note: Some colonies removed for LTB & BGLB tests.)						d340e1	
ii__ Incubated at 35.0 ± 0.5 ºC for 4 hours						d340e2	
iii__ 366-nm blue-light fluorescent halos around MF colonies confirm that Total Coliform is E. coli						d340e3	
f. SM 9222B/SM 92222I: NW E. coli by Membrane Filtration							
i__ Positive samples determined by SM 9222B subject to SM 9222I using NA-MUG	40 CFR 136.3 Table IA					d340	
g. SM 92222D/SM9020B, 9b: Fecal Coliform by Membrane Filtration							
i__ Inoculate at least 10 colonies from filter into LTB						d340f1	
ii__ Incubated at 35.0 ± 0.5 ºC for 24 hours (48 hours if no gas production after 24 hr.)						d340f2	
iii__ Positive cultures from LTB (gas formation) inoculated into EC medium						d340f3	
iv__ EC tubes incubated at 44.5 ± 0.2 ºC for 24 hours						d340f4	
v__ Positives verified monthly (by picking at least 10 blue colonies from one positive sample); and false negatives picked and verified (SM9020B, 9.b.2)						d340f5	
vi__ Count is adjusted based on these results.						d340f6	
Note: May inoculate m-FC colonies directly into EC medium. However, if gas is observed in LTB, but not in the corresponding EC tube, another EC tube must be inoculated & tested with the positive culture from the LTB tube.							

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Constant and Consistent Test Conditions:							
Laboratory Facility:							
1. Floors and work surfaces are non-absorbent and easy to clean and disinfect.	M5,1.7.3.7(a)					00d354r	
2. Work surfaces are adequately sealed.						00d353r	
3. Sufficient storage space is provided						00d355ar	
4. Laboratory is clean and free from dust accumulation.						00d355cr	
Laboratory Equipment:							
1. Temperature Measuring Devices:							
a. The temperature measurement devices have the appropriate quality needed to achieve the specification in the test method.	M5,1.7.3.7(b)(i)					00d356r	
b. The temperature measurement devices are verified to national or international standards for temperature at least annually. Note: See ELAP Certification Manual Item 231 for required frequency.	M5,1.7.3.7(b)(i)					00d357r	
c. Temperature measuring device verification may be accomplished using a single point that represents the method mandated temperature and use conditions.	M5,1.7.3.7(b)(i)					0d357a	
d. The graduation and range of the temperature measuring devices are appropriate for the required accuracy of measurement.	M5,1.7.3.7(b)(i)					00d358	
2. Sterilization Equipment:							
a. The performance of each autoclave is initially evaluated by establishing its functional properties and performance, i.e. heat distribution characteristics established with respect to typical uses.	M5, 1.7.3.7(b)(ii)(a)(1)					00d360r	
b. Autoclaves shall meet specified temperature tolerances. Note: Pressure cookers cannot be used for sterilization of growth media.	M5, 1.7.3.7(b)(ii)(a)(1)					00d361r	
c. Proper sterilization temperature is demonstrated by use of continuous temperature recording device or use of a maximum registering thermometer each autoclave cycle.	M5, 1.7.3.7(b)(ii)(a)(2)					00d361a	
d. Effective sterilization is demonstrated using appropriate biological indicators at least once each month the autoclave is used.	M5, 1.7.3.7(b)(ii)(a)(2)					00d361b	
e. The biological indicator used in the autoclave shall be effective at the sterilization temperature and time needed to sterilize lactose-based media.	M5, 1.7.3.7(b)(ii)(a)(2)					00d361dr	
f. Temperature sensitive tape is used each autoclave run to indicate the contents have been processed.	M5, 1.7.3.7(b)(ii)(a)(2)					00d361c	

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g. Records of autoclave operation maintained for every cycle include the following: i.___ Date, ii.___ Contents, iii.___ Maximum temperature reached, iv.___ Time in sterilization mode, v.___ Total run time (may be recorded as time in and time out), vi.___ Analyst's initials, and vii.___Pressure	M5, 1.7.3.7(b)(ii)(a)(3)					00d361er or 0d362ar 0d362br 0d362cr 0d362dr 0d362er 0d362fr 0d362gr	
h. Annual autoclave maintenance, internally or by service contract, is performed and records are maintained.	M5, 1.7.3.7(b)(ii)(a)(4)					00d363zr	
i. Annual autoclave maintenance includes a pressure check and verification of temperature device. Note: If it has been determined that the autoclave has no leaks, pressure checks can be documented using the formula PV=nRT.	M5, 1.7.3.7(b)(ii)(a)(4)					0d363ar	
j. The autoclave mechanical timing device is checked quarterly against a stopwatch and the actual time elapsed is documented.	M5, 1.7.3.7(b)(ii)(a)(5)					0d363br	
k. Ovens used for sterilization are checked for sterilization effectiveness monthly with appropriate biological indicator.	M5,1.7.3.7(b)(ii)(b)					0d367ar	
l. Records of oven operation maintained for every cycle include the following: i.___ Date, ii.___ Cycle time, iii.___ Temperature, iv.___ contents, and v.___ Analyst's initials.	M5,1.7.3.7(b)(ii)(b)					d367bar d367bbr d367bcr d367bdr d367ber	
m. Temperature sensitive tape is used each oven run to indicate the contents have been processed.	M5,1.7.3.7(b)(ii)(b)					d367bfr	
3. Volumetric Equipment:							
a. Volumetric equipment with movable parts such as automatic dispensers, dispensers/diluters, and mechanical hand pipettes are verified by the laboratory for accuracy quarterly and documented.	M5,1.7.3.7(b)(iii)(a)					00d364r	
b. Volumetric equipment such as filter funnels, bottles, non-Class A glassware, and other marked containers are verified by the laboratory once per lot prior to first use.	M5,1.7.3.7(b)(iii)(b					00d365r	

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c. The volume of disposable volumetric equipment such as sample bottles, disposable pipettes, and micropipette tips are checked by the laboratory once per lot.	M5,1.7.3.7(b)(iii)(c)					0d365ar	
d. Verification of volume is within 2.5% of expected volume. Note: This verification can be volumetric as compared to Class A or gravimetric.	M5,1.7.3.7(b)(iii)(d)					0d365br	
4. UV Instruments:							
a. UV instruments used for sanitization are tested quarterly for effectiveness with an appropriate UV light meter or by agar spread plate irradiation test.	M5,1.7.3.7(b)(iv); ELAP Certification Manual Item 231					0d366ar	
b. Bulbs are replaced 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.	M5,1.7.3.7(b)(iv); ELAP Certification Manual Item 231					00d366r	
5. Incubators:							
a. The uniformity of temperature distribution and time required to achieve equilibrium conditions in incubators and water baths is established prior to first use after installation and service. The equilibrium check includes the time required after test sample addition to re-establish equilibrium conditions under full capacity load for intended use. Note: Position, space between and height of stacks of Petri dishes established. Dishes are not to be stacked more than 4 high. SimPlate plates can be stacked higher than 4.	M5,1.7.3.7(b)(v)(a)					000d32a	
b. When samples are under test, temperatures of incubators and water baths are recorded twice daily separated by **at least 4 hours.** Data loggers, continuous temperature monitoring devices, or other temperature monitoring equipment can be used if it is calibrated in accordance with the TNI V1M2 Section 5.5.13.1 and ELAP Certification Manual Item No. 231 requirements. **Note**: "Under test" is defined as the time period that the sample is in the incubation phase of the method. **Note:** There is no intent to take incubator temperatures when there are no samples under test.	M5,1.7.3.7(b)(v)(b)					000d32r 0d367cr	
6. Labware:							
a. The laboratory has a documented procedure for washing labware, if applicable. Note: Labware is glassware and plasticware.	M5,1.7.3.7(b)(vi)(a)					0d367dr 0d367er	
b. Only detergents designed for labware are used.	M5,1.7.3.7(b)(vi)(a)						

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c. Glassware used is made of borosilicate or other non-corrosive material, free of chips and cracks, and has readable measurement marks. d. The laboratory tests labware that is washed and reused for the possible presence of residues which may inhibit or promote growth of microorganisms by performing the Inhibitory Residue Test initially, and each time the lab changes detergent formulation or washing procedures. e. Washed labware is tested at least once daily, each day of washing, for possible acid or alkaline residue by testing one piece of glassware with a suitable pH indicator such as bromothymol blue. Records of tests are maintained.	M5,1.7.3.7(b)(vi)(a) M5,1.7.3.7(b)(vi)(b) M5,1.7.3.7(b)(vi)(c) M5,1.7.3.7(b)(vi)(c)					00d368r 00d369r	
Original containers of reagents and media are labeled with an expiration date.	M2,5.6.4.2(b)					51026r	
Media, solution and reagent requirements: **a.** ____ **Heterotrophic Plate Count Medium** (SM9215A, 6, SM9215B, 3a, SM9215C, 2-3, and SM9215D, 2-3): ___ Plate count agar autoclaved at 121 ºC for 15 minutes. R2A agar heated and sterilized at 121 ºC for 15 minutes. ___ Final pH 6.8-7.2 for Plate Count Agar, 7.2 for R2A Agar. ___ Sterile agar medium melted not more than once. ___ Melted agar used within 3 hours; agar tempered at 44-46 ºC before pouring. ___ A separate "temperature" container exposed to same heating and cooling as medium. (Do not depend on the sense of touch.) ___ Blood agar and chocolate agar are not used with dialysis related product water (ANSI/AAMI RD52:2004, 7.2.3 and RD62:2006, 5.1.1). **b.** ____ **Phosphate buffer** (SM9050C): ___ Stock buffer autoclaved at 121 C for 15 minutes. ___ Stock buffer final pH 7.2 ± 0.5 ___ Dilution rinse water prepared from stock buffer & $MgCl_2$. ___ A commercially available preparation can also be used.						00d335a 00d335b	
c. ____ **Peptone water** (SM9050B, 1b): ___ 10% peptone stock solution autoclaved or filter sterilized. ___ 0.1% peptone water prepared as dilution rinse water. ___ Final pH 6.6-7.0.						00d335c	

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d. ____ m-Endo Medium (SM9222B, 2): ___ Medium brought to a boil, but not boiled, removed immediately; and not autoclaved. ___ Ethanol used is not denatured. ___ Prepared in sterilized flask. ___ Final pH 7.0-7.4 for m-Endo Agar LES; 7.0-7.4 for m-Endo medium. ___ Uninoculated media discarded if growth or surface sheen observed.						00d335d	
e. ____ Lauryl Tryptose (Lauryl Sulfate) or Lactose Broth (SM9221B, SM9222B, 1a): ___ Formulated so that concentration is single strength after sample addition. ___ Autoclaved at 121 ºC for 12-15 minutes. ___ Final pH 6.6-7.0. ___ Inverted vials in sterilized media, one-third to one-half covered by media, & free of air bubbles.						00d335e	
f. ____ Brilliant Green Lactose Bile Broth (SM9221B, SM9222B, 2a): ___ Autoclaved at 121 ºC for 12-15 minutes. ___ Final pH 7.0-7.4.						00d335f	
g ____ Presence-Absence Test Medium (SM9221D, 1a): ___ Autoclaved at 121 ºC for 12 minutes, with space allowed between bottles. ___ Final pH 6.6-7.0. Note: Media 6x formulation strength may be used if media is filter sterilized instead of autoclaved.	40 CFR 141.21(f)(3)					00d335g	
h. ____ EC Medium (SM9221E, 1a): ___ Autoclaved at 121 ºC for 12-15 minutes. ___ Final pH 6.7-7.1. ___ Inverted tubes one-third to one-half covered by media & free of air bubbles.						00d335h	
i. ____ MMO-MUG Medium (Colilert, Colisure, E*Colite, Readycult, Fluorocult, or Colitag) (SM9223B, 1): ___ Commercial preparation used. ___ Protected from light. ___ Not autoclaved.						00d335i	

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j. ____ EC Medium + MUG (EPA 1104, 7): ___ Autoclaved at 121 ºC for 12-15 minutes. ___ Final MUG concentration 50 ug/ml. ___ Final pH 6.7-7.1. ___ Inverted vial in test tube not used. ___ Checked for absence of fluorescence prior to use (with 366-nm UV light).						00d335j	
k. ____ Nutrient Agar Medium + MUG (EPA 1105, 7): ___ Autoclaved in 100-mL volumes at 121 ºC for 15 minutes. ___ Final MUG concentration 100 ug/ml. ___ Final pH 6.6-7.0.						00d335k	
l. ____ A-1 Medium (SM9221E, 2a): ___ Autoclaved at 121 ºC for 10 minutes. ___ Final pH 6.8-7.0. ___ Inverted tubes one-third to one-half covered by media & free of air bubbles. (Note: May be stored in the dark at room temperature, but must be used within 1 week.)						00d335l	
m. ____Chromocult (used with Membrane Filtration): ___ Not autoclaved or overheated. ___ Final pH 6.6-7.0.						00d335m	
n. ____ Coliscan (used with Membrane Filtration): ___ Not autoclaved or overheated. ___ Final pH 6.6-7.0.						00d335n	
o. ____ m-ColiBlue-24 (used with Membrane Filtration): ___ Inverted 2-3 times to mix contents before opening broth ampule. ___ Final pH 6.8-7.2.						00d335o	
p. ____ Enterolert: ___ Commercial preparation used. ___ Protected from light. ___ Not autoclaved.						00d335p	
q. ____ mEI (EPA 1600, 7.5): ___ Filter-sterilized solution. ___ Final pH 6.9-7.3.						00d335q	

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r. ____m-FC Broth or Agar (SM9222D, 1a & EPA-600/8-78-017, Part II-B, 5.2.1): ___ Medium brought to boiling point, removed immediately, not autoclaved, and final pH of 7.2-7.6. (Note: m-FC Broth or Agar medium may be used without 1% rosalic acid provided no background growth.) **s ____SimPlate:** ___ Commercial preparation used. ___ Stored at 2-30 ºC and protected from light. ___ Commercial preparation used. ___ pH 6.7 - 7.3						00d335rr 00d335s	
ISO 11731:2017(E), 8.2 – 8.5: Legionella <u>*Concentration of Water Samples*</u> 1__ Filtered sample using vacuum filtration or positive pressure filtration, or centrifuged sample where concentration by filtration is not possible. 2__ Filtered an appropriate volume of sample based on particulate content or desired detection level. 3__ MF and direct plating: Filtered water sample (without treatment, after acid treatment, and if required, after heat treatment) through **cellulose nitrate or mixed cellulose esters membrane** filter, and placed filter (right-side up) directly onto culture media, ensuring no air bubble is trapped. 4__ MF followed by washing: Filtered water through **polycarbonate or polyethersulfone** membrane filter. Note: Placed filter right side down in a screw cap, sterile container with or without sterile beads. 5__ Washed filter using 5 to 10 ml of sterile diluent, or sample, and vortexed for at least 2 minutes, or alternatively, placed the container in an ultrasonic bath, ensuring the level of diluent is below the level of the water in the bath, for an optimum time interval for maximum recovery. Note: Filters may be cut into pieces using sterile scissors to aid elution. Also, refer to NOTES 1-3 in method. 6__ Divided concentrate into one portion untreated, one portion with heat, and one portion for treatment with acid solution.						 0d31k1 0d31k2 0d31k3 0d31k4 0d31k5 0d31k6	

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Sample Pre-Treatment							
7__ Heat: Added sample (concentrated or unconcentrated) into a sterile container and placed in a water bath at 50 ± 1 ºC for 30 ± 2 min. Note: Small volumes (<= 5 ml) should be used.						0d31k7	
8__Acid: Diluted one volume of the sample (concentrated or unconcentrated) with nine volumes of an acid solution, mixed well and left to stand for 5.0 ± 0.5 min.						0d31k8	
OR							
9__Acid: Transferred around 30 ml acid solution onto membrane filter, left for 5.0 ± 0.5 min, and rinsed the filter with at least 20 ml of the diluent.						0d31k9	
Plating and Inoculation							
10__ For samples expected with high concentration of Legionella (>10^4 cfu/l) and low concentration of interfering microorganisms: plated sample directly and inoculated and spread 0.1 ml to 0.5 ml sample onto one plate of BCYE and one plate of BCYE+AB agar.						0d31k10	
11__ For samples expected with low concentration of Legionella and low concentration of interfering microorganism: placed untreated, filtered sample onto one plate of BCYE agar. For acid treated filters, the samples are placed on one or more plates of BCYE+AB agar or GVPC or MWY agar.						0d31k11	
OR							
12__Samples expected with low concentration of Legionella and low concentration of interfering microorganism: inoculated and spread 0.1 ml to 0.5 ml of each concentrated portion of untreated, heat treated and acid treated sample from membrane filtration with washing onto one plate of BCYE agar and on one or more plates of BCYE+AB agar or GVPC or MWY agar.						0d31k12	
13__ Samples expected with a high concentration of interfering microorganisms: spread 0.1 ml to 0.5 ml of each portion of untreated, heat treated, and acid treated subsample onto one plate of GVPC or MWY agar.						0d31k13	
14__ Samples expected with an extremely high concentration of interfering microorganisms: spread 0.1 ml to 0.5 ml of each portion of subsample, that has been heat treated then acid treated and mixed well by a vortex mixer or in an ultrasonic water bath, onto one plate of GVPC or MWY agar.						0d31k14	

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Incubation							
15__ Plates inverted and incubated plates at 36 ± 2 ºC for 10 d in a humid atmosphere to prevent desiccation of plates. Note: Inoculated media left to stand until inoculated volume is absorbed. **Note**: *Environmental samples (including but not limited to surface, potable, non-potable, industrial, waste, and cooling tower samples) showing new growth at 7 days must be incubated up to 10 days to ensure colony is large enough for isolation.* The laboratory must have language in their SOP regarding their incubation duration procedures.						0d31k15	
Examination of Plates							
16__ Plates inspected for the first time on day 2, 3, 4, or 5 followed by a final inspection at the end of the incubation period (i.e. day 7 or day 10, dependent on the nature of the sample), and the # of each colony type recorded. Check the plates on day 2 to determine if dilutions are needed. Note: With outbreak investigations, it is advisable for samples with expected high concentration of interfering microorganisms to check the plates on day 2.						0d31k16	
Subculturing/Confirmation							
17__ Subculture 3 presumptive colonies from plate(s) showing the highest counts when there is only one colony type. First inoculate BCYE-cys (or alternate media in note 6.1.2) and then BCYE.						0d31k17	
18__ Subculture at least 1 colony of each type if more than 1 presumptive type of colony is present. First inoculate BCYE-cys (or alternate media in note 6.1.2) and then BCYE.						0d31k18	
19__ Subcultured plates incubated at 36 ± 2 ºC for 2 d to 5 d in a humid atmosphere to prevent desiccation of plates. Note: It is acceptable to stop the incubation at day 2 for those samples that are easily confirmed.						0d31k19	
20__ With outbreak investigations, subculture and incubated at least 5 presumptive colonies if only one morphology is present, or 2 presumptive colonies for each type of morphology if multiple morphologies present.						0d31k20	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
Recording Results							
21__ Record the results of all plates. Regard as Legionella those colonies that grow on BCYE agar but fail to grow on BCYE-cys agar.						0d31k21	
22__ Record volume filtered.						0d31k22	
23__ Record volume concentrated and final volume.						0d31k23	
24__ Record the inoculated volume.						0d31k24	
Note: Record issues can also be cited using a code in the Quality System checklist, Section 13 – Control of Records.							
Legionella Reagents and Media:							
a__ Phosphate Buffered Saline (ISO 11731, 6.2 Annex C):						00d335y	
____ Commercially available preparation used							
___ Reconstituted according to manufacturer's instructions							
___ Final pH 7.5							
___ Sterility check performed once per lot using double-strength non-selective medium, 35 ºC, 24 hour						00d335t	
b__BCYE (ISO 11731:2017(E), Annex B.1):							
___ L-cysteine and iron solutions prepared fresh, sterilized through filtration, and stored at -20 ± 3 °C for not more than 3 months.							
___ ACES buffer is prepared by mixing 2 solutions – 1) ACES granules dissolved in 500 ml distilled water using a water bath (45-50 °C) and 2) KOH pellets dissolved in 480 ml distilled water using gentle shaking.							
___ Charcoal, yeast extract and α-ketoglutarate added sequentially to ACES buffer.							
___ H2SO4or KOH used to adjust pH to 6.8 ± 0.2.							
___ Agar added and mixed to ACES solution, autoclaved at 121 ± 3 °C for 15 ± 1 min, and cooled in a water bath to 48 ± 3 °C.							
___ L-cysteine and iron solutions added aseptically, mixing well between additions.							
___ Final pH is 6.8 ± 0.2 at 25 °C.							
___ Stored at 5 ± 3 °C in airtight containers and protected from light for 3 months.						00d335u	
c ___BCYE-cys (ISO 11731:2017(E), Annex B.2):							
___ Prepared as noted above for BYCE, except that L-cysteine is omitted.							
___ Stored at 5 ± 3 °C in airtight containers in the dark for 3 months.							

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
d __BCYE+AB (ISO 11731:2017(E), Annex B.3): ___ Prepared as noted above for BCYE, except that 3 antibiotics supplements are added (Polymyxin B sulfate, Sodium cefazolin, and Pimaricin syn Natamycin). ___ Added Polymyxin B sulfate to 100 ml of water to achieve a concentration of 14,545 IU/ml. Sterilize the solution by filtration through 0.2 um or lower pore size filter. ___ Added 180 mg of Sodium cefazolin to 20 ml of water. Sterilize the solution by filtration through 0.2 um or lower pore size filter. ___ Added 1.75 g of Pimaricin to 100 ml of water. Sterilize the solution by filtration through 0.2 um or lower pore size filter ___ Prepared antibiotic supplements are stored in sterile containers at -20 ± 3 °C for not more than 3 months. ___ Final pH 6.8 ±0.2 at 25 °C.						00d335v	
e ___GVPC (ISO 11731:2017(E), Annex B.4): ___ Prepared as noted above for BYCE except that ammonia-free glycine and 3 antibiotic supplements are added. ___ Ammonia-free glycine added after α-ketoglutarate. ___ H2SO4 or KOH used to adjust pH to 6.8 ± 0.2 at 25 °C. ___ Stored at 5 ± 3 °C in airtight containers in the dark for up to 4 weeks. ___ 3 antibiotics - Polymyxin B sulfate, Vancomycin HCl and Cycloheximide - prepared fresh, sterilized by filtration, and stored at -20 ± 3 °C for up to 3 months when frozen, and thawed at room temperature for use. ___ 3 antibiotics are added and mixed well to the final medium after the aseptic addition of L-cysteine and iron solutions.						00d335w	
f ___Acid Solution (ISO 11731:2017(E), Annex D): ___ Prepared using HCl and KCl. ___ pH is adjusted to 2.2 ± 0.2 using KOH. ___ Stored in the dark at room temperature for no longer than 1 month.						00d335x	

Relevant Aspect of Standards	TNI 2016/ELAP/ Regulation/Method Reference	Y	N	N/A	S	ELAP citation codes	Comments
g ___Diluents – Page's Saline, Diluted Ringer's Solution, and Phosphate-buffered Saline (ISO 11731:2017(E), Annex C): ___ Page's Saline – 5 chemicals (NaCl, $MgSO_4 \cdot 7H_2O$, $CaCO_2 \cdot 2H_2O$, Na_2HPO_4, and KH_2PO_4) added to distilled water, dissolved, mixed well and autoclaved at 121 ± 3 °C for 15 ± 1 min. ___ Diluted Ringer – Use a commercially available preparation (1:10 dilution of ¼ strength Ringer's solution). pH 7.0 ± 0.2. ___ Phosphate-buffered saline – Use a commercially available preparation at pH 7.5 ___ Sterile tap water						00d335y	
h___ Modified Wadowsky Yee (ISO 11731:2017(E). Annex B.5): ___ Prepared as noted above for BCYE, except the 3 antibiotics supplements are added (Polymyxin B sulfate, Vancomycin hydrochloride, Anisomycin), two indicators (Bromothymol blue, Bromocresol purple), and ammonium-free glycine. ___ Polymyxin B sulfate, Vancomycin hydrochloride - sterilized by filtration with a 0.2 um or lower pore size, and stored at -20 ± 3 °C for not more than 3 months. ___ Anisomycin – prepared fresh solution ___ Indicators - sterilized by filtration with a 0.2 um or lower pore size, and stored at 5 ± 3 °C for a maximum of 1 year. ___ Final pH 6.8 ±0.2 at 25 °C.						00d335aa	
i ___ Agars – Blood, Nutrient and Tryptic soy agar (ISO 11731:2017(E), Annex B.6, B.7 and B.8): ___ Blood Agar – pH adjusted to 6.8 ± 0.2 at 25 °C, autoclaved at 3 °C for 15 ± 1 min, cooled in a water bath (48 ± 3 °C), poured to a depth of 4 mm, and stored at in the dark at 5 ± 3 °C for up to 4 weeks. ___ Nutrient Agar – pH adjusted to 6.8 ± 0.2 at 25 °C, autoclaved at 121 ± 3 °C for 15 ± 1 min, cooled at 48 ± 3 °C, poured to a depth of 4 mm, and stored in the dark 5 ± 3 °C for up to 8 weeks. ___ TSA - pH adjusted to 6.8 ± 0.2 at 25 °C, autoclaved at 121 ± 3 °C for 15 ± 1 min, cooled at 48 ± 3 °C, poured to a depth of 4 mm, and stored in the dark 5 ± 3 °C for up to 8 weeks.						00d335bb	