NATIONAL ENVIRONMENTAL LABORATORY ACCREDITATION CONFERENCE (NELAC)

ON-SITE LABORATORY ASSESSMENT

MICROBIOLOGY CHECKLIST (24 PAGES TOTAL)

LABORATORY: _____

Physical Address: _____

_____

Mailing Address: _____
(if different from above)

_____

Telephone Number: _____ Facsimile Number: _____

E-mail address: _____

INSPECTED BY: (Name) (Affiliation)

_____ _____

_____ _____

_____ _____

INSPECTION DATES: _____

LABORATORY TECHNICAL DIRECTORS AND MANAGEMENT:
(Name) (Title)

_____ _____

_____ _____

_____ _____

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GENERAL INSTRUCTIONS: Before each item is a blank line and a NELAC Standard citation in **Bold Numerals**.

Place a check mark (__----) in the blank if the laboratory meets the NELAC Standard referenced.

Place an X-mark (X) in the blank if the Standard is not met and the laboratory must devise an acceptable Plan of Correction and estimated completion date. **The NELAC Standard reference must be cited in in the on-site assessment report.**

Mark **"N/A"** in the blank if the NELAC Standard is not applicable to this laboratory, either because of the nature of its business mission, because of the analytical tests it performs, or because of the situation never occurring

Notes: 40 CFR Part 136.3, Table 1A mandates the use of test methods **SM9213D, SM9221B, SM9221E, SM9222B, SM9222D, SM9221F, SM9222G, SM9230B, SM9230C; EPA-600/8-78-017; EPA 1103.1, 1106.1, 1600, 1603, 1604 (2002 versions for all EPA mtds.); USGS B-0025-85, B-0050-85, B-0055-85; AOAC991.15; HACH m-ColiBlue 24; ASTM D5259-92, D5392-93, D6503-99; IDEXX Colilert, Colilert-18, Enterolert**

40 CFR Part 141.21(f) mandates the use of test methods **SM9215B, SM9221B, SM9221D, SM9221E, SM9222B, SM9223B (with UV for E. coli), EC+MUG (EPA 1104), Nutrient Agar+MUG (EPA 1105), MI Agar (EPA 1604), E*Colite, m-ColiBlue24, ReadyCult, ChromoCult, & Colitag**

40 CFR Part 141.74(a) mandates the use of test methods **SM9215B, SM9221B, SM9221E, SM9222B, SM9222D, SM9223B, EPA 1604, & Simplate**

40 CFR Part 503.8 mandates the use of test methods **SM9221E, SM9260D, & J. WPC Fed. 46, 2163**

The use of **APHA Standard Methods (SM)** implicitly requires compliance with applicable quality assurance requirements in **SM9020**

ALL references to SM refer to the **20th Edition** unless otherwise specified

If the laboratory appears to meet a particular NELAC Standard but does not have the documentation to back up its claim, use the following:

____ **5.0** Does the laboratory have **all items** identified in NELAC Chapter 5 Quality Systems **available** for on-site inspection or data audit

____ **5.1.1** Does the laboratory demonstrate compliance with requirements in **mandated test methods or regulations** that are more stringent than the corresponding NELAC Standards

Note: SDWA **Total Coliform Rule (TCR)** holding times are **30 hours**
SDWA **Surface Water Treatment Rule (SWTR)** holding times are **8 hours** & **<10 C**
SDWA **Long Term Stage 2** (LT2) Enhanced Surface Water Treatment Rule holding times are **30 hours** & **<10 C**
CWA holding times are **6 hours & thermal preservation at 4 C**

Note: In SDWA TCR HPC may be monitored in lieu of Residual Chlorine; if so, **R2A Medium** must be used & **HPC MCL is 500 CFU/mL**; SM9215B, SM9215C, & SM9215D are approved methods for TCR

Note: SDWA TCR MCL is **no more than 5% positive samples** if over > 40 samples per month, **No more that 1 positive sample** if < 40 samples per month

Note: SWTR requires **<10% positive samples** during previous 6 months to avoid filtration; Positive samples mean **Total Coliform > 100/100 mL** or **Fecal Coliform > 20/100 mL**

Note: SM9020B, 4i requires usage of **opened bottles of media** with **6 months**

Note: SM9020B, 3a requires **calibration of thermometers** or temperature-recording instruments against NIST-traceable references **semiannually**

MICROBIOLOGY LABORATORY TOUR

____ **5.5.8.3.1(a)(2)** Has the laboratory **checked samples** for **proper preservation** (e.g. pH, absence of free chlorine) prior to or during sample preparation or analysis

Note: These checks are not required for chlorinated water systems as long as:

(a) Sufficient Na2S2O3 was added to each sample container to dechlorinate at least 5 mg/L Chlorine in Drinking Water samples & at least 15 mg/L Chlorine in Non-Potable Water samples;

(b) The laboratory must have records showing that Chlorine was measured in the field & the actual concentration is documented; AND

(c) The laboratory must check one sample container from each commercial lot or prepared batch (for adequate Na2S2O3), to prove that 5 mg/L Chlorine in Drinking Water & 15 mg/L Chlorine in Non-Potable Water can be neutralized

____ **5.5.5.2.1(d)** Is the **support equipment acceptability** for use according to the **needs of the analysis** or the application for which the equipment is being used

____ **Heterotrophic Plate Count bacteria in PCA** incubation at **35 degrees Celsius (SM9215A)**

____ **Total Coliform bacteria** incubation at **35.0 +/- 0.5 degrees Celsius** (**SM9221B, SM9221D, SM9222B, EPA-600/8-78-017, & B-0025-85**)

____ **Fecal Coliform bacteria** incubations at **35.0 +/- 0.5 & 44.5 +/- 0.2 degrees Celsius** (**SM9221E, SM9222D, B-0050-85, EPA 1680, 1681, & EPA-600/8-78-017**)

____ **Total Coliform & Escherichia coli (E. coli)** incubation at **35.0 +/- 0.5 degrees Celsius** (**SM9223B; Colilert, Colilert-18, Colisure, MI Agar (EPA 1604), E*Colite, m-ColiBlue24, Colitag, AOAC991.15)**

____ **E. coli** incubation at **44.5 +/- 0.2 degrees Celsius** (**EC with MUG (SM9222G, SM9221F) or 35.0 +/- 0.5 degrees Celsius (Nutrient Agar with MUG**)

____ **E. coli** incubation at **35.0 +/- 0.5 degrees Celsius** for 2 hours then **44.5 +/- 0.2 degrees Celsius** for 22 hours (**SM9213D, D5392-93, EPA 1103.1, 1603)**

____ **Fecal Streptococcus & Enterococcus bacteria** incubation at **35.0 +/- 0.5 degrees Celsius** (**SM9230B, SM9230C, EPA 1600, EPA-600/8-78-017)**

____ **Fecal Streptococcus & Enterococcus** incubation at **41.0 +/- 0.5 degrees Celsius** (**SM9230C, Enterolert, EPA 1106.1, 1600, D5259-92, D6503-99**)

____ **Enterococcus** incubation at **45.0 +/- 0.5 degrees Celsius** (**EPA 1600**)

____ **Salmonella** incubated at **35 degrees Celsius** (**SM9260D**) or at **37 degrees & 40.0 +/- 0.2 degrees Celsius** (**J. WPCF 46, 2163**)

____ **Salmonella** incubated at **42 +/- 0.5 degrees Celsius (EPA 1682)** for MSRV & **36.0 +/- 1.5 degrees Celsius** for XLD, TSI, LIA, & Urease broths

____ **Total Coliform & Escherichia coli (E. coli)** incubation at **36.0 +/- 1.0 degrees Celsius (Readycult, Chromocult)**

____ **Total Coliform & Escherichia coli (E. coli)** incubation at **32-37 degrees Celsius (Coliscan)**

____ Temperature incubation for **alternate test methods & alternate test organisms**
LIST:

____ **5.5.5.2.1(d)** Is the following **support equipment** associated with microbiological testing checked with NIST traceable materials (where available)

- ____ pH meter
- ____ Balance(s)
- ____ Conductivity meter
- ____ Chlorine meter
- ____ Refrigerator(s) for sample storage and/or media storage
- ____ Water Baths
- ____ Incubators

QUALITY OF STANDARDS, REAGENTS, AND MEDIA

____ **D.3.6** Does the laboratory ensure that the **quality of reagents & media is appropriate** for the test concerned

____ **D.3.6(a)** Does the laboratory **only use culture media** from commercial **dehydrated powders** or purchased **ready-to-use**

Note: Preparation from basic ingredients is permitted if the commercial media is demonstrated not to provide adequate results or if the media is not available commercially; media prepared from basic ingredients must be tested for performance prior to first use (selectivity, sensitivity, sterility, growth promotion, growth inhibition), with the detailed testing criteria information documented & defined

____ **D.3.6(b)** Does the laboratory use reagents, commercial dehydrated powders, & media **within the shelf-life** of the product

____ **D.3.6(b)** **5.5.6.4(b)** Are **all** original containers of reagents & media **labeled** with an **expiration date**

____ **D.3.6(c)** Is the laboratory reagent water used in the preparation of **media solutions & buffers free** from **bactericidal & inhibitory substances**

____ **D.3.6(c)** Is the laboratory reagent water tested **monthly, when maintenance is performed** on the water treatment system, or **at start-up** when the period of disuse exceeds one month, for **chlorine residual, specific conductance, & Heterotrophic Plate Count**

____ **D.3.6(c)** Does the laboratory test its Microbiology reagent water **annually** for **toxic metals & Bacteriological Water Quality** (to determine presence of toxic agents or growth promoting substances)

Note: In the absence of any mandated test method requirements, the Bacteriological Water Quality Test is **not required** for laboratories that have documentation to show that their water source **meets the criteria**, as specified in the method, for **Type I or Type II** reagent water

____ **D.3.6(c)** Does the laboratory maintain records on **all water quality checks** (for 5 years) & meet the following criteria for acceptance (**SM9020B**, 4d & **EPA-600/8-78-017**, Part IV-A, 5.2):

pH 5.5-7.5 (measured each use)
Residual Chlorine < 1.0 mg/L (monthly)
Conductivity < 2.0 umho/cm at 25 degrees Celsius (with each use)
Heterotrophic Plate Count < 1000 colony forming units per mL (monthly)
Bacteriological ratio 0.8 – 3.0 (annually, **EPA-600/8-78-017** only)
Cd, Cr, Cu, Ni, Pb, Zn each < 0.05 mg/L, collectively < 0.1 mg/L (annually)
NH3, Organic Nitrogen < 0.1 mg/L (monthly check)
TOC < 1 mg/L (monthly)
Student's t < 2.78 for Use Test (quarterly & for new water source)

____ **D.3.6(d)** Does the laboratory have records on **media preparation in the laboratory** that includes the date of preparation, preparer's initials, type & amount of media prepared, manufacturer, & lot number, final pH of the media, & expiration date

____ **D.3.6(d)** Does the laboratory's documentation on media **purchased pre-prepared, ready-to-use** include manufacturer, lot number, type & amount of media received, date of receipt, pH of the media, and expiration date

____ **D.3.6(d)** Are the media, solutions, & reagents **prepared, used, & stored** according to a documented procedure that **follows the manufacturer's instructions or the test method**

____ Heterotrophic Plate Count Medium (**SM9215A**, 6; **SM9215B**, 3a; **SM9215C**, 3; & **SimPlate**): (R2A or PCA)
Autoclaved at 121 degrees Celsius for 15 minutes
Final pH 6.8-7.2 for Plate Count Agar (PCA) (nutritionally rich medium) (approved for SDWA SWTR & only for Pour Plate Method)
Adjusted pH to 7.0-7.4 for R2A Agar (low nutrient medium) (approved for SDWA TCR)
Final pH 6.7-7.3 for SimPlate (multiple enzyme technology)
Sterile agar medium melted not more than once
Melted agar used within 3 hours, agar tempered at 44-46 C before pouring
Medium predried for water-weight loss of 2-3 g prior to use (Spread-Plate Method)

____ Phosphate buffer (**SM9050C**, 1a; **EPA-600/8-78-017**, Part II-B, 7.1; & **EPA 9131**, 5.2):
Stock buffer autoclaved at 121 degrees Celsius for 15 minutes
Stock buffer final pH 6.7-7.7
Dilution rinse water prepared from stock buffer & MgCl2

____ Peptone water (**SM9050C**, 1b; **EPA-600/8-78-017**, Part II-B, 7.2; & **EPA 9131**, 5.2):
10% peptone stock solution autoclaved or filter-sterilized
0.1% peptone water prepared as dilution rinse water
Final pH 6.8 (recommended)

____ Tryptic Soy, Trypticase Soy, and Tryptone non-selective media (TSB) (enrichment media for various mtds.)
Autoclaved at 121 degrees Celsius for 12-15 minutes
Final pH 7.1-7.5 (manufacturer instructions)

____ m-Endo Medium (**SM9222B**, 2; **EPA-600/8-78-017**, Part II-B, 5.2.2 & 5.2.4; & **EPA 9132**, 5.2):
Medium brought to a boil, then removed immediately (not autoclaved)
Ethanol used is not denatured
Prepared in sterilized flask
Final pH 7.0-7.4 (manufacturer's instructions)
Uninoculated media discarded if growth or surface sheen observed
RCRA: Unopened media kept no longer than 2 years

____ Lauryl Tryptose (Lauryl Sulfate) (**SM9221B**, 1a; **EPA-600/8-78-017**, Part II-B, 5.3.1; **EPA 9131**, 5.3; **EPA 1103.1**, 7.9; **EPA 1603**, 7.15):
Lactose Broth allowed if parallel testing study on file showing equivalent results with LTB
Bromcresol Purple may also be added
Formulated so that concentration is single-strength after sample addition
Autoclaved at 121 degrees Celsius for 12-15 minutes
Final pH 6.6-7.0 (manufacturer's instructions)
Inverted vials in sterilized media, one-third to one-half covered by media, & free of air bubbles
RCRA: Unopened media kept no longer than 2 years

____ Brilliant Green Lactose Bile Broth (**SM9221B**, 2a; **EPA-600/8-78-017**, Part II-B, 5.3.2; & **EPA 9131**, 5.4):
Brilliant Green Agar (**SM9260D**):
Autoclaved at 121 degrees Celsius for 12-15 minutes
Final pH 7.0-7.4 (manufacturer's instructions)

____ Presence-Absence Test Medium (Clark's P/A) (**SM9221D**, 1a):
Autoclaved at 121 degrees Celsius for 12 minutes, with space allowed between bottles
Final pH 6.6-7.0 (manufacturer's instructions)
Discarded if liquid evaporation exceeds 10% of original volume

____ EC Medium (**SM9221E**, 1a; **EPA 1680**, 7.7 & **EPA-600/8-78-017**, Part II-B, 5.3.4; **EPA 1103.1**, 7.14; **EPA 1603**, 7.11):
Autoclaved at 121 degrees Celsius for 12-15 minutes
Final pH 6.7-7.1 (manufacturer's instructions)
Inverted tubes one-third to one-half covered by media & free of air bubbles

____ MMO-MUG Medium (Colilert, Idexx-18, or Quantitray: **SM9223B**, 1); or (**Colisure**)
Commercial preparation used
Colilert: o-Nitrophenyl-b-D-Galactopyranoside & 4-Methylumbelliferyl-b-D-Glucoronide (MUG)
Colisure: Chlorophenol Red b-D-Galactopyranoside & 4-Methylumbelliferyl-b-D-Glucoronide
Protected from light
Not autoclaved
Final pH 7.0-7.6 (Colilert instructions)

____ MI Agar (**EPA 1604**), **m-Coliblue24**, **Coliscan**, or **ChromoCult** (MF); or
E*Colite, **Colitag**, **ReadyCult** (MMO-MUG)
Commercial preparation used & manufacturer's directions followed
MI Medium: 4-Methylumbelliferyl-b-D-Galactopyranoside & Indoxyl-b-D-Glucoronide;
Cefsulodin antibiotic inhibits growth of gram-negative background organisms
Final pH 6.75-7.15 for MI agar, 6.85-7.25 for MI broth
m-Coliblue24: 2,3,5-Triphenyl-Tetrazolium Chloride (TTC) & 5-Bromo-4-Chloro-3-Indolyl-b-D-Glucoronide, final pH 6.8-7.2
Chromocult Agar: SalmonGal & X-Gluc, final pH 6.6-7.0
Coliscan: RedGal & Indoxyl-b-D-Glucoronide, final pH 6.8-7.2
Colitag: o-Nitrophenyl-b-D-Galactopyranoside, 4-Methylumbelliferyl-b-D-Glucoronide, & TMAO
ReadyCult & E*Colite: 5-Bromo-4-chloro-3-indolyl-b-D-Galactopyranoside & 4-Methylumbelliferyl-b-D-Glucoronide (ReadyCult final pH 6.7-7.0)

____ EC Medium + MUG (**EPA 1104**, 7; **SM9221F**, 1a; **SM9222G**, 1c2):
Autoclaved at 121 degrees Celsius for 12-15 minutes
Final MUG concentration 50 ug/mL
Final pH 6.7-7.1 (manufacturer's instructions)
Inverted vial in test tube not used
Checked for absence of fluorescence prior to use (with 6-watt, 366-nm UV light)

____ Nutrient Agar (**EPA 1103.1**, 7.10; **EPA 1603**, 7.7)
Nutrient Agar + MUG (**EPA 1105**, 7; **SM9222G**, 1c1):
Autoclaved in 100-mL volumes at 121 degrees Celsius for 15 minutes
Final MUG concentration 100 ug/mL
Final pH 6.6-7.0 (manufacturer's instructions)

____ m-FC Broth or Agar (**SM9222D**, 1a & **EPA-600/8-78-017**, Part II-B, 5.2.1):
Medium brought to boiling & removed immediately; not autoclaved
Final pH 7.2-7.6 (manufacturer's instructions)

____ A-1 Medium (**SM9221E**, 2a & **EPA 1681**, 7.6):
Autoclaved at 121 degrees Celsius for 10 minutes
Final pH adjusted to 6.8-7.0
Inverted tubes one-third to one-half covered by media & free of air bubbles
Note: Can be stored in the dark at room temperature, but must be used within 1 week

____ m-E Agar (**SM9230C**, 2a; **EPA 1106.1**, 7.5 or 7.6; **D5259-92**, 8.4):
Medium must be sterilized, use manufacturer's procedure (contains Sodium Azide, TTC, & Nalidixic Acid)
Final pH 6.9-7.3 (manufacturer's instructions)

____ EIA Substrate (Esculin Iron Agar) (**SM9230C**, 2b; **EPA 1106.1**, 7.6 or 7.7; **D5259-92**, 8.5):
Final pH 6.9-7.3 (manufacturer's instructions)
Autoclaved at 121 degrees Celsius for 15 minutes after pH is adjusted

____ m-Enterococcus Agar (**SM9230C**, 2c):
Medium not autoclaved (contains Sodium Azide & TTC)

____ m-EI Agar (**EPA 1600**, 7.5):
m-E medium with Indoxyl-b-D-Glucoside
Autoclaved at 121 degrees Celsius for 15 minutes
Final pH 6.9-7.3 (m-E instructions)

____ Brain Heart Infusion Broth & Agar (BHI) (**SM9230C**, 2d & 2e; **EPA-600/8-78-017**, Part II-B, 5.4.5 & 5.4.6; **EPA 1106.1**, 7.8 & 7.10; **EPA 1600**, 7.6 or 7.7 & 7.8 or 7.9):
Autoclaved at 121 degrees Celsius for 15 minutes
Final pH 7.2-7.6 (or manufacturer's instructions)

____ Brain Heart Infusion Broth with 40% Bile (**EPA-600/8-78-017**, Part II-B, 5.4.9):
Autoclaved at 121 degrees Celsius for 15 minutes
Final pH 7.2-7.6 (BHI instructions)
10% oxgall (bile) filter sterilized, then added to BHI broth

____ Bile Esculin Agar (BEA) (**SM9230C**, 2f; **EPA 1106.1**, 7.11; **EPA 1600**, 7.9 or 7.10):
Autoclaved at 121 degrees Celsius for 15 minutes
Final pH 6.4-6.8 (or manufacturer's instructions)

____ Brain Heart Infusion Broth with 6.5% NaCl (**EPA 1106.1**, 7.9; **EPA 1600**, 7.7 or 7.8):
Autoclaved at 121 degrees Celsius for 15 minutes
Final pH 7.2-7.6 (BHI instructions)
NaCl added to BHI broth

____ Azide Dextrose Broth (**SM9230B**, 1a & **EPA-600/8-78-017**, Part II-B, 5.4.2; **EPA 1106.1**, 7.12; **EPA 1600**, 7.11):
Medium sterilized at 121 degrees Celsius & 12 PSI for 15 minutes
Final pH 7.0-7.4 (or manufacturer's instructions)

____ Pfizer Selective Enterococcus Agar (**SM9230B**, 1b & **EPA-600/8-78-017**, Part II-B, 5.4.4):
(also known as Bile Esculin Azide Agar, BEAA)
Autoclaved at 121 degrees Celsius for 15 minutes
Final pH 6.9-7.3 (or manufacturer's instructions)

____ KF Streptococcus Agar (**EPA-600/8-78-017**, Part II-B, 5.4.1):
Sterilized by boiling for 5 minutes; not autoclaved
Final pH 7.0-7.4

____ Dulcitol Selenite Broth (**SM9260D & J. WPCF 46, 2163**) (found in **EPA-600/8-78-017**, Part II-B, 5.5.3):
Medium not autoclaved
Final pH 6.7-7.1

____ Tetrathionate Broth (**SM9260D**, alternate medium to Dulcitol Selenite) (found in **EPA-600/8-78-017**, Part II-B, 5.5.2):
Medium not autoclaved
Final pH 7.6-8.0

____ Modified Semisolid Rappaport-Vassiliadis Agar (MSRV) (**EPA 1682**, 7.7)
(a) 2% Novobiocin stock solution filter-sterilized through 0.22-um porosity filter
(b) Basal medium agar containing Malachite Green, not autoclaved, final pH 5.0-5.4
(a) & (b) combined
Novobiocin & Malachite Green inhibit growth of non-Salmonella species

____ Xylose Lysine Desoxycholate Agar (XLD) (**SM9260D & J. WPCF 46, 2163**) (**EPA 1682**, 7.8) (also found in **EPA-600/8-78-017**, Part II-B, 5.5.7):
Medium not autoclaved
Final pH 7.2-7.6

____ Triple Sugar Iron Agar (TSI) (**SM9260D & J. WPCF 46, 2163**) (**EPA 1682**, 7.9) (also found in **EPA-600/8-78-017**, Part II-B, 5.5.9):
Sterilized at 118-121 degrees Celsius & 12 PSI for 15 minutes
Final pH 7.1-7.5

____ Lysine Iron Agar (LIA) (**SM9260D**) (**EPA 1682**, 7.10) (also found in **EPA-600/8-78-017**, Part II-B, 5.5.10):
Autoclaved at 121 degrees Celsius for 12 minutes
Final pH 6.5-6.9

____ Urease Test Broth (**EPA 1682**, 7.11):
Contains Urea, Phenol Red, & Yeast
Medium not autoclaved
Final pH 6.7-6.9

____ Salmonella O Antiserum Polyvalent Groups A-I & Vi (**EPA 1682**)

____ m-TEC Agar (**SM9213D**, 3a1; **EPA 1103.1**, 7.6; **D5392-93**, 8.6)
Autoclave at 121 C & 15 PSI for 15 minutes
Final pH 7.1-7.5 (or manufacturer's instructions)

____ Urea Substrate (**SM9213D**, 3a2; **EPA 1103.1**, 7.7; **D5392-93**, 8.7)
Contains Urea & Phenol Red
pH adjusted to 3.0-4.0
Color appearance should be straw-yellow

____ modified m-TEC Agar (**EPA 1603**, 7.6)
Contains the chromogen 5-Bromo-6-chloro-3-indolyl-b-D-glucuronide
Autoclave at 121 C & 15 PSI for 15 minutes
Final pH 7.1-7.5 (or manufacturer's instructions)

____ Simmons Citrate Agar (**EPA 1103.1**, 7.12; **EPA 1603**, 7.9)
Contains Citric Acid & Bromthymol Blue
Autoclave at 121 C & 15 PSI for 15 minutes
Final pH 6.7-7.1 (or manufacturer's instructions)

____ Enterolert (**D6503-99**)
Commercial preparation used & manufacturer's directions followed
(MUG is to the glucosidase activity and not the glucuronidase activity)

____ Kovac's Indole Reagent (**READYCULT; EPA 1103.1**, 7.16; **EPA 1603**, 7.13)
Contains p-Dimethylaminobenzaldehyde

____ Cytochrome Oxidase Reagent (**EPA 1103.1**, 7.15; **EPA 1603**, 7.12)
Contains N,N,N'N'-Tetramethyl-p-Phenylenediamine Dihydrochloride

Alternate Media / Reagents & preparation requirements:

____ Storage of prepared media (**SM9020B**, 4i4 & **SM9050A**, 1; **EPA-600/8-78-017**, Part IV-A, 7.9; & **EPA 9131**, 8.3.7):
Unused Membrane Filter broth refrigerated & used within 96 hours
Membrane Filter agar plates, tight-fitting covers, refrigerated & used within 2 weeks
Media in tubes/containers with loose-fitting closures refrigerated & used within 2 weeks
Broth media in tubes/containers with screw caps used within 3 months
(refrigeration required for 19th ed. SM and earlier editions)
Poured HPC agar in plates sealed in plastic bags, refrigerated, & used within 2 weeks
HPC agar stored in screw-cap flask or container refrigerated & used within 3 months
Refrigerated fermentation tube media incubated overnight prior to use;
media indicating growth not used
OR
Fermentation tube media stored at 25 C used within 2 weeks, evaporative losses < 1 mL

COMMENTS:

SELECTIVITY; CONSTANT AND CONSISTENT TEST CONDITIONS

____ **D.3.7(a)** Does the laboratory use **reference cultures of microorganisms** for positive & negative controls obtained from a **recognized national collection,** organization, or manufacturer recognized by the NELAP Accrediting Authority
Note: Microorganisms can be single-use preparations or cultures maintained by documented procedures that demonstrate continued purity & viability of the organism

____ **D.3.7(a)(1)** Are reference cultures of microorganisms **revived** (if freeze-dried) or transferred from slants & subcultured **only once** to provide **reference stocks**

____ **D.3.7(a)(1)** Are reference stocks **preserved by a technique** that maintains the **desired characteristics** of the strain

____ **D.3.7(a)(1)** Are the **working stocks** of microorganisms for routine work **prepared** from the reference stocks

____ **D.3.7(a)(1)** Are reference stocks that have been thawed **not re-frozen & re-used**

____ **D.3.7(a)(2)** Are microorganism working stocks **not sequentially subcultured** more than **5 times**

____ **D.3.7(a)(2)** Are working stocks of microorganisms **not subcultured to replace** reference stocks

____ **D.3.8(a)** Are the laboratory floors & work surfaces where Microbiology testing takes place **non-absorbant and easy to clean** & disinfect

____ **D.3.8(a)** Are **work surfaces** adequately **sealed**

____ **D.3.8(a)** Is the laboratory storage spaces for Microbiology testing **sufficient, clean, & free from accumulation of dust**

____ **D.3.8(a)** Does the laboratory **prohibit plants, food, & drink** from the Microbiology work area

____ **D.3.8(b)(1)** Are the available **temperature monitoring devices** that are used in incubators, autoclaves, refrigerators, or other equipment where temperature accuracy has a direct effect on the Microbiological analysis of **appropriate quality** to achieve **specifications** in the test method (e.g. no separations in liquid column for liquid-in-glass thermometers)

____ **D.3.8(b)(1)** Is the **scale of graduations** for each temperature measuring device appropriate for the **required accuracy of measurement**

____ **D.3.8(b)(1)** Is each temperature measuring device (e.g. liquid-in-glass thermometers, thermocouples, platinum resistance thermometers) **calibrated at least annually** to national or international standards for temperature

____ **D.3.8(b)(2)(i)** Has the laboratory evaluated the **functional properties & performance** (e.g. heat distribution characteristics) **for each autoclave** with respect to typical uses

____ **D.3.8(b)(2)(i)** Is the autoclave capable of **meeting specified temperature tolerances**

____ **D.3.8(b)(2)(i)** Are **pressure cookers not used** for sterilization of growth media

____ **D.3.8(b)(2)(ii)** Does the laboratory **demonstrate sterization temperature** by using a **continuous temperature recording device or maximum registering thermometer** with each cycle

____ **D.3.8(b)(2)(ii)** Does the laboratory use **appropriate biological indicators once per month** to determine **effective sterilization**

____ **D.3.8(b)(2)(ii)** Does the laboratory use **temperature sensitive tape** with the contents of **each autoclave run** to indicate that the autoclave contents have been processed

____ **D.3.8(b)(2)(iii)** Does the laboratory record the date, contents, maximum temperature reached, pressure, time in sterilization mode, total run time (may be documented as time in & time out), and analyst's initials for **every cycle of autoclave operations**

____ **D.3.8(b)(2)(iv)** Does the laboratory perform **autoclave maintenance annually** (either internally or by service contract) which includes a **pressure check & calibration of the temperature device**
Note: Records of this maintenance are to be kept in equipment logs

____ **D.3.8(b)(2)(v)** Does the laboratory check the **autoclave mechanical timing device quarterly** against a stopwatch, and document the **actual elapsed time**

____ **D.3.8(b)(3)(i)** Does the laboratory **calibrate volumetric equipment with movable parts**, such as automatic dispensers, dispensers/diluters, & mechanical hand pipettes **quarterly**

____ **D.3.8(b)(3)(ii)** Does the laboratory **calibrate volumetric equipment** such as filter funnels, bottles, non-Class A glassware, & **other marked containers once per lot** prior to first use

____ **D.3.8(b)(3)(iii)** Does the laboratory check the volume of **disposable volumetric equipment** such as sample bottles, disposable pipettes, & micropipette tips **once per lot**

____ **D.3.8(b)(4)** If used for sanitation, are **UV instruments tested quarterly** for effectiveness with an appropriate **UV light meter** or by **plate count agar spread plates**

Note: UV bulbs must be replaced if output is **less than 70% of the original** for light tests (254 nm) or if count reduction is **less than 99%** for a plate containing 200-300 organisms

____ **D.3.8(b)(5)** Are **conductivity meters, oxygen meters, pH meters, hygrometers**, & other support equipment **calibrated** according to the **method-specified requirements**

COMMENTS:

____ **D.3.8(b)(6)(i)** Has the laboratory established the **stability, uniformity of temperature distribution, & time to re-establish thermal equilibrium conditions** (after test sample additions) in incubators & water baths

____ **D.3.8(b)(6)(i)** Does the laboratory document temperatures of incubators & water baths **twice daily, at least 4 hours apart**, on each day of use

____ **D.3.8(b)(6)(ii)** Are **ovens used for sterilization** checked for sterilization **effectiveness monthly** with appropriate biological indicators

____ **D.3.8(b)(6)(ii)** Does the laboratory maintain records of **each sterilization cycle for the oven** that include date, cycle time, temperature, contents, & analyst's initials

____ **D.3.8(b)(7)(i)** Does the laboratory have a documented procedure for **washing labware** if applicable

____ **D.3.8(b)(7)(i)** Does the laboratory use **detergents designed for laboratory use** for washing labware

____ **D.3.8(b)(7)(ii)** Is the laboratory's glassware used for Microbiological analysis **made of borosilicate** or other non-corrosive material, **free of chips & cracks**, and have **readable measurement marks**

____ **D.3.8(b)(7)(iii)** Is labware that is **washed & reused** tested for possible presence of residues which **may inhibit or promote growth** of microorganisms by performing the **Inhibitory Residue Test annually**

____ **D.3.8(b)(7)(iii)** Does the laboratory perform the Inhibitory Residue Test each time it **changes the lot of detergent or washing procedures**

____ **D.3.8(b)(7)(iv)** Does the laboratory test washed labware **at least once daily**, each day of washing, for possible **acid or alkaline residues** by testing at least one piece of labware with a **suitable pH indicator** such as bromothymol blue

Note: Records of these tests must be maintained

COMMENTS:

MICROBIOLOGY TEST METHODS

____ **5.5.4.1.2(a)** Does the laboratory have an **in-house methods manual** for each accredited **analyte** or **method**
Note: This manual may consist of copies of published or referenced test methods

____ **5.5.4.1.2(b)** Does the laboratory **clearly indicate** in its methods manual **any modifications** made to the referenced test method and **describe any changes or clarifications** where the referenced test method is ambiguous or provides insufficient detail

Does each test method in the in-house methods manual include or reference, where applicable:

____ **5.5.4.1.2(b)(1)** **Identification** of the test method
____ **5.5.4.1.2(b)(2)** Applicable **matrix or matrices**
____ **5.5.4.1.2(b)(3)** **Method Detection Limit**
____ **5.5.4.1.2(b)(4)** **Scope & application**, including components to be analyzed
____ **5.5.4.1.2(b)(5)** **Summary** of the test method
____ **5.5.4.1.2(b)(6)** **Definitions**
____ **5.5.4.1.2(b)(7)** **Interferences**
____ **5.5.4.1.2(b)(8)** **Safety**
____ **5.5.4.1.2(b)(9)** **Equipment & supplies**
____ **5.5.4.1.2(b)(10)** **Reagents & standards**
____ **5.5.4.1.2(b)(11)** Sample **collection, preservation, shipment, & storage**
____ **5.5.4.1.2(b)(12)** **Quality control**
____ **5.5.4.1.2(b)(13)** **Calibration & standardization**
____ **5.5.4.1.2(b)(14)** **Procedure**
____ **5.5.4.1.2(b)(15)** **Data Analysis & Calculations**
____ **5.5.4.1.2(b)(16)** Method **performance**
____ **5.5.4.1.2(b)(17)** Pollution **prevention**
____ **5.5.4.1.2(b)(18)** **Data assessment & acceptance criteria** for quality control measures
____ **5.5.4.1.2(b)(19)** **Corrective actions** for out-of-control data
____ **5.5.4.1.2(b)(20)** Contingencies for **handling out-of-control or unacceptable data**
____ **5.5.4.1.2(b)(21)** **Waste management**
____ **5.5.4.1.2(b)(22)** **References**
____ **5.5.4.1.2(b)(23)** **Tables, diagrams, flowcharts, validation data**

____ **D** Does the laboratory ensure that the **essential standards** outlined in Appendix D are incorporated into the method manuals and/or Quality Manual

COMMENTS:

MICROBIOLOGY TEST METHODS ASSESSED: _____

____ **5.5.4.2.2(a) C.1** Has the laboratory performed a **satisfactory demonstration of method capability** prior to the acceptance & institution of this test method

Note: See Appendix D.3.3(a) for the **specific procedural requirements** for Microbiology testing

Note: The 4-replicate procedure below is **required** for the 2005 versions of **EPA 1103.1 & 1106.1** (recommended procedure in 2005 versions of EPA 1600, 1603, 1680, 1681, & 1682)

____ **C.1** Does the laboratory **document** in its Quality Manual **other adequate approaches** to **Demonstration of Capability** if this procedure is **not required** by the mandated test method or regulation and if the laboratory **elects not to perform** this procedure

____ **C.1(b)** Are the analytes diluted in a volume of **clean quality system matrix** sufficient to prepare **4 aliquots** at the **specified concentration** or to a concentration approximately **1-4 times** the **limit of quantitation**

____ **C.1(c)** Are **at least 4 such aliquots prepared & analyzed** according to the test method

Note: These analyses may occur either concurrently or over a period of days

____ **C.1(d)** Does the laboratory **calculate the mean recovery** in the appropriate reporting units & the **standard deviation** of the population sample (n-1) in the same units for **each parameter of interest** using **all of the analysis results obtained**

Note: When it is not possible to assess mean & standard deviation, such as **for presence-absence & logarithmic values,** the laboratory must assess performance against established & documented criteria

____ **C.1(e)** Are the mean and standard deviation for each parameter **compared** to the corresponding **acceptance criteria for precision & accuracy** in the test method (if applicable) or in laboratory-generated acceptance criteria (if the method or analyte is non-standard)

Note: Acceptance criteria specified in the following test methods (Sec. 9.3 in each method):

EPA 1103.1: Mean recovery 76-124% for lab-prepared spike, 68-96% for BioBall; Precision <41% RSD for lab-prepared spike, <25% RSD for BioBall

EPA 1106.1: Mean Recovery 15-136% for lab-prepared spike, 86-102% for BioBall; Precision <21% RSD for lab-prepared spike, <12% for BioBall

EPA 1600: Mean Recovery 31-127% for lab-prepared spike, 85-106% for BioBall; Precision <28% RSD for lab-prepared spike, <14% RSD for BioBall

EPA 1603: Mean Recovery 46-119% for lab-prepared spike, Detect-144% for BioBall; Precision <36% RSD for lab-prepared spike, <61% RSD for BioBall

EPA 1680: Mean Recovery 65-221%; Precision <84% RSD

EPA 1681: Mean Recovery 1-312%; Precision <96% RSD

EPA 1682: Mean Recovery 0-254% for lab-prepared spike, 22-126% for BioBall; Precision <92% RSD for lab-prepared spike, <69% RSD for BioBall

____ **C.1(e)** Does the laboratory consider the performance unacceptable & **not analyze actual samples** for parameters that **fail the acceptance criteria**

____ **C.1(f)** When one or more parameters **fail** at least one of the **acceptance criteria**, does the analyst:

- **Locate & correct** the source of the problem, then **repeat the test** for all parameters of interest, OR
- **Repeat the test** for all parameters that failed to meet criteria

Note: Repeated failure from employing the second option above indicates a general problem with the entire measurement system, and the analyst must then perform the first option above

____ **5.5.4.2.2(d) C.2** Does the laboratory use the **NELAC-specified certification statement** to document the **completion of each Demonstration of Capability** (initial & continuing)

____ **C.2** Are copies of these certification statements retained in the **personnel records** of each **employee performing the test method**

____ **5.5.2.6(c)(3)** Does each Analyst have **documentation** of **continued proficiency** by at least **one of the following once per year**:

- Acceptable performance of a **blind sample** (single blind to the analyst)
- Another **demonstration of capability or initial measurement system evaluation**
- Successful performance of a blind performance sample on a **similar test method** using the **same technology** (acceptable limits must be determined prior to analysis)
- At least **4 consecutive** laboratory **control samples** with **acceptable levels** of precision & accuracy (acceptable limits for precision & accuracy must be determined prior to analysis)
- Analysis of **authentic samples** that have been analyzed by **another trained analyst** with **statistically identical results**

Note: Acceptance criteria specified in the following test methods (Sec. 9.4 in each method):
EPA 1103.1: Mean recovery 54-146% for lab-prepared spike, 58-106% for BioBall
EPA 1106.1: Mean Recovery 14-137% for lab-prepared spike, 80-108% for BioBall
EPA 1600: Mean Recovery 27-131% for lab-prepared spike, 78-113% for BioBall
EPA 1603: Mean Recovery 38-127% for lab-prepared spike, Detect-144% for BioBall
EPA 1680: Mean Recovery 37-391%
EPA 1681: Mean Recovery 1-371%
EPA 1682: Mean Recovery 0-287% for lab-prepared spike, 1-147% for BioBall

____ **5.5.4.2.2(d)** Does the laboratory **retain all associated supporting data** necessary to **reproduce the analytical results** summarized in the appropriate certification statement

____ **5.5.4.2.2(e) C.1** Does the laboratory **complete a demonstration of capability each time** there is a **change** in **instrument type, personnel, or test method**

____ **5.5.4.2.2(f)** Does the laboratory **fully document** the achievement of **demonstration of capability requirements** for each **specialized work cell**
Note: A work cell is defined as a group of analysts with specifically defined tasks that together perform the test method

____ **5.5.4.2.2(g)** Does the laboratory demonstrate & document acceptable performance through **acceptable continuing performance checks** (e.g laboratory control samples) **each time** that **membership** in a work cell **changes**

____ **5.5.4.2.2(g)** Do the **new members** of the work cell **work with experienced analysts** in the specialty area

____ **5.5.4.2.2(g)** Does the laboratory **repeat a Demonstration of Capability** with the new work cell if the **first 4 continuing performance checks** following the change in personnel **produce a failure** in any sample batch acceptance criteria

____ **5.5.4.2.2(g)** Does the laboratory **repeat a Demonstration of Capability** if the entire **work cell is changed or replaced**

____ **5.5.4.2.2(h)** Is the **performance of the work cell** as a group **linked to the training records** of the **individual members** of the work cell

COMMENTS: If applicable, list all test species & test methods where the above Standards are not being met.

STERILITY CHECKS AND BLANKS

____ **D.3.1(a)** Does the laboratory demonstrate that filtration equipment & filters, sample containers, media, & reagents **have not been contaminated** through improper handling or preparation, inadequate sterilization, or environmental exposure

____ **D.3.1(a)(2)** For each filtration series in the filtration technique, is **one beginning & one ending sterility check** conducted for each laboratory sterilized unit used in a filtration series or, for pre-sterilized single-use funnels, one per lot

Note: The filtration series may include single or multiple filtration units that have been sterilized prior to beginning the series

____ **SM9222B, EPA 9132, & EPA-600/8-78-017** – Total Coliform bacteria by Membrane Filtration (MF) (m-Endo, then LTB & BGLB)

____ **SM9222D & EPA-600/8-78-017** – Fecal Coliform bacteria by MF (Non-potable Water only) (m-FC, then LTB & EC)

____ **SM9230C, EPA 1106.1, D5259-92** – Enterococcus bacteria by MF (Non-potable Water only) (m-E, then BHI & BEA)

____ **SM9230C & EPA-600/8-78-017** – Fecal Streptococcus bacteria by MF (Non-Potable Water only) (m-Enterococcus, then BHI & BEA)

____ **EPA 1600** – Enterococcus bacteria by MF (Non-potable Water only) (m-EI)

____ **MI Agar (EPA 1604)** – Total Coliform bacteria & E. coli by membrane filtration

____ **m-ColiBlue24** – Total Coliform bacteria & E. coli by membrane filtration

____ **ChromoCult** - Total Coliform bacteria & E. coli by membrane filtration (Drinking Water only)

____ **SM9213D, EPA 1103.1, D5392-93** – E. coli by membrane filtration (Non-Potable Water only) (m-TEC)

____ **EPA 1603** – E. coli by membrane filtration (Non-Potable Water only) (modified m-TEC)

____ **Coliscan** – Total Coliform & E. coli by membrane filtration (Drinking Water only)

____ Additional Test Methods:

____ **D.3.1(a)(2)** Is the membrane filtration series considered **ended** when more than **30 minutes elapses** between successive filtrations

____ **D.3.1(a)(2)** Is a sterility blank analyzed **every 10 samples** (unless filtration units are sanitized by UV light after each filtration)

Note: During a filtration series filter funnels must be rinsed with three 20-30 mL portions of sterile rinse water after each sample filtration

____ **D.3.1(a)(3)** For pour-plate technique does the laboratory make a **sterility blank** of the medium by **pouring at least one uninoculated plate** for each lot of prepared, ready-to-use media & for each batch of medium prepared in the laboratory

____ **D.3.1(a)(4)** Does the laboratory perform **sample container sterility checks** on at least one container for each lot of purchased, pre-sterilized containers, or on one container per sterilized batch for containers prepared & sterilized in the laboratory, with **nonselective growth media**

Note: Incubate at 35 C for 24 hours & check for growth

____ **D.3.1(a)(5)** Does the laboratory perform a **sterility blank on each batch of dilution water** prepared in the laboratory, & on **each batch** of prepared, ready-to-use dilution water, with **nonselective growth media**

Note: Incubate at 35 C for 24 hours & check for growth

____ **D.3.1(a)(6)** Does the laboratory check at least one filter from **each new lot** of **membrane filters** for sterility with **non-selective growth media**

Note: 24 hours incubation at 35 degrees Celsius & check for growth

POSITIVE AND NEGATIVE CONTROLS; TEST VARIABILITY / REPRODUCIBILITY

____ **D.3.1(a)(1)** Is a **sterility blank** analyzed for **each lot** of pre-prepared, ready-to-use medium & for **each batch** of medium prepared in the laboratory
Note: This blank must be analyzed **prior to first use** of the medium

____ **D.3.1(b)** Does the laboratory test **each lot** of prepared, ready-to-use medium & **each batch** of medium
D.3.4(a) prepared in the laboratory with at least one **pure culture** of a known **positive reaction**
Note: This positive culture control must be analyzed **prior to first use** of the medium and test organisms need to respond in an acceptable & predictable manner

____ **D.3.1(c)** Does the laboratory test **each lot** of prepared, ready-to-use medium & **each batch** of medium prepared in the laboratory with at least one or more **known negative culture controls** (non-target organisms) as appropriate to the method
Note: This negative culture control must be analyzed **prior to first use** of the medium

Sterility Control	**Positive Control**	**Negative Control**	
____	____		Heterotrophic Plate Count Agar (PCA, R2A, or Simplate)
____	____		Non-selective Medium (Tryptic Soy, Trypticase Soy, or Tryptone) (TSB)
____	____	____	m-Endo Broth or Agar
____	____	____	Lauryl Tryptose (Lauryl Sulfate) or Lactose Medium (LTB)
____	____	____	Brilliant Green Bile Broth (BGLB) or Agar
____	____	____	Presence-Absence Medium (Clark's, LTB with Bromcresol Purple)
____	____	____	MMO-MUG Medium (Colilert, Idexx-18 or Quantitray)
____	____	____	Colisure
____	____	____	EC Medium
____	____	____	EC Medium + MUG (EC+MUG)
____	____	____	Nutrient Agar
____	____	____	Nutrient Agar Medium + MUG (NA+MUG)
____	____	____	A-1 Medium
____	____	____	m-FC Broth or Agar
____	____	____	m-Enterococcus Agar
____	____	____	m-E Agar
____	____	____	m-EI Agar
____	____		Brain Heart Infusion (BHI) Broth & Agar
____	____	____	Brain Heart Infusion Broth with 40% Bile
____	____	____	Brain Heart Infusion Broth with 6.5% NaCl
____	____	____	Bile Esculin Agar
____	____	____	Azide Dextrose Broth
____	____	____	Pfizer Selective Enterococcus Agar (or Bile Esculin Azide Agar)
____	____	____	MI Agar
____	____	____	E*Colite
____	____	____	m-ColiBlue24
____	____	____	ReadyCult
____	____	____	ChromoCult
____	____	____	Enterolert
____	____	____	Colitag
____	____	____	Coliscan
____	____	____	Modified Semisolid Rappaport-Vassiliadis Agar (MSRV)
____	____	____	Xylose Lysine Desoxycholate Agar (XLD)
____	____	____	Triple Sugar Iron (TSI)
____	____	____	Lysine Iron Agar (LIA)
____	____	____	Urease Test Broth
____	____	____	m-TEC
____	____	____	Simmons Citrate Agar

____ **D.3.2** If the test method specifies **colony counts** (e.g. membrane filtration, HPC), does the laboratory verify the ability of individual analysts to count colonies at least **once per month** by having **two or more analysts count colonies** from the **same plate**

Note: Counts must be **within 10%** to be acceptable

Note: An analyst in a 1-person laboratory may do **repetitive counting** on the same plate, with **no more than 5% difference** between the counts

METHOD EVALUATION AND DATA REDUCTION

____ **D.3.3(a)** Has the laboratory **demonstrated proficiency** with the test method **prior to its first use**

Note: This can be done by analyzing at least **10 spiked samples** whose quality system matrix is representative of those normally submitted to the laboratory, by **passing one proficiency test series** provided by an approved PT Provider, or by **comparison to a method** already approved for use in the laboratory

____ **SM9215B** - Heterotrophic Plate Count by Pour-Plate Method
____ **SM9221B & EPA-600/8-78-017** - Total Coliform bacteria by Most Probable Number
____ **SM9221E, EPA 1680, & EPA-600/8-78-017** – Fecal Coliform bacteria by EC Medium
____ **SM9221E & EPA 1681** – Fecal Coliform bacteria by A-1 Medium (NPW or SCM only)
____ **SM9230B & EPA-600/8-78-017** – Fecal Streptococcus bacteria (Non-potable Water only)
____ **EPA 1600** – Enterococcus bacteria (Non-potable Water only)
____ **SM9260D, EPA 1682, & JWPC Fed.** – Salmonella bacteria (CWA Sludge Rule)
____ **EPA 9131** – Total Coliform bacteria by Multiple Tube Fermentation (RCRA program)
____ **SM9222B & EPA-600/8-78-017** – Total Coliform bacteria by Membrane Filtration (MF)
____ **SM9222D & EPA-600/8-78-017** – Fecal Coliform bacteria by MF (Non-potable Water only)
____ **SM9230C & EPA-600/8-78-017** – Fecal Streptococcus bacteria by MF (Non-potable Water only)
____ **SM9230C, EPA 1106.1, D5259-92** – Enterococcus bacteria by MF (Non-Potable Water only)
____ **EPA-600/8-78-017** – Fecal Streptococcus & Enterococcus by plate count (Non-potable Water only)
____ **EPA 9132** – Total Coliform bacteria by Membrane Filtration (RCRA program)
____ **SM9223B** – Total Coliform & E. coli (Colilert & Colilert-18 formulations)
____ **Colisure** – Total Coliform & E. coli (Drinking Water only)
____ **m-Coliblue 24** – Total Coliform & E. coli (MF)
____ **E*Colite** – Total Coliform & E. coli (Drinking Water only)
____ **MI Agar (EPA 1604)** – Total Coliform & E. coli (MF)
____ **ReadyCult** – Total Coliform & E. coli (Drinking Water only)
____ **ChromoCult** – Total Coliform & E. coli (Drinking Water only, MF)
____ **Enterolert & D6503-99** – Enterococcus bacteria by MPN (Non-Potable Water only)
____ **SM9213D, EPA 1103.1, D5392-93** – E. coli by 2-step MF (Non-Potable Water only)
____ **EPA 1603** – E. coli by single-step MF (Non-Potable Water only)
____ **Colitag** – Total Coliform & E. coli (Drinking Water only)
____ **Coliscan** – Total Coliform & E. coli (Drinking Water only)
____ **SimPlate** – Heterotrophic Plate Count by Enzyme Substrate method

Additional Test Methods:

____ **D.3.3(a)** Does the laboratory record & retain all Microbiological validation data as long as the **pertinent test method is in force** and for at least **5 years** past the date of its **last use**

____ **D.3.3(b)** Does the laboratory participate in **proficiency testing required by NELAP** & use the results to evaluate its ability to produce acceptable data

____ **5.5.9.2(d) D** Does the laboratory's **Microbiology data** indicate that the **quality control protocols** in the test methods manual are being followed

____ **D** Are all quality control measures **assessed & evaluated** on an **on-going basis**

____ **D.3.5** Does the laboratory follow the **calculations, data reduction, & statistical interpretations** specified in **each test method**
(**SM9020B**, 9b – adjust MF colony counts on Non-potable Water samples based on % verification)

____ **D.3.6(d)** Does the laboratory **use media, solutions, & reagents** according to a documented procedure following the **manufacturer's instructions** or the **test method**

____ **SM9215A**, 5 & 7 & 8: Heterotrophic Plate Count
All samples analyzed in **duplicate plates**
PCA incubated at 35.0 +/- 0.5 degrees Celsius for 48 +/- 3 hours (Pour Plate Method)
R2A incubated at 20-28 degrees Celsius for 5-7 days (Pour Plate, Spread Plate & Membrane Filtration methods)
Colonies counted with a **dark-field colony counter**, or one with equivalent magnification & illumination

____ **SM9221B**, 1b; **EPA-600/8-78-017**, Part III-B, 4; & **EPA 9131**, 7.1: Total Coliform Multiple Tube Fermentation with Lauryl Tryptose Medium
Drinking Water: **100 +/- 2.5 mL** sample analyzed (Total Coliform Rule)
Non-Potable Water: 3-dilution, 5-tube (for each dilution) technique for each sample
Incubated at 35.0 +/- 0.5 degrees Celsius for 24 +/- 2 hours
Drinking Water: If no gas detected after 24 hours, incubate for another 24 hours
All water samples producing turbid cultures with no gas production are invalidated, with another sample requested
RCRA program: If no gas detected after 24 hours, incubate & re-examine after 48 +/- 3 hours

____ **SM9221D**, 1b: Total Coliform with Presence/Absence Medium
100 +/- 2.5 mL sample analyzed (SDWA Total Coliform Rule)
Incubated at 35.0 +/- 0.5 degrees Celsius for 24 hours
If purple color indicator does not turn yellow, incubate for another 24 hours
All samples producing turbid cultures with no color change invalidated, with another sample requested

____ **SM9221E**, 1b & **EPA-600/8-78-017**, Part III-C, 5: Fecal Coliform Most Probable Number with EC Medium
3-dilution (sample volumes), 5-tube (per sample volume) technique for each sample
Each tube inoculated from positive culture grown on m-Endo or LTB medium
Incubated at 44.5 +/- 0.2 degrees Celsius for 24 +/- 2 hours
Gas formation indicates Fecal Coliform; no further verification needed

____ **EPA 1680 & 1681**, 11 & 12: Fecal Coliform Most Probable Number
At least 4 dilutions & 5 tubes per dilution required
EPA 1680: Enrichment in LTB medium (35 C for 24 +/- 2 hours (for positive results) or for 48 +/- 3 hours (for negative results) for Fecal Coliform)
EPA 1680: Gas-positive tubes from LTB inoculated into EC, incubated at 44.5 C for 24 +/- 2 hrs
EPA 1681: Direct inoculation into A-1 medium, incubation at 35.0 +/- 0.5 degrees Celsius for 3 hours, then at 44.5 +/- 0.2 C for 21 +/- 2 hours
EPA 1681: Gas formation indicates Fecal Coliform; no further verification needed

____ **SM9221E**, 2b: Fecal Coliform Most Probable Number with A-1 Medium
3-dilution (sample volumes), 5-tube (per sample volume) technique for each sample
Direct inoculation with sample possible
Incubated at 35.0 +/- 0.5 degrees Celsius for 3 hours (+/- 15 min), then at 44.5 +/- 0.2 C for 21 +/- 2 hours
Gas formation indicates Fecal Coliform; no further verification needed

____ **SM9222B**, 5a-5d; **EPA-600/8-78-017**, Part III-B, 2; & **EPA 9132**, 7.0: Total Coliform by Membrane Filtration
Can use m-Endo Broth or Agar; agar plates stored inverted, broth plates inverted or upright, per method requirements
Drinking Water: 100 mL sample filtered (Total Coliform Rule)
Non-Potable Water: Filter 3 different sample volumes so that at least one dilution will give 20-200 colonies
Non-Potable Water: Enrichment required if sample has residual Chlorine present
Non-Potable Water: Incubated at 35.0 +/- 0.5 degrees Celsius for 22-24 hours (m-Endo)
RCRA: Incubated at 35.0 +/- 0.5 degrees Celsius for 2 hr in LTB, then for 21 +/- 1 hr in m-Endo
Golden-green, metallic sheen colonies may indicate Total Coliform presence (due to aldehyde formation)

____ **SM9223B**, 2-3: Total Coliform & Escherichia coli by MMO-MUG (Colilert, Colilert-18, & Quantitray)
Colisure: Total Coliform & Escherichia coli by MMO-MUG
100 mL sample analyzed
COLILERT: Incubated at 35.0 +/- 0.5 degrees Celsius for 24 hours; incubated for additional 4 hours if color change or fluorescence indeterminate
COLISURE: Incubated at 35.0 +/- 0.5 degrees Celsius for 24-48 hours; must allow sample to equilibrate at room temperature prior to incubation start time
IDEXX-18: Incubated at 35.0 +/- 0.5 degrees Celsius for 18 hours; first 20 minutes MUST be in 35 C water bath, or else first 7-10 minutes in a 44.5 C water bath
NOTE: Pre-warming step not required for IDEXX-18 used with MPN Quantitray
Color change indicates Total Coliform (yellow color from colorless for Colilert & Idexx-18, yellow changing to red-magenta for Colisure); 366-nm blue-light fluorescence indicates E. coli

____ **SM9222D**, 2a-2d & **EPA-600/8-78-017**, Part III-C, 2: Fecal Coliform by Membrane Filtration
Filter volumes or dilutions that will give 20-60 fecal coliform colonies per membrane
Incubated at 44.5 +/- 0.2 degrees Celsius for 24 +/- 2 hours
Blue colonies (any shade of blue) may indicate Fecal Coliform

____ **SM9230B**, 2 & **EPA-600/8-78-017**, Part III-D, 4: Fecal Streptococcus Most Probable Number with Azide Dextrose Broth
3-dilution (sample volumes), 5-tube (per sample volume) technique for each sample
Incubated at 35.0 +/- 0.5 degrees Celsius for 24 +/- 2 hours
If no turbidity formation, reincubate & read again at end of 48 +/- 3 hours

____ **SM9230C**, 3a; **EPA 1106.1**, 11; & **D5259-92**, 12: Enterococcus Membrane Filtration with mE Agar
Filter 3 different sample volumes to give 20-60 colonies on the membrane surface
Incubated at 41.0 +/- 0.5 degrees Celsius for 48 +/- 3 hours
Pink to red colonies may indicate Enterococci (due to breakdown of 2,3,5-Triphenyl-Tetrazolium Chloride (TTC))
Filter transferred to EIA substrate; incubated at 41 C for 20-30 minutes
Brown precipitate on bottom of the plate indicates Enterococci

____ **SM9230C**, 3b: Fecal Streptococcus Membrane Filtration with m-Enterococcus Medium
Filter 3 different sample volumes to give 20-60 colonies on the membrane surface
Incubated at 35.0 +/- 0.5 degrees Celsius for 48 hours
Pink to red colonies may indicate Fecal Streptococcus (due to breakdown of 2,3,5-Triphenyl-Tetrazolium Chloride (TTC))

____ **EPA-600/8-78-017**, Part III-D, 2: Fecal Streptococcus Membrane Filtration with KF Streptococcus Agar
Filter 3 different sample volumes to give 20-100 colonies on the membrane surface
Incubated at 35.0 +/- 0.5 degrees Celsius for 48 hours

____ **EPA-600/8-78-017**, Part III-D, 5: Fecal Streptococcus Pour Plate Method
3 different sample dilutions analyzed to produce 30-300 colonies in the plate
Medium added TO the sample
KF Streptococcus Agar: Incubated at 35.0 +/- 0.5 degrees Celsius for 48 +/- 3 hours
Pfitzer Selective Enterococcus Agar: Incubated at 35.0 +/- 0.5 degrees Celsius for 18-24 hours

____ **SM9260D**, 1 & **J. WPCF 46, 2163**: Multiple Tube Enrichment Technique for Salmonella with Dulcitol Selenite Broth or Tetrathionate Broth
3 different sludge sample sizes used (3-dilution, 5-tube technique if water)
SM9260D: Incubated at 35 degrees Celsius for 24 hours
J. WPCF 46, 2163: Incubated at 40.0 +/- 0.2 degrees Celsius for 24 hours, then for additional 24 hours if no growth observed

____ **EPA 1682**, 11 & 12: Multiple Tube enrichment for Salmonella in TSB & MSRV
At least 3 dilutions & 5 tubes per dilution required
TSB: incubate at 24 +/- 2 hr at 36 +/- 1.5 degrees Celsius
MSRV: incubate at 42 +/- 0.5 degrees Celsius for 16-18 hours
Whitish halo in MSRV indicate presumptive positive results

____ **MI Agar (EPA 1604**, 11**)**: Total Coliform & Escherichia coli by Membrane Filtration
Filter volumes or dilutions that will give 20-60 E. coli colonies per membrane (recommended)
Incubated at 35 +/- 0.5 C for 24 +/- 2 hours
Fluorescent colonies or halos (366 nm) indicate Total Coliform; blue colonies indicate E. coli
Read results from the top of the plate with the lid off
Total Coliform TNTC & E. coli colonies are countable: Count E. coli colonies & report Total Coliform positive due to TNTC
E. coli TNTC or both target organisms > 200 colonies & uncountable: Report E. coli positive & Total Coliform positive due to TNTC

____ **m-ColiBlue24** (HACH 10029, 11): Total Coliform & Escherichia coli by Membrane Filtration
Incubated at 35 +/- 0.5 C for 24 +/- 4 hours
Red colonies indicates Total Coliform; blue to purple colonies indicates E. coli
(Red colonies are non-specific reaction w/ 235-Triphenyltetrazolium Chloride (TTC))

____ **E*Colite**: Total Coliform & Escherichia coli by MMO-MUG
3 compartments in specialized sample bag: Sample, Medium, Disinfectant
Incubated at 35 +/- 0.5 C for 28-48 hours; must use entire 48-hour period before sample results reported as negative;
Color change from yellow to blue or blue-green (aqua-blue) indicates Total Coliform
Blue fluorescence (under 366 nm UV light) indicates E. coli
Red color indicates faulty bag seal; must discard sample & request another sample

____ **EPA 1600**, 11: Enterococcus Membrane Filtration with m-EI Agar
Filter different sample volumes to give 20-60 colonies on membrane surface
Incubated at 41.0 +/- 0.5 C for 24 +/- 2 hours
Colonies with Blue halo (regardless of colony color) are Enterococci

____ **ChromoCult**: Total Coliform & Escherichia coli by Membrane Filtration
Incubated at 36 +/- 1 C for 24 +/- 1 hours
Salmon to red colonies indicate Coliforms; dark blue to violet colonies indicate E. coli

____ **ReadyCult**: Total Coliform & Escherichia coli by MMO-MUG
Incubated at 36 +/- 1 C for 24 +/- 1 hours
Color change from slightly yellow to blue-green (aqua-blue) indicates Total Coliform
Blue fluorescence (under 366 nm UV light) indicates E. coli

____ **Enterolert & D6503-99**
Incubated at 41.0 +/- 0.5 C for 24-28 hours
Blue fluorescence (under 366 nm UV light) indicates Enterococci

____ **SM9213D**, 3b; **EPA 1103.1**, 11; & **D5392-93**, 12: E. coli Membrane Filtration with m-TEC Agar
Filter different sample volumes to give 20-80 colonies on membrane surface
Incubated at 35.0 +/- 0.5 C for 2 +/- 0.5 hr, then at 44.5 +/- 0.2 C for 22 +/- 2 hr
Transferred to Urea Substrate Medium, room temperature for 15-20 minutes
Yellow, yellow-green, or yellow-brown colonies are E. coli (red or purple colonies, NOT E. coli)

____ **EPA 1603**, 11: Escherichia coli single-step Membrane Filtration with modified m-TEC Agar
Filter different sample volumes to give 20-80 colonies on membrane surface
Incubated at 35.0 +/- 0.5 C for 2 hr, then at 44.5 +/- 0.2 C for 22 hr
Red or magenta colonies are E. coli

____ **Colitag**: Total Coliform & Escherichia coli by MMO-MUG
Incubated at 35.0 +/- 0.5 C for 24 +/- 2 hours
Yellow color indicates Total Coliform; 366-nm blue-light fluorescence indicates E. coli

____ **Coliscan**: Total Coliform & Escherichia coli by MF
Incubated at 32-37 C for 24-28 hours
Pink-magenta colonies indicate Total Coliform; Purple-blue colonies indicate E. coli

____ **SimPlate**: Heterotrophic Plate Count by Enzyme Substrate method
Incubated at 35.0 +/- 0.5 C for 45-72 hours
Unit-Dose: 10-mL sample size, when small HPC counts are expected
Multi-Dose: use when expected HPC number is not known
Blue fluorescence (under 6-watt, 366 nm UV light) indicates Heterotrophic bacteria

Additional Test Methods:

____ **D.3.4(b)** Does the laboratory **verify target organism identity** as specified by the test method (by use of the completed test or by secondary verification tests such as a catalase test)

____ **SM9221B**, 2b; **SM9221D**, 2b; **EPA-600/8-78-017**, Part III-B, 4.6.3; & **EPA 9131**, 7.2: Total Coliform
Each positive culture from LTB (gas formation or color change) 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)
Incubated at 35.0 +/- 0.5 degrees Celsius for 24 +/- 2 hours
If no gas formation, re-incubate for additional 24 hours (total of 48 +/- 3 hours)
Gas formation in BGLB confirms Total Coliform for purposes of MPN calculation or Presence-Absence reporting
Drinking Water: samples also tested according to SM9221E or EPA 1104 below
(if no positive samples, 1 known positive sample analyzed & confirmed quarterly)
Non-Potable Water: 10% of confirmed positive samples verified through Completed Test

____ **SM9222B**, 5f & **EPA-600/8-78-017**, Part III-B, 3: Total Coliform MF colonies
Non-Potable Water: Inoculate > 10 colonies from positive sample monthly into LTB & BGLB
Non-Potable Water: Verify atypical colonies of different morphological types to determine false negatives
Drinking Water: Inoculate all colonies (can swab entire filter) into 1 LTB tube & 1 BGLB tube
(if no positive samples, 1 known positive sample analyzed & confirmed quarterly)
Incubate at 35.0 +/- 0.5 degrees Celsius for 48 hours
Gas production in LTB & BGLB confirms Total Coliform
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
Drinking Water: Positive cultures from LTB or membrane filter colonies also tested according to SM9221E, EPA 1104, or EPA 1105 below
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

____ **SM9221E**, 1b: Fecal Coliform with EC Medium (A-1 is not allowed for Drinking Water samples)
Incubated at 44.5 +/- 0.2 degrees Celsius for 24 +/- 2 hours
Gas formation confirms that the Total Coliform is a Fecal Coliform

____ **EPA 1104**, 11: E. coli by EC + MUG Tube Procedure
Incubated at 44.5 +/- 0.2 degrees Celsius for 24 +/- 2 hours
366-nm blue-light fluorescence confirms that the Total Coliform is E. coli

____ **EPA 1105**, 11: E. coli by Nutrient Agar + MUG Membrane Filter Procedure
Membrane filter transferred in its entirety to NA + MUG medium
Note: some colonies removed for LTB & BGLB tests
Incubated at 35.0 +/- 0.5 degrees Celsius for 4 hours
366-nm blue-light fluorescent halos around MF colonies confirm that Total Coliform is E. coli
Verify 5% of Drinking Water samples with Citrate test & with Indole test (44.5 C)
Verify 1 positive Non-Potable Water sample Monthly with Citrate test & with Indole test (44.5 C)
(E. coli yields no growth on Citrate but is indole-positive)

____ **SM9020B**, 9a(2): Total Coliform & E. coli by Enzyme Substrate tests (e.g., Colilert)
5% Drinking Water samples & 10% Non-Potable Water positive samples verified (MPN results)
Total Coliform verified with LTB or with ONPG test & Cytochrome Oxidase test (for indophenol)
E. coli verified with EC MUG test

____ **Readycult**: E. coli Verification
Immediate formation of red ring when KOVAC's indole reagent added to the broth

____ **SM9020B**, 9b(2) (refers to **SM9222D**) & **EPA-600/8-78-017**, Part III-C, 4: Fecal Coliform MF Colonies

SM: Verify > 10 Blue Colonies from one positive sample Monthly

SM: Verify atypical colonies of different morphological types to determine false negatives

Inoculate at least 10 colonies from filter into LTB

Incubated at 35.0 +/- 0.5 degrees C for 24 +/- 2 hr (48 +/- 3 hr if no gas production after 24 hr)

Positive cultures from LTB (gas formation) inoculated into EC medium

EC tubes incubated at 44.5 +/- 0.2 degrees Celsius for 24 hours

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

____ **SM9230B,** 3 & **EPA-600/8-78-017**, Part III-D, 4.6.7-4.6.9: Fecal Streptococcus MPN

Streak positive cultures (turbidity in azide dextrose medium and/or sediment button at bottom of culture tube) onto Pfizer Selective Enterococcus Agar (Bile Esculin Azide Agar) plates

Incubated at 35.0 +/- 0.5 degrees Celsius for 24 hours

Brownish-black colonies w/ brown halos confirms Fecal Strep. for purposes of MPN calculation

____ **SM9230C**, 5; **D5259-92**, 13: & **EPA 1106.1 & 1600**, 15 (2002 version) or 12 (2005 version): Enterococcus Membrane Filter Colonies

SM9020B, 9b5: Verify >10 Colonies Monthly

Transfer (pink-to-red m-E or m-Enterococcus colonies, or black or reddish brown precipitate on EIA) (any m-EI blue colonies or with blue halos) to BHI Broth & BHI Agar

BHI Broth incubated at 35.0 +/- 0.5 degrees Celsius for 24 +/- 2 hours

BHI Agar incubated at 35.0 +/- 0.5 degrees Celsius for 48 +/- 3 hours

SM9230C: Catalase test with hydrogen peroxide on BHI Agar culture; proceed with further Enterococcus verifications if no gas bubbles form

All Methods: Gram stain on BHI Agar culture; enterococcus are gram-positive ovoid cocci mostly in pairs or short chains, 0.5-1.0 um diameter

Inoculate cultures from the BHI Broth into fresh BHI Broth, Bile Esculin Agar (BEA), & BHI Broth with 6.5% NaCl

BHI Broth incubated at 45.0 +/- 0.5 degrees Celsius for 48 +/- 3 hours (look for turbidity)

BEA incubated at 35.0 +/- 0.5 degrees Celsius for 48 +/- 3 hr (look for brown or black precipitate from esculin hydrolysis)

BHI Broth with 6.5% NaCl incubated at 35.0 +/- 0.5 degrees Celsius for 48 +/- 3 hr (turbidity)

Growth in all 3 media confirms the membrane filter colonies as Enterococci; colony counts are adjusted proportionally

____ **SM9230C**, 5: Fecal Streptococcus Membrane Filter Colonies

SM9020B, 9b4: Verify >10 Colonies Monthly

Transfer (light & dark red m-Enterococcus colonies) to BHI Broth & BHI Agar

BHI Broth incubated at 35.0 +/- 0.5 degrees Celsius for 24 hours

BHI Agar incubated at 35.0 +/- 0.5 degrees Celsius for 48 hours

Catalase test with hydrogen peroxide on BHI Agar culture; proceed with further verifications if no gas bubbles form

Gram stain on BHI Agar culture; Fecal Streptococcus are gram-positive ovoid cocci mostly in pairs or short chains, 0.5-1.0 um diameter

Inoculate cultures from the BHI Broth into fresh BHI Broth & Bile Esculin Agar (BEA)

BHI Broth incubated at 45.0 +/- 0.5 degrees Celsius for 48 hours (look for Turbidity)

BEA incubated at 35.0 +/- 0.5 degrees Celsius for 48 hours (look for brown or black precipitate from esculin hydrolysis)

Growth in both media confirms the membrane filter colonies as Fecal Streptococci; colony counts are adjusted proportionally

____ **Chromocult & Coliscan**: E. coli Verification of membrane filter colinies

E. coli dark blue to violet colonies immediately change to cherry red color when KOVAC's indole reagent is added to these colonies

____ **EPA-600/8-78-017**, Part III-D, 3: Fecal Streptococcus Membrane Filter Colonies on KF Agar
Transfer pink & red KF Streptococcus Agar colonies to BHI Broth & BHI Agar
BHI Broth incubated at 35.0 +/- 0.5 degrees Celsius for 24 hours
BHI Agar incubated at 35.0 +/- 0.5 degrees Celsius for 48 hours
Catalase test with hydrogen peroxide on BHI Agar culture; proceed with further verifications if no gas bubbles form
Inoculate cultures from the BHI Broth into fresh BHI Broth & BHI Broth with 40% Bile
BHI Broth incubated at 45.0 +/- 0.5 degrees Celsius for 48 hours
BHI Broth with 40% Bile incubated at 35.0 +/- 0.5 degrees Celsius for 48 hours
Growth in both media confirms the membrane filter colonies as Fecal Streptococcus; colony counts are adjusted proportionally

____ **SM9020B**, 9b3 or **SM9222B**, 5f2b; **EPA 1103.1 & 1603**, 12 (2005 version) or 15 (2002 version); & **D5392-93**, 14: E. coli MF verification
SM: At least one positive sample verified monthly
Yellow to yellow-brown m-TEC colonies inoculated into Nutrient Agar & Trypticase Soy Broth
Red or magenta Modified m-TEC colonies inoculated into Nutrient Agar & Trypticase Soy Broth
Nutrient Agar & Trypticase Soy Broth both incubated at 35 +/- 0.5 C for 24 +/- 2 hours
Deposit growth from Nutrient Agar into filter paper saturated with Cytochrome Oxidase reagent, test is positive if purple spot forms within 15 seconds
Transfer growth from Trypticase Soy into Simmons Citrate Agar, Tryptone Broth, & EC Broth
Simmons Citrate Agar and Tryptone Broth incubated at 35 +/- 0.5 C for 48 hours;
Positive Simmons Citrate test indicates intense blue color on the agar slant
(**EPA 1103.1 & 1603** 2005 versions incubate Tryptone at 35 +/- 2 C for 18-24 hr & Simmons Citrate Agar at 35 +/- 2 C for 4 days)
EC Broth fermentation tube incubated at 44.5 +/- 0.2 C for 24 hours
Add Kovacs Indole Reagent to the Tryptone Broth culture; deep red color in top alcohol layer indicates a positive test
E. coli produces gas in the EC tube, is indole-positive, is oxidase-negative, and does not utilize citrate (the medium remains green)
ALTERNATIVE: Use a commercial multi-test identification system that includes lactose fermentation, o-Nitrophenyl-b-D-galactopyranoside, & cytochrome oxidase test reactions

____ **SM9260D**, 1; **EPA 1682**, 12; **J. WPCF 46, 2163**: Salmonella MPN Verification
Turbid cultures from Dulcitol Selenite or Tetrathionate Broths streaked to Brilliant Green Agar (**SM9260D** only) & Xylose Lysine Desoxycholate Agar (**all** methods)
Both agars incubated at 35 degrees Celsius for 24 hours (**SM9260D**)
XLD agar incubated at 36 +/- 1.5 C for 18-24 hours (**EPA 1682**)
Pink-edged, clear, black-centered colonies on XLD: Salmonella
Flat, mucoid, grayish alkaline, pink erose-edged colonies on XLD: Pseudomonas aeruginosa
Pinkish-white colonies with red background on BG Agar: Salmonella
One colony from each agar inoculated into Triple Sugar Iron Agar & Lysine Iron Agar (**SM9260D**)
Both agars incubated at 35 degrees Celsius for 24 hours
Colonies from XLD inoculated into TSI Agar, LSI Agar, & Urease Test Broth (**EPA 1682**)
All media incubated at 36 +/- 1.5 degrees Celsius for 24 +/- 2 hours
Positive TSI test is acid butt (yellow), alkaline slant (red), with or without H2S production (black): indicates Salmonella, but black H2S production also indicates Salmonella
Positive LIA test is alkaline butt (purple), alkaline slant (purple), with or without H2S (black): indicates Salmonella, but black H2S production also indicates Salmonella
Urease test: negative results indicate Salmonella (medium remains orange in color)
(Positive test would have a color change to pink or purple-red)
Serological tests with Salmonella "O" Polyvalent Antiserum & with Salmonella Vi Antigen from TSI culture: agglutination reaction indicates positive result
Salmonella: MSRV positive, XLD positive, either TSI or LIA positive, Urease negative, & Polyvalent-O positive

Verification Procedures for Alternate Tests: