



**ILLINOIS DEPARTMENT OF PUBLIC HEALTH
Water Microbiology Laboratory Evaluation Form**

Laboratory _____ Laboratory Number _____
Certification Officer(s) _____ Evaluation Date _____

Address:

Street _____ City _____ State _____ ZIP Code _____

Telephone _____ Fax _____ E-mail _____

Mailing Address (if different):

Street _____ City _____ State _____ ZIP Code _____

Laboratory Personnel

Position/Title	Name	Education Level / Degree	Experience/ time at current position (years)
Supervisor			
Analyst			
Analyst			
Analyst			
Analyst			
Analyst			
Analyst			
Analyst			
Analyst			
Analyst			
Analyst			
Analyst			
Analyst			
Analyst			

LABORATORY FACILITIES

S=Satisfactory X=Unsatisfactory U=Undetermined NA=Not Applicable

1. Minimum 150 square feet of floor space per analyst (465.320a) _____
2. Floors of impervious material (465.320b) _____
3. Ample floor space for stationary equipment (e.g., autoclave, incubator, oven) (465.320c) _____
4. Storage space free of dust and insects for glassware, media and portable equipment (465.320c) _____
5. Potable, non-potable source and recreational water in separate room from sewage (465.320d) _____
6. Separate bench for preparation and sterilization of media, glassware and equipment (465.320e) _____
7. Walls have smooth, easily cleaned finish; ceilings maintained in good condition (465.320f) _____
8. Minimum of 6 linear feet of usable bench space per analyst (465.320g) _____
9. Bench tops of impervious material and level (465.320h) _____
10. Minimum of 100 foot candles light at all working surfaces (465.320i) _____
11. All water supply outlets protected by backflow prevention device (465.320j) _____
12. Laboratory well ventilated and free of dust, drafts and extreme temperature changes (465.320k) _____
13. Temperature maintained between 60°F and 80°F (465.320k) _____
14. Laboratory has provisions for the disposal of microbiological waste (465.320u) _____
15. No mobile laboratories allowed (465.320t) _____
16. Laboratory not located in structure used as residence (465.320s) _____

LABORATORY EQUIPMENT, SUPPLIES AND MATERIALS

1. Service contracts or in-house protocols on laboratory equipment; service records include equipment, date, name of servicing person and service provided (465.400m). _____

2. Balances

Manufacturer/Model _____

Manufacturer/Model _____

- a. Top loading or trip pan balance clean, not corroded (465.330a). _____
- b. Balances used for weighing 2 grams or more detects 100 mg at a 150-gram load (465.330a1). _____
- c. Analytical balances used for weighing less than 2 grams sensitive to 1 mg at a 10 gram load (465.330a2). _____
- QC** d. Balance(s) calibrated monthly using NIST Echelon I or II, or equivalent ASTM 1, 2, 3 weights using minimum of three weights that bracket the weighing requirements of the laboratory (465.400b). _____
- QC** e. Certificate listing correction data accompanies NIST or ASTM weights (465.400b). _____
- QC** f. NIST or ASTM weights recertified every five years (465.400b). _____
- QC** g. Electronic balances calibrated annually by service representative; certificate of calibration maintained (465.400b). _____

3. Temperature Monitoring Devices

Manufacturer/Certification Number _____

- a. NIST certified thermometer [graduated in 0.2° or less and accompanied by its certification papers (465.330k5)]. _____
- b. NIST checked at ice point annually (465.400c). _____
- c. NIST calibrated every five years, mercury NIST at each temperature of use (465.400c). _____
- QC** d. Calibration of thermometers and automatic temperature recording devices checked annually at temperature of use against a certified thermometer (465.330k5). _____
- e. Laboratory thermometers do not vary more than $\pm 1^{\circ}\text{C}$ from certified thermometer (465.330k5). _____
- f. No separation in the liquid column (465.330k8). _____
- g. Glass or electronic thermometers graduated in no greater than 0.5°C units for use in 35°C incubators (465.330k1). _____
- h. Glass or electronic thermometers graduated in no greater than 0.2°C units for use in 44.5°C water baths (465.330k2). _____
- i. Glass or electronic thermometers graduated in no greater than 1.0°C units for use in 55°C-65°C incubators (465.330k3). _____
- j. Electronic thermometers with thermo couplings and continuous recording devices sensitive to no greater than 0.5°C for 35°C incubators, 0.2 °C for 44.5°C water baths, 1.0°C for 55°C-65°C incubators (465.330k4). _____
- k. Maximum registering thermometer or data logger graduated in increments no greater than 1°C (465.330k6). _____
- l. All thermometers tagged with correction factor, date calibrated, temperature calibrated, initials (465.400c). _____

4. pH Meter

Manufacturer/Model _____

- a. Accuracy of ± 0.1 units; scale graduation ± 0.1 units (465.330c). _____
- b. Electrodes maintained according to manufacturer's recommendations (465.400a). _____
- c. pH buffer solution aliquots used only once (465.400a). _____

QC d. Commercial buffer solutions dated when received and discarded before expiration date (465.400a). _____

QC e. pH meter standardized each day of use with pH 7.0 and either pH 4.0 or pH 10.0 standard buffers; record of the standardization including percent slope maintained (percent slope 95%-105%)(465.400a). _____

5. Agar Tempering Water Bath

Manufacturer/Model _____

- a. Appropriate size for holding melted media (465.330j). _____

QC b. Temperature maintained at $45 \pm 1^\circ\text{C}$ (465.330j). _____

6. Incubator Unit(s)

Manufacturer/Model (35°) _____

Manufacturer/Model (44.5°) _____

Manufacturer/Model (spore) _____

- a. Maintains uniform temperature of $35^\circ\text{C} \pm 0.5^\circ\text{C}$, $44.5^\circ\text{C} \pm 0.2^\circ\text{C}$, (465.330g); water bath circulating with cover (465.330g). _____

QC b. Temperatures recorded continuously or recorded twice daily (at times separated by at least four hours) (465.400d). _____

- c. Thermometers on top and bottom shelves of the use area (465.400d). _____

- d. Temperature readings from walk-in incubators with a continuous reading device supplemented by readings from thermometers placed on shelves other than where the device is located (465.400d). _____

- e. Thermometer bulb immersed in liquid (465.400d). _____

- f. Culture dishes and tubes in aluminum block incubator fit snugly (465.330g). _____

7. Refrigerator

Manufacturer/Model _____

- a. Temperature maintained at 1°C to 5°C on top shelf (465.330i). _____

- b. Thermometer graduated in 1°C increments or less (465.330i). _____

- c. Thermometer bulb immersed in liquid (465.330i) (465.400v). _____

QC d. Temperature recorded daily (465.400v). _____

- e. Refrigerator unit visibly clean and outdated materials discarded (465.400v). _____

8. Autoclave

Manufacturer/Model _____

- a. Separate pressure and temperature gauges with sensor on exhaust (465.330e3). _____

- b. Operational safety valve (465.330e2). _____

- c. Sterilization temperature ($121 \pm 1^\circ\text{C}$) maintained during cycle (465.330e4). _____

- d. Entire cycle completed within 45 minutes when a 12 minute-15 minute sterilization period used (465.330e4). _____
- e. Depressurizes slowly to ensure media do not boil over and bubbles do not form in fermentation tubes (465.330e5). _____
- f. Spore strips or ampules used monthly. Incubate according to manufacturer's instructions (465.400s). _____
- g. Maximum registering thermometer or data logger used each cycle (465.400s). _____
- h. Data logger with external probe/maximum registering thermometer placed in a container of water unless otherwise specified in manufacturer's instructions (465.400s). _____
- QC** i. Automatic timing mechanism checked accuracy with stopwatch quarterly (plus or minus 1-minute for each 15-minute time period) (465.400s). _____
- j. Records include date, contents, sterilization time and temperature, total time in autoclave, analyst's initials (465.400e). _____

9. Hot Air Oven

Manufacturer/Model _____

- a. Minimum temperature of 175°C maintained (465.330f). _____
- b. Thermometer graduated in no more than 10°C increments (465.400f). _____
- c. Thermometer bulb in sand or oven equipped with temperature recording device (465.400f). _____
- QC** d. Spore strips used monthly (465.400s). _____
- e. Maximum registering thermometer or data logger used each cycle (465.400s). _____
- QC** f. Records include date, contents, sterilization time and temperature, total time in oven, analyst's initials (465.400f). _____

10. Colony Counter

Manufacturer/Type _____

- a. Dark field colony counter available to count heterotrophic plate count colonies (465.360t9). _____

11. Microscope

Manufacturer/Model _____

- a. Binocular dissecting microscope (10-15x) with external daylight fluorescent light source at an angle of 60° to 80° above the colonies to count MF colonies (465.330l). _____
- b. Mechanical hand tally (465.330m). _____

12. Conductivity Meter

Manufacturer/Model _____

- a. Readable in ohms or mhos; range capable of determining conductivity or resistivity of lab pure water (465.330d). _____
- QC** b. Calibrated monthly according to manufacturer's instructions using certified traceable low level standard of 20 micromhos or less; meter reading within 2% of the value of the standard; in-line units must be able to be calibrated (465.400z). _____

13. Inoculating Equipment

- a. Presterilized cotton swabs or applicator sticks sterilized by dry heat (465.330n). _____
- b. Metal loops of 22-gauge to 24-gauge chrome or platinum-iridium wire or presterilized plastic loops; loop diameter at least 3mm (465.330n). _____

14. Membrane Filtration (MF) Equipment

Manufacturer/Type _____

- a. MF units of stainless steel, glass or autoclavable plastic (465.330o). _____
- b. Units do not leak, not scratched or corroded (465.330o). _____
- c. Forceps tips without corrugations (465.330r). _____
- QC** d. Multi-use MF units initially calibrated with Class A graduated cylinder; tolerance $\pm 2.5\%$ (465.400ff). _____
- QC** e. Each lot of single use MF units checked for calibration with Class A graduated cylinder; $\pm 2.5\%$ tolerance (465.400ee). _____

15. Membrane Filters and Pads

Manufacturer/Type _____

- a. Membrane filters from cellulose ester material, white, grid marked, 47mm diameter, 0.45 μ m pore size (465.330p). _____
- b. Alternate pore size used if manufacturer gives performance data equal to or better than the 0.45 μ m membrane filter (465.330p). _____
- c. Membrane filters recommended by manufacturer for water analysis (465.400g). _____
- d. Membrane filters and pads purchased presterilized or autoclaved before use (465.330p) (465.330q). _____
- QC** e. One certificate per lot number of membrane filters on file; date of receipt recorded (465.400g). _____
- f. Membrane filters not brittle or distorted, no gridline inhibition (465.400g). _____
- QC** g. Run positive control on each new lot (465.400g). _____

16. Culture Dishes

- a. Pre-sterilized plastic or sterilized glass dishes used (465.340e). _____
- b. Loose-lid dishes incubated in a tight-fitting container (465.340e). _____
- c. Glass culture dishes sterilized in stainless steel or aluminum canisters or in heavy aluminum foil or char-resistant paper (465.340e). _____
- d. Open packs of disposable culture dishes resealed between uses (465.340e). _____
- e. Dishes clear, flat bottomed and free from bubbles and scratches (465.340e). _____

17. Culture Tubes, Containers, and Closures

- a. Tubes and containers borosilicate glass or other corrosion-resistant glass (465.340f). _____
- b. Tubes and containers of sufficient size that medium plus sample does not exceed 3/4 full (465.340f). _____
- c. Closures stainless steel, plastic, aluminum or screw cap with non-toxic liner (465.340f). _____
- d. Cotton and foam plugs not allowed (465.340f). _____

18. Pipettes

- a. Reusable pipettes sterilized in stainless steel or aluminum canisters (465.340d). _____
- b. Packs of disposable sterile pipettes resealed between major use periods (465.340d). _____
- c. Pipettes not etched or chipped, graduation markings legible (465.340a). _____
- d. Pipettes and pipettors have a tolerance of 2.5% or less (465.340d) (465.400u). _____
- e. Micropipettes are fixed volume, tips sterile, calibrated annually with 10 weighings, or for volumes greater than or equal to 1 ml checked with a Class A graduated cylinder (465.400u). _____
- f. Pipetting devices used; mouth pipetting not permitted (465.340d). _____
- g. Pipette aid clean and dry; no pipette aids allowed that were previously used outside of the certified laboratory (465.340d). _____

19. Dilution Bottles

- a. Dilution bottles of borosilicate glass or other corrosion resistant glass or of autoclavable plastic (465.340g). _____
- b. Graduation level distinctly marked at 99 ml (465.340g). _____

QC c. Plastic screw caps with leak proof liner free of toxicity by test (465.340g). _____

20. Sample Containers

- a. Capacity at least 120 ml (4 oz) to allow at least one inch head space (465.340h). _____
- b. Sample bottles wide mouth plastic with a non-toxic cap liner, borosilicate glass with a ground glass stopper, pre-sterilized containers, including single-service sterilized plastic bottles, or sampling bags with sodium thiosulfate (465.340h). _____
- c. Tops of glass-stoppered bottles covered with aluminum foil or char-resistant paper prior to sterilization (465.340h). _____
- d. Glass bottles sterilized by autoclaving or dry heat; plastic bottles sterilized by autoclaving (465.350a1, 465.350a5). _____
- e. Empty container moistened before autoclaving (465.350a6). _____

21. Miscellaneous Supplies

- a. Glass made of borosilicate or other corrosion-resistant glass (465.340a). _____
- b. Free of chips, cracks or excessive etching (465.340a). _____
- c. Plastic items are non-toxic (465.340a). _____
- d. Graduated cylinders and other pre-calibrated containers used to measure sample volume have clearly marked volumes of 2.5% tolerance or less (465.340b). _____
- e. Media preparation utensils borosilicate glass or stainless steel, clean and dry, free from foreign residues or dried medium (465.340c). _____

22. Ultraviolet Lamp for Funnel Disinfection

QC a. Lamps cleaned monthly with a soft cloth moistened with ethanol (465.400w). _____

QC b. Lamp tested quarterly by exposing agar spread plates to the light for two minutes; alternatively lamps checked upon first use and quarterly with light meter (465.400w). _____

QC c. Lamps replaced if less than 99% kill or if emission < 70% of initial output (465.400w). _____

GENERAL LABORATORY PRACTICES

1. Sterilization and Sanitation Procedures (465.350a1)

a. <u>Item</u>	<u>Minimum duration of autoclaving at 121°C ± 1°C</u>	
Membrane filters and pads	10 minutes	_____
Carbohydrate media	12-15 minutes	_____
Contaminated test material	30 minutes	_____
Membrane filter assemblies	15 minutes	_____
Sample collection bottles	15 minutes	_____
Individual glassware	15 minutes	_____
Dilution water blanks	15 minutes	_____
Rinse water volumes of 500-1000 mL	45 minutes	_____
Rinse water volumes > 1000 mL	Time adjusted for volume	_____

- QC** b. MF filters and pads and all media immediately removed from autoclave after sterilization cycle 465.350a2). _____
- c. Total exposure of media to heat not over 45 minutes (465.350a3). _____
- d. Membrane filter assemblies autoclaved at start of each filtration series (series ends when 30 minutes or more elapses between sample filtrations) (465.350a4) (465.360l2). _____
- e. UV sterilizer or boiling water used for at least two minutes between sample filtrations (465.350a4). _____

2. Laboratory Pure Water

Type _____ System Used _____ or Brand Purchased _____

- a. Laboratory pure water used to prepare media, reagents and dilution/rinse water (465.380b). _____
- b. Laboratory pure water tested to assure the following minimum criteria are met; manufacturer's test results shall not be used to establish compliance (465.380a): _____

	<u>Parameter</u>	<u>Limits</u>	<u>Frequency</u>	
QC	Conductivity	>0.5megohms resistance or <2micromhos/cm @ 25°C	monthly	_____
QC	Total chlorine residual ¹	<0.1 mg/L	monthly	_____
QC	Heterotrophic plate count ²	<500/ml	monthly	_____
QC	Metals-Cd, Cr, Cu, Pb, Ni, Zn	Not greater than 0.05 mg/L per contaminant. Collectively, no greater than 0.1 mg/L.	annually	_____
QC	Bacteriological quality ³	Ratio of growth rate 0.8-3.0	annually	_____

¹ DPD Method

² Pour Plate Method SM 9215B or SimPlate

³ not required for water with conductivity <1 micromhos/cm @ 25°C or resistivity >1 megohms

Chemical Quality Testing Lab _____ Date _____

Microbiological Testing Lab _____ Date _____

3. Buffer Solutions

- a. Stock Phosphate Buffer (465.350c1)

QC pH 7.2 ± 0.5

1. Prepared in lab (34gKH₂PO₄/L lab pure water)

Preparation Date _____ or _____

2. Purchased commercially prepared

Lot Number _____ Expiration Date _____

- b. Stock Magnesium Chloride Solution (465.350c1)

1. Prepared in lab (81gMgCl₂·6/L lab pure water)

Preparation Date _____ or _____

2. Purchased commercially prepared

Lot Number _____ Expiration Date _____

- c. Laboratory prepared buffers and solutions autoclaved or filter sterilized, labeled and dated (465.350c2).

- d. Commercially prepared buffers and solutions: date received, expiration date, proof of sterility and pH of phosphate buffer shall be recorded (465.350c5).

- e. Buffers and solutions stored at 1°C-5°C (465.350c2).

- f. Stock phosphate buffer and magnesium chloride solution free of turbidity (465.350c3).

4. Dilution / Rinse Water

- a. Dilution/rinse water prepared by adding 1.25mL stock buffer and 5mL MgCl₂ solution per liter of reagent water (465.350c4).

- b. Commercially prepared rinse water (465.350c5).

Brand _____ Lot Number _____ Exp. Date _____

- c. Purchased dilution blanks used by manufacturer's expiration date (465.400dd)

Brand _____ Lot Number _____ Exp. Date _____

- QC** 1. Each batch or lot of dilution blanks or rinse water checked for sterility by adding 50 mL of water to 50 mL of double strength non-selective broth then checked for growth after 24 and 48 hours (465.400bb).

2. Final pH of dilution water blanks is 7.2 ± 0.2 (465.400cc).

- QC** 3. Volume of dilution blanks accuracy verified by checking 1 of 25 per batch or lot using a Class A graduated cylinder volume 99 ± 2 mL (465.400dd).

4. Used by manufacturer's expiration date (465.400dd).

5. Sample Containers

- a. Stock sodium thiosulfate solution free of turbidity (465.370f).

- b. 0.1 mL of 3% solution sodium thiosulfate added to sample containers prior to sterilization to neutralize up to 5 mg/L (465.370f).

- QC** c. At least one bottle per lot or batch of sterilized bottles prepared with sodium thiosulfate checked for a sufficient amount of dechlorinating agent (465.400k).

- QC** d. Sterility of each lot or batch of sample bottles determined using non-selective broth; broth checked for growth at 24 and 48 hours (465.400j). _____
- QC** e. Pre-calibrated sample containers checked by measuring the volume of one container per lot with class A graduated cylinder. $\pm 2.5\%$ tolerance (465.400l). _____
6. Glassware Washing
- a. Distilled or deionized water used for final rinse (465.400h). _____
- b. Detergent designed for laboratory use (465.400h). Brand _____
- QC** c. Inhibitory residue test performed on clean glassware before initial use of detergent and whenever different formulation, lot number, container or washing procedure (465.400h). _____
- QC** d. Piece of glassware or plastic ware from each batch checked with bromothymol blue; corrective action taken if necessary (465.400i). _____

MEDIA

1. General

- a. Either commercially prepared or commercially dehydrated media used (465.350d1). _____
- QC** b. Records kept of kind, amount, date received and date opened for containers of media (465.400y). _____
- c. Media used on first in first out basis (465.400y). _____
- d. Date received and date opened (initial use) written on containers (465.400y). _____
- e. Dehydrated media stored in cool, dry location (465.350d3). _____
- f. Opened bottles of dehydrated media kept in desiccator. Yes _____ No _____
- g. Media that have passed manufacturer's expiration date discarded (465.400y). _____
- h. Open dehydrated media discarded after six months (by manufacturer's expiration date if stored in desiccator) (465.400y). _____
- i. Media discarded if visible deterioration is observed; e.g. clumping, color change, visible growth, sheen (465.400y) (465.360m6). _____
- j. Prepared agar refrigerated, placed in tightly closed container, dish or plastic bag; laboratory prepared MF agar used within two weeks; laboratory prepared MF broth used within 96 hours (465.360m6). _____
- k. Multiple Tube Fermentation (MTF) broth with loose-fitting caps used within one week (465.350d6). _____
- QC** l. Lab prepared MTF broth with screw caps used within three months stored in the dark; evaporation <1.0 mL per 10 mL (465.350d7). _____
- m. Refrigerated sterilized MTF broth incubated overnight at 35°C; tubes with growth or gas bubbles discarded (465.350d6). _____
- QC** n. Media preparation records include type of medium, lot number, date of preparation, sterilization time and temperature, final pH, initials (465.400n). _____
- QC** o. Media dispensing apparatus, when used, checked for accuracy (465.400t). _____
- QC** p. Each new lot/batch of medium checked with known positive and negative culture controls before use (465.400p). _____
- q. If lactose broth used, 25 parallel tests with lauryl tryptose broth (LTB) conducted before first use; results differ <10% (465.400r). _____
- r. M-Endo broth, M-Endo agar LES and m-FC media prepared in sterile flask; brought just to boiling point, not autoclaved (465.360m1) (465.360m5). _____
- s. Rosolic acid (1% in 0.2N NaOH; not autoclaved) added to m-FC media when heavy background anticipated (SM9222D) (SM9222D-97). _____
- t. MI agar melted in boiling water bath or according to manufacturer's recommendation, not autoclaved (EPA Manual). _____
- u. Filter sterilized cefsulodin added to tempered MI agar (EPA Manual). _____
- QC** v. Commercially prepared media records include: date received, type of medium, lot number, sample performance and pH (465.400o). _____

2. Heterotrophic Plate Count Agar

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH 7.0 ± 0.2 (465.400n) _____

3. Lauryl Tryptose Broth (Lauryl Sulfate), Lactose Broth

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH single strength 6.8 ± 0.2 (465.400n) _____
Final pH double strength 6.7 ± 0.2 (465.400n) _____
Final pH Triple strength 6.6 ± 0.2 _____

4. Brilliant Green Lactose Bile Broth

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH 7.2 ± 0.2 (465.400n) _____

5. EC Broth

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH 6.9 ± 0.2 (465.400n) _____

6. EC Broth - MUG

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH 6.9 ± 0.2 (465.400n) _____

7. Nutrient Agar with MUG

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH 6.8 ± 0.2 (465.360s1) _____

8. M-Endo Media (broth, broth/agar added, Les Endo)

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH 7.2 ± 0.2 (465.400n) _____

9. M-FC Broth or Agar

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH 7.4 ± 0.2 (465.400n) _____

10. Presence-Absence Broth

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH 6.8 ± 0.2 (465.400n) _____

11. SimPlate

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
Final pH 7.0 ± 0.3 (465.360t2) _____

12. Colilert

Manufacturer _____ Lot Number _____ Exp. Date _____
 Final pH 7.3 ± 0.3 _____

13. Colilert 18

Manufacturer _____ Lot Number _____ Exp. Date _____
 Final pH 7.3 ± 0.3 _____

14. Colisure

Manufacturer _____ Lot Number _____ Exp. Date _____
 Final pH 7.3 ± 0.3 _____

15. E*Colite Test

Manufacturer _____ Lot Number _____ Exp. Date _____
 Final pH 6.9 ± 0.2 _____

16. ReadyCult

Manufacturer _____ Lot Number _____ Exp. Date _____
 Final pH 6.8 ± 0.2 _____

17. Modified Colitag

Manufacturer _____ Lot Number _____ Exp. Date _____
 Final pH 6.8 ± 0.2 _____

18. m-Colibblue24

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
 Final pH 7.0 ± 0.2 (465.360m2) _____

19. MI Agar

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
 Final pH 6.95 ± 0.2 (465.360m3) _____

20. MI Broth

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
 Final pH 7.05 ± 0.2 (465.360m3) _____

21. Non-Selective Broth (used to check sterility of bottles and rinse water/ dilution blanks) Broth _____

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____
 Final pH per manufacturer's instructions _____

METHODOLOGY

1. General

- a. Methodology as specified in the Revised Total Coliform Rule (RTCR), the Surface Water Treatment Rule (SWTR), the Groundwater Rule (GWR), or the Enhanced Long Term 2 Surface Water Treatment Rule (LT2) used, as applicable.
- b. Water sample shaken vigorously at least 25 times in a complete up and down movement (465.360c).
- c. All total coliform-positive cultures tested for the presence of *E. coli* (465.360o) (RTCR).
- d. All samples analyzed under the RTCR and the GWR are 100 mL.

QC e. PT sample satisfactorily analyzed annually for each certified method (465.390c1) (465.200).

2. Membrane Filter Procedures

RTCR (Detect)			SWTR (Count)		
Total Coliform	SM 20 th Ed 9222B		Total Coliform	SM 20 th Ed 9222A,B	
	SM 21 st Ed 9222B			SM 21 st Ed 9222A,B	
	SM online 9222B-97			SM online 9222A,B-97	
Total Coliform and <i>E. coli</i>	m-ColiBlue Manufacturer's Instructions		Fecal Coliform	SM 22 nd Ed. 9222D	
	EPA 1604 (MI Medium)			SM online Ed. 9222 D-97	
			Total Coliform and <i>E. coli</i>	EPA 1604 (MI Medium)	

New Construction (Detect)		
Total Coliform	SM 20 th Ed 9222B	
	SM 21 st Ed 9222B	
	SM online 9222B-97	

GWR (Detect)		
<i>E. coli</i>	EPA 1604 (MI Medium)	
	m-ColiBlue 24 Manufacturer's Instructions	

- a. Absorbent pads saturated with broth (2 ml), excess discarded; or 4ml of agar medium used (465.360j4).
- QC** b. Sterility check conducted at start and end of each filtration series; if control indicates contamination, all data rejected and another sample obtained (465.360j2).
- c. Funnel rinsed at least twice with 20ml-30 ml portions of sterile buffered rinse water (465.360j3).
- d. MF removed with sterile forceps; grasped outside effective filtration area.
- e. MF rolled onto medium so air bubbles are not trapped.
- f. M-Endo incubated at 35°C ± 0.5°C for 22 to 24 hours, m-ColiBlue 35°C ± 0.5°C for 24 hours, MI 35°C ± 0.5°C for 24 ± 2 hours; M-FC 44.5°C ± 0.2°C for ± 2 hours.
- g. Dishes with loose-fitting lids incubated in high humidity chambers.
- h. All samples either confluent growth or too numerous to count (TNTC) invalidated, unless total coliform-positive and a new sample obtained (RTCR) (465.360l).
- i. All samples under the SWTR that are TNTC or confluent growth (coliform count cannot be determined) invalidated (465.360o).
- j. M-Endo or LES-Endo verification performed.

1. RTCR: All sheen colonies verified (up to 5 typical and 5 atypical) or membrane swabbed; transferred to EC-MUG (see SM 9221F), LTB, and brilliant green lactose broth (BGLB) in that order (465.360p).

2. SWTR: All analysts transferred 10 colonies into LTB and EC broth (EC) tubes monthly; verification data used to adjust counts.
3. LTB and BGLB incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 hours to 48 hours.
4. Growth and gas production in LTB and BGLB verified for total coliform.
- k. MI: Total Coliforms - fluorescent colonies under UV light; *E.Coli* - blue colonies under normal light.
- l. m-ColiBlue24: Total Coliforms - red colonies. *E.Coli* - blue to purple colonies.
- m. M-FC dishes anchored below water surface to maintain critical temperature.
- n. For source water samples (SWTR), if more than one analyst in laboratory, all analysts counted the total and fecal coliform colonies on same membrane monthly; colony counts agreed within 10% (465.390c4).

QC

3. Standard Total Coliform Fermentation Technique

RTCR (Detect)		
Total Coliform	SM 20 th Ed 9221B.1,B.2	
	SM 21 st Ed 9221B.1,B.2	
	SM online 9221B.1,B.2-99	

SWTR (Count)		
Total Coliform	SM 20 th Ed 9221A,B,C	
	SM 21 st Ed 9221A,B,C	
	SM online 9221A,B,C-99	

New Construction (Detect)		
Total Coliform	SM 20 th Ed 9221B.1,B.2	
	SM 21 st Ed 9221B.1,B.2	
	SM online 9221B.1,B.2-99	

- a. Concentration of inoculated medium correct (465.360h2).
- b. Incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hours.
- c. If no gas detected, incubated for another 24 hours.
- d. All turbid gas-negative cultures invalidated and another sample obtained (RTCR).
- e. Cultures from gas-positive tubes incubated in BGLB and [EC (see 9221E) for SWTR] or [EC-MUG (see 9221F) for RTCR].

4. Presence-Absence (P-A) Coliform Test (modification of Standard Total Coliform Technique)

RTCR (Detect)		
Total Coliform	SM 20 th Ed 9221D.1,D.2	
	SM online 9221D.1,D.2-99	

- a. Triple strength P-A Broth, 50 ml, used.
- b. Sample incubated in screw-capped, 250 ml culture vessel.
- c. Incubated at $35 \pm 0.5^{\circ}\text{C}$ and observed for yellow color after 24 hours and, if necessary, 48 hours.
- d. Yellow cultures confirmed in BGLB (and EC-MUG).
- e. All non-yellow cultures are invalidated, and another sample obtained.

5. Fecal Coliform Fermentation Broth Methods

SWTR (Count)		
Fecal Coliform (A-1 Broth)	SM 20 th Ed 9221E	
	SM 21 st Ed 9221E	
	SM 22 nd Ed 9221E	
	SM online 9221E-99	

SWTR (Count)		
Fecal Coliform (EC Broth)	SM 20 th Ed 9221E	
	SM 21 st Ed 9221E	
	SM 22 nd Ed 9221E	

a. A-1 Broth (EPA Manual)

1. Three sample volumes of source water (e.g., 10, 1, 0.1 mL), or five or 10 tubes/sample volume used. _____
2. Incubated three hours at 35°C ± 0.5°C. _____
3. Tubes transferred to 44.5°C ± 0.2°C water bath and incubated additional 21 ± 2 hours. _____
4. Any gas detected in inverted vial of tube that has turbid growth reported as positive for fecal coliform. _____
5. Water level of water bath above level of media in culture tubes. _____

b. EC Broth (EPA Manual)

1. Initially, MTF test conducted, (presumptive phase). _____
2. Three sample volumes of source water (e.g., 10, 1, 0.1 mL), or five or 10 tubes/sample volume used. _____
3. Incubated at 44.5°C ± 0.2°C for 24 ± 2 hours. _____
4. Any gas detected in inverted vial of tube that has turbid growth reported as positive for fecal coliform. _____
5. Water level of water bath above level of media in culture tubes. _____

6. Verification Procedures

a. *Escherichia coli* Procedure following lactose fermentation methods

RTCR (Detect)		
E. coli	SM 20 th Ed 9221F.1	
	SM 21 st Ed 9221F.1	

GWR (Detect)		
E. coli	SM 22 nd Ed 9221F	

1. Positive culture from LTB transferred to EC-MUG. _____
2. Water level of water bath above upper level of media in culture tubes. _____
3. Incubated at 44.5°C ± 0.2°C for 24 ± 2 hours. _____
4. Fluorescence considered *E. coli* positive. _____

b. *Escherichia coli* Partition Method following Membrane Filtration Methods

RTCR (Detect)		
<i>E. coli</i>	SM 20 th Ed 9222G.1c(2)	
	SM 21 st Ed 9222G.1c(2)	

GWR (Detect)		
<i>E. coli</i>	SM 20 th Ed 9222G	

1. Positive culture from m-Endo, m-Endo LES or m-FC transferred to EC-MUG. _____
2. Water level of water bath above upper level of media in culture tubes. _____
3. Incubated at $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ for 24 ± 2 hours. _____
4. Fluorescence considered *E. coli* positive. _____

c. *Escherichia coli* NA-MUG Procedure following membrane filtration methods

RTCR (Detect)		
E. coli	SM 20 th Ed 9222G.1c(1)	
	SM 21 st Ed 9222G.1c(1)	

1. Membrane filter transferred from 22 hours - 24 hours m-Endo plate or 22 hours - 26 hours m-FC plate to NA-MUG. _____
2. Sheen (m-Endo) or blue (m-FC) colonies marked on lid; incubated additional four hours at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. _____
3. Fluorescent colonies reported as *E. coli*. _____

7. Enzyme Substrate Methods

a. Colilert, Colilert 18, Colisure

RTCR (Detect)		
Total Coliform and E. coli	SM 20 th Ed 9223B	
	SM 21 st Ed 9223B	
	SM online 9223B-97	

SWTR (Count)		
Total Coliform and E. coli	SM 20 th Ed 9223B	
	SM 21 st Ed 9223B	
	SM online 9223B-97	

b. ReadyCult®, E*Colite®, Modified Colitag™

RTCR (Detect)		
Total Coliform and E. coli	ReadyCult® Manufacturer's Instructions	
	E*Colite® Manufacturer's Instructions	
	Modified Colitag™ Manufacturer's Instructions	

- c. Colilert/Colilert 18 with Quantitray or Quantitray 2000, or MTF (5-10 tubes) used for SWTR samples; dilution water, if used, is sterile deionized water or distilled water (465.360k14). _____
- d. Colilert Test: samples incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 hours, up to 28 hours; yellow, coliform positive (465.360k13). _____
- e. Colilert 18 Test: samples incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 18 hours, up to 22 hours; yellow, coliform positive (465.360k13). _____
- f. Colisure Test: samples incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 hours, up to 48 hours; red/magenta, coliform positive (465.360k16). _____
- g. E*Colite Test: samples incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 28 hours; blue/blue-green, coliform positive; add 20 hours incubation if no fluorescence; if red, sample discarded and resample requested (465.360k17). _____
- h. ReadyCult Test: samples incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 ± 1 hours; blue-green, coliform positive (465.360k18). _____
- i. Modified Colitag Test: samples incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hours; yellow, coliform positive (465.360k19). _____

- j. Total Coliform-positive samples exposed to UV light (365-366nm); blue fluorescence, *E. coli* positive; (Readycult optional for *E.coli*: Kovac's indole reagent added, immediate red ring, *E. coli* positive) (465.360k9). _____
- k. Enzyme tests not used to verify/confirm coliform on membrane filters or in broth cultures (465.360ik10). _____
- l. Media protected from light and only from commercially available source (465.360k2 k3). _____
- QC** m. Media and bottles checked for fluorescence before use (465.360k4,k6). _____
- QC** n. For Quanti-Tray, sealer checked monthly for leakage (465.360i15). _____
- o. Reference comparators provided by manufacturer discarded by expiration date. (465.360i12). _____
- p. Pre-incubation sample instructions followed: _____
1. If testing for presence/absence, shake to begin dissolving granules. If using a Quanti-Tray for enumeration, granules must be completely dissolved prior to adding sample to the Quanti-Tray. _____
 2. Colilert-18 Quanti-Tray and Colisure: sample allowed to reach room temperature before incubation. _____
 3. Colilert -18 P/A: sample pre-warmed to 35°C ± 0.5 C water bath 20 minutes or 44.5°C for 7-10 minutes. _____
 4. Colilert P/A, Quanti-Tray, Readycult, Colitag: 24-hour incubation time includes time to bring sample temperature to 35°C±0.5 C _____
 5. Large sample load brought to room temperature before incubation (465.360k7). _____

8. Heterotrophic Plate Count (HPC)

SWTR (Count)		
Heterotrophic Plate Count	Pour Plate SM 22 nd Ed 9215B	
	SimPlate Manufacturer's Instructions	

- a. Work area disinfected. _____
- b. No more than 1ml or less than 0.1ml of sample plated. _____
- c. Refrigerated medium stored up to three months (465.360t4). _____
- d. At least two replicate plates per dilution prepared for each sample (465.360t5). _____
- e. Agar tempered to 44°C - 46°C before plating; melted agar used within three hours; temperature control bottle containing media used (465.360t3). _____
- f. Center of media in containers not more than 2.5 cm from some surface (465.360t3). _____
- g. Pipette tips not dragged across exposed ends of pipets in the pipet container or across lips and necks of dilution bottles. _____
- h. Pipettes not inserted more than 2.5 cm below surface of sample or dilution. _____
- i. When removing sample portions, pipette held at a 45° angle with the bottom of the tip touching the inside neck of the sample or dilution bottle. _____
- j. When pipetting measured portions, the tip of the pipet rests on the inside bottom of the petri dish. _____
- k. After flow has stopped, touched off once on a dry spot for 1 ml portions, if pipette is not a blow-out type; remaining volume gently blown out from cotton-plugged blow-out-type pipette for 1 ml portions after flow has stopped; 0.1ml portions not touched off. _____
- l. 10ml -12 ml agar poured per plate (465.360t6). _____

- m. Sample and agar mixed carefully (465.360t6). _____
- n. Plates incubated in inverted position, no more than four high at 35°C ± 0.5°C for 48 ± 3 hours (465.360t6). _____
- QC** o. Media control for each bottle of agar used (poured last); air control exposed for 15 minutes, pipet, petri dishes, and dilution water controls for each series of samples (air plate started before making first dilution or plating first sample) (465.390d1,d2,d3,d4). _____
- QC** p. Agar weight loss determined quarterly, less than 15%. _____
- q. Counts reported for plates having 30 colonies -300 colonies (If 1.0 ml of undiluted sample results in fewer than 30 colonies, actual count reported) (465.360t9). _____
- r. If more than one analyst in laboratory, each counted the colonies on same plate monthly; colony counts agreed within 10% (465.390c4) (SWTR). _____
- s. SimPlate Unit Dose: 10 mL added to dehydrated medium or 9 mL sterile diluent and 1 mL sample, poured into center of plate, distributed evenly, excess drained into absorbent pad (465.360t7). _____
- t. SimPlates incubated in inverted position at 35°C ± 0.5°C for 48 hours ± 3 hours. _____
- u. Wells that fluoresce under UV light counted; count converted with Idexx Unit Dose MPN table (465.360t7). _____
- v. 10 mL sample most probable number (MPN) read directly, 1 mL sample multiplied by 10 (465.360t7). _____
- w. SimPlate Multiple Dose: 100 mL sterile diluent added to dehydrated medium, 1.0 mL of sample and 9 mL of reconstituted medium added to center of plate, plate swirled to mix, distributed evenly, excess drained into absorbent pad (465.360t8). _____
- x. SimPlates incubated in inverted position at 35°C ± 0.5°C for 48 hours ± 3 hours. _____
- y. Wells that fluoresce counted under UV light; count converted with Idexx Multi-Dose MPN table; if dilution made, MPN value multiplied by dilution factor (465.360t7). _____

SAMPLE COLLECTION, HANDLING, AND PRESERVATION

1. Minimum of 1 inch air space for mixing sample; if too full, poured into a larger sterile container and mixed properly (465.370d). _____
2. Sample Identification
Identification included sample source, location, time and date of collection, collector's name and organization (if not the water supply), persons transporting sample (if not the sampler), transportation condition, sample type and total chlorine residual where applicable (465.370e). _____
3. Form completed in indelible ink immediately after collecting sample (465.370e). _____
4. Date and time recorded of arrival at laboratory and name of person receiving sample (465.370g). _____
5. Each sample assigned a laboratory number; repeat or replacement sample has original sample number recorded (465.370g2). _____
6. For drinking water samples, time between sample collection and placement of analyzed sample in incubator less than or equal to 30 hours (465.370i). _____
7. For water samples tested under the SWTR, time between sample collection and placement of analyzed sample in incubator shall not exceed eight hours (465.370j). _____
8. For water samples tested under the SWTR, samples shall be held at <10C (465.370l). _____
9. Potable water samples for HPC delivered within six hours after collection and analyzed within two hours of receipt (465.370k). _____

DATA HANDLING

1. All forms used in the laboratory for both sample reporting and quality control reviewed and approved by the certification officer (465.410e). _____
2. All records initialed or signed by person/s responsible for recording any or all of the data or performing the tests (465.410a). _____
3. Data transferred from a bench sheet, verification of accuracy performed; initialed or signed (465.410d). _____
4. Quality control records maintained for five years (465.390a). _____

QUALITY ASSURANCE PROGRAM

1. Written QA plan maintained and available to analysts in area where analytical work takes place (465.390a) (465.390b). _____
2. QA plan: (USEPA Certification Manual Chapter III)
 - a. Laboratory organization and responsibility
 1. Chart or table showing laboratory organization. _____
 2. Job descriptions of personnel. _____
 3. Description of training provided to keep personnel updated on regulations and methodology. _____
 - b. SOPs with dates of last revision (465.390b)
 1. SOPs accurately reflect all phases of current laboratory activities. _____
 2. List of SOPs maintained. _____
 3. SOPs reviewed annually and as changes are made. _____
 4. SOPs have signature pages and revisions dated. _____
 - c. Field sampling procedures (as applicable)
 1. Process described that is used to identify sample collectors, sample procedures and locations, required preservation, proper containers, correct sample container cleaning procedures, sample holding times from collection to analysis, and sample shipping and storage conditions. _____
 2. Procedure to complete forms including all required information. _____
 3. Description of how samples are checked upon arrival (e.g., proper containers, temperature, proper preservation). _____
 4. Written sampling procedure available to samplers. _____
 - d. Laboratory sample receipt and handling procedures
 1. Documentation procedures described (e.g., ink only, entries dated and signed). _____
 2. Rejection criteria established; procedure for notification of sample originators. _____
 - e. Analytical procedures (may reference SOP)
 1. Complete method cited. _____
 2. Quality control procedures described (may reference SOP). _____
 - f. Data reduction, validation, reporting and verification (may reference SOP)
 1. Data reduction process described: method of conversion of raw data to coliforms/100mL, heterotrophic bacteria counts to CFU/mL. _____
 2. Data validation process described. _____
 3. Reporting procedures described, including procedures and format. _____
 4. Data verification process described. _____
 - g. Type of quality control (QC) checks and the frequency of their use (may reference SOPs). _____
 - h. List schedules of internal and external system and data quality audits and inter laboratory comparisons (PTs) (may reference SOPs). _____
 - i. Corrective action contingencies. _____
 - j. Record keeping procedures. _____

RECORD MAINTENANCE

1. Sample report forms include identification of sample origin, date, time and place of sampling, name of sample collector, date and time of receipt and analysis, laboratory identification (name and certification number), person(s) responsible for performing analyses, analytical method used and results of analysis (465.420c).

 - a. Drinking water results reported as present or absent for total coliform and *E. coli* for compliance with the RTCR.

 - b. Surface water results enumerated for total coliform and *E.coli*.

 - c. Ground water results are reported for *E. coli* as present or absent for monitoring under the GWR.

2. Sample report forms retained five years (465.420b).

3. All required records in ink (465.420a).

4. Any change lined through so that original entry is visible; changes initialed and dated; documentation supporting all corrections on records shall be maintained (465.420a).

ACTION RESPONSE TO LABORATORY RESULTS

1. Positive presumptive results on public water supply (PWS)samples reported to the PWS and to the state regulatory agency that has jurisdiction over the PWS as preliminary (465.430a).

2. Upon completion of tests, the adjusted results are reported to the PWS and to the state regulatory agency that has jurisdiction over the PWS (465.430a).

3. The public water supply and the state regulatory agency that has jurisdiction over the PWS are notified of invalid sample results (465.430a).

4. If any sample is positive for *E. coli*, the system shall notify the state regulatory agency and PWS by the end of the day (465.430b).

References:

III. Rules for the Certification and Operation of Environmental Laboratories, Title 77, Chapter I, Sec.465, Subchapter d.

Standard Methods for the Analysis of Water and Wastewater (SM), Edition as cited

EPA Manual for the Certification of Laboratories Analyzing Drinking Water, 5th Edition

EPA Manual for the Certification of Laboratories Analyzing Drinking Water, 5th Edition, Supplement 1

40 CFR 141,142, National Primary Drinking Water Regulations; Revisions to the Total Coliform Rule (RTC)

40 CFR 141.70-141.75, National Drinking Water Regulations; Surface Water Treatment Rule (SWTR)

40 CFR 9, 141,142, National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule 40 CFR 9, 141, 142 National Primary Drinking Water Regulations: Ground Water Rule (GWR)

QC = Records must be maintained

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