ILLINOIS DEPARTMENT OF PUBLIC HEALTH
Water Microbiology Laboratory Evaluation Form

Laboratory _____ Laboratory Number _____

Certification Officer(s) _____ Evaluation Date _____

Street Address _____

City _____ State _____ ZIP Code _____

Telephone _____ E-mail _____

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			
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). _____
17. No food or drink for consumption allowed in laboratory area (465.320v). _____

LABORATORY EQUIPMENT, SUPPLIES, AND MATERIALS

1. **Service Contracts**

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). _____

QA 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). _____

QA e. Certificate listing correction data accompanies NIST or ASTM weights (465.400b). _____

QA f. NIST or ASTM weights recertified every five years (465.400b). _____

QA 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). _____

QA 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 ± 1°C from certified thermometer (465.330k5). _____

f. No infrared thermometers allowed (465.330k9). _____

g. No separation in the liquid column (465.330k8) _____

h. Glass or electronic thermometers graduated in no greater than 0.5°C units for use in 35°C incubators (465.330k1). _____

i. Glass or electronic thermometers graduated in no greater than 0.2°C units for use in 44.5°C water baths (465.330k2). _____

j. Glass or electronic thermometers graduated in no greater than 1.0°C units for use in spore incubators (465.330k3). _____

k. Electronic thermometers with thermocouplings 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 spore incubators (465.330k4). _____

l. Maximum registering thermometer or data logger graduated in increments no greater than 1°C (465.330k6). _____

m. 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). _____

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

QA 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 (calculated by pH meter) maintained (percent slope 95%-105%) (465.400a). _____

5. **Agar Tempering Water Bath**

Manufacturer/Model _____

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

QA b. Temperature maintained at 45 ± 1°C (465.330j). _____

6. **Incubator Unit(s)**

Manufacturer/Model (35°C) _____

Manufacturer/Model (44.5°C) _____

Manufacturer/Model (spore) _____

a. Maintains uniform temperature of 35 ± 0.5°C, 44.5 ± 0.2°C, (465.330g); water bath circulating with cover (465.330g). _____

QA 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. For temperature monitoring systems, follow the manufacturer's instructions. _____

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

Manufacturer/Model _____

7. **Refrigerator**

a. Temperature maintained at 1 - 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). _____

d. For temperature monitoring systems, follow manufacturer's instructions. _____

QA e. Temperature recorded daily (465.400v). _____

f. 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 ± 1°C) maintained during cycle (465.330e4). _____

d. Entire cycle completed within 45 minutes when a 12–15-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 or when autoclave is in use if less frequent. Incubate according to manufacturer's instructions (465.400s). _____

g. Maximum registering thermometer, data logger, or internal digital thermometer used each cycle (465.400s). _____

h. Data logger or internal digital thermometer must be calibrated annually by a qualified service representative not affiliated with laboratory. Records maintained (465.400c). _____

i. Data logger with external probe/maximum registering thermometer placed in a container of water unless otherwise specified in manufacturer's instructions (465.400s). _____

QA j. Automatic timing mechanism checked accuracy with stopwatch quarterly (plus or minus 1-minute for each 15-minute time period) (465.400s). _____

k. 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). _____

QA d. Spore strips used monthly (465.400s). _____

e. Maximum registering thermometer or data logger used each cycle (465.400s). _____

QA 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 (465.360s9). _____

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). _____

QA 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). _____

QA d. Multi-use MF units initially calibrated with Class A graduated cylinder; tolerance ± 2.5% (465.400ff). _____

QA e. Each lot of single use MF units checked for calibration with Class A graduated cylinder; ± 2.5% tolerance (465.400ee). _____

15. **Membrane Filters and Pads**

Manufacturer/Type _____

a. Membrane filters from cellulose ester material, white, grid marked, 47mm diameter, 0.45µm pore size (465.330p). _____

b. Alternate pore size used if manufacturer gives performance data equal to or better than the 0.45µm 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). _____

QA 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). _____

QA 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). _____

QA 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 (required only if doing optional UV sterilization – See 1.e below)**

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

QA 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). _____

QA 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. Item	Minimum duration of autoclaving at 121 ± 1°C	
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	_____

QA 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 more than 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) (465.360k2). _____

e. UV sterilizer or boiling water used for at least two minutes between sample filtrations (Optional) (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). _____

	Parameter	Limits	Frequency	
QA	Conductivity	>0.5megohms resistance or <2micromhos/cm @ 25°C	monthly	_____
QA	Total chlorine residual[1]	<0.1 mg/L	monthly	_____
QA	Heterotrophic plate count [2]	<500/mL	monthly	_____
QA	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	_____
QA	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)

QA pH 7.2 ± 0.5 _____

1. Prepared in lab (34gKH_2PO_4/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 (81g$MgCl_2$.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 1-5°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 1.25 mL stock buffer and 5 mL $MgCl_2$ 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 _____ _____

QA 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). _____

QA 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). _____

QA 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). _____

QA 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). _____

e. Pre-calibrated sample containers checked by measuring the volume of one container per lot with class A graduated cylinder. A ±2.5% tolerance is required (465.400l). _____

QA f. Each lot of sample containers checked for fluorescence before use (465.360.j.6). _____

6. **Glassware Washing**

a. Distilled or deionized water used for final rinse (465.400h). _____

b. Detergent designed for laboratory use (465.400h). Brand _____ _____

QA c. Inhibitory residue test performed on clean glassware before initial use of detergent and whenever detergent formulation or washing procedure changes (465.400h). _____

QA 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). _____

QA 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) (465.360l5). _____

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 (465.360l5). _____

k. Multiple Tube Fermentation (MTF) broth with loose-fitting caps used within one week (465.350d6). _____

QA 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). _____

QA n. Media preparation records include type of medium, lot number, date of preparation, sterilization time, and temperature, final pH, initials (465.400n). _____

QA o. Media dispensing apparatus, when used, checked for accuracy (465.400t). _____

QA 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 (465.360l1) (465.360l4). _____

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 (USEPA Certification Manual 5th edition). _____

u. Filter sterilized cefsulodin added to tempered MI agar USEPA Certification Manual 5th edition. _____

QA v. Commercially prepared media records include date received, type of medium, lot number, sample performance, pH, and fluorescence check (465.400o) (465.360j4). _____

2. Heterotrophic Plate Count Agar

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 7.0 ± 0.2 (465.400n) (465.400y) _____

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) (465.400y) _____

Final pH double strength 6.8 ± 0.2 (465.400n) (465.400y) _____

Final pH triple strength 6.8 ± 0.2 (465.400n) (465.400y) _____

4. Brilliant Green Lactose Bile Broth

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 7.2 ± 0.2 (465.400n) (465.400y) _____

5. EC Broth

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 6.9 ± 0.2 (465.400n) (465.400y) _____

6. EC Broth – MUG

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 6.9 ± 0.2 (465.400n) (465.400y) _____

7. Nutrient Agar with MUG

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 6.8 ± 0.2 (465.360r1) (465.400n) (465.400y) _____

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) (465.400y) _____

9. M-FC Broth or Agar

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 7.4 ± 0.2 (465.400n) (465.400y) _____

10. SimPlate

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 7.0 ± 0.3 (465.360s2) (465.400y) _____

11. Colilert

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 7.3 ± 0.3 (465.400n) (465.400y) _____

12. Colilert 18

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 7.3 ± 0.3 (465.400n) (465.400y) _____

13. Colisure

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 7.3 ± 0.3 (465.400n) (465.400y) _____

14. E*Colite Test

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 6.9 ± 0.2 (465.400n) (465.400y) _____

15. Readycult

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 6.8 ± 0.2 (465.400n) (465.400y) _____

16. Modified Colitag

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 6.8 ± 0.2 (465.400n) (465.400y) _____

18. m-Coliblue24

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 7.0 ± 0.2 (465.360l2) (465.400n) (465.400y) _____

19. MI Agar

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 6.95 ± 0.2 (465.360l3) (465.400n) (465.400y) _____

20. MI Broth

Manufacturer _____ Lot Number _____ Date Opened _____ Exp. Date _____

Final pH 7.05 ± 0.2 (465.360l3) (465.400n) (465.400y) _____

21. Non-Selective Broth (used to check sterility of bottles and rinse water/ dilution blanks) (465.400n) (465.400y) 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 (465.360b). _____

c. All total coliform-positive cultures tested for the presence of *E. coli* (465.360p) (RTCR). _____

d. All samples analyzed under the RTCR and the GWR are 100 mL. _____

e. Date and time of analysis start, and completion must be recorded (465.420c6). _____

QA f. PT sample satisfactorily analyzed annually for each certified method. (465.390c1) (465.200).
[*Example: Colilert-18 P/A, Colilert P/A, Colilert-18 Quanti-Tray, and Colilert Quanti-Tray are all considered different methods and thus each require an annual PT.*] _____

4. Membrane Filter Procedures

GWR (Detect)		
E. coli	EPA 1604 (MI Medium)	
	m-ColiBlue 24 Manufacturer's Instructions, SM 23rd Ed. 9222 J	

RTCR (Detect)		
Total Coliform	SM 21st Ed. 9222 A, B, C	
	SM 23rd Ed. 9222 A, B, C	
	SM online Ed. 9222 B, C-97	
Total Coliform and *E. coli*	m-ColiBlue Manufacturer's Instructions, SM 23rd Ed. 9222 J	
	EPA 1604 (MI Medium)	

SWTR (Count)		
Total Coliform	SM 21st Ed. 9222 A, B, C	
	SM 23rd Ed. 9222 A, B, C	
	SM online Ed. 9222 A, B, C-97	
Fecal Coliform	SM 21st Ed. 9222 D	
	SM 22nd Ed. 9222 D	
	SM 23rd Ed. 9222 D	
	SM online Ed. 9222 D-97, 9222D-06	
Total Coliform and *E. coli*	EPA 1604 (MI Medium)	

a. Absorbent pads saturated with broth (2 mL), excess discarded; or 4ml of agar medium used (465.360k4). _____

QA b. Sterility check conducted at start and end of each filtration series; if control indicates contamination, all data rejected and another sample obtained (465.360k2). _____

c. Funnel rinsed at least twice with 20ml-30ml portions of sterile buffered rinse water (465.360k3). _____

d. MF removed with sterile forceps; grasped outside effective filtration area. (465.360k5). _____

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. (465.360l6). _____

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) (465.360m). _____

i. All samples under the SWTR that are TNTC or confluent growth (coliform count cannot be determined) invalidated (465.360n). _____

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 (465.390c2).
3. LTB and BGLB incubated at 35°C ± 0.5°C for 24 - 48 hours (465.360h4).
4. Growth and gas production in LTB and BGLB verified for total coliform (465.360o).

k. MI: Total Coliforms - fluorescent colonies under UV light; *E. coli* - blue colonies under normal light (465.360I6)

l. m-ColiBlue24: Total Coliforms - red colonies and blue to purple colonies. *E. coli* – only blue to purple colonies (465.360I6).

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). **QA**

3. **Standard Total Coliform Fermentation Technique**

RTCR (Detect)		
Total Coliform	SM 21st Ed. 9221 B.1, B.2, 9221 D.1, D.2	
	SM 22nd Ed. 9221 B.1, B.2	
	SM 23rd Ed. 9221 B.1, B.2	
	SM online 9221 B.1, B.2-99, B-2-06, 9221 D.1, D.2-99	

SWTR (Count)		
Total Coliform	SM 21st Ed. 9221 A, B, C	
	SM 22nd Ed. 9221 A, B, C	
	SM 23rd Ed. 9221 A, B, C	
	SM online 9221 A, B, C-99, C-06	

a. Concentration of inoculated medium correct (465.360h2).

b. Incubated at 35°C ± 0.5°C for 24 ± 2 hours (465.360h4).

c. If no gas detected, incubated for another 24 hours (465.360h4).

d. All turbid gas-negative cultures invalidated, and another sample obtained (RTCR) (465.360i1).

e. Cultures from gas-positive tubes incubated in BGLB and [EC (see 9221E) for SWTR] or [EC-MUG (see 9221F) for RTCR].

4. **Fecal Coliform Fermentation Broth Methods**

SWTR (Count)		
Fecal Coliform (A-1 Broth)	SM 21st Ed. 9221 E	
	SM 22nd Ed. 9221 E	
	SM 23rd Ed. 9221 E	
	SM online 9221 E-99, E-06	

SWTR (Count)		
Fecal Coliform (EC Broth)	SM 21st Ed. 9221 E	
	SM 22nd Ed. 9221 E	
	SM 23rd Ed. 9221 E	
	SM online 9221 E-99, E-06	

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 ± 0.5°C.
3. Tubes transferred to 44.5 ± 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 ± 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. _____

5. **Verification Procedures**

a. *E. coli* Procedure following lactose fermentation methods.

RTCR (Detect)		
E. coli	SM 21st Ed. 9221 F.1	
	SM 22nd Ed. 9221 F.1	
	SM 23rd Ed. 9221 F.1	
	SM on-line 9221 F-06	

GWR (Detect)		
E. coli	SM 22nd Ed. 9221 F	
	SM 23rd Ed. 9221 F	
	SM on-line 9221 F-06	

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 ± 0.2°C for 24 ± 2 hours. _____
4. Fluorescence considered *E. coli* positive. _____

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

RTCR (Detect)		
E. coli	SM 21st Ed. 9222 G.1c(1,2)	
	SM 23rd Ed. 9221 I, 9222 H	

GWR (Detect)		
E. coli	SM 23rd Ed. 9222 I	

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 (465.360.f). _____
3. Incubated at 44.5°C ± 0.2°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 21st Ed. 9222 G.1c(1)	

1. Membrane filter transferred from 22 - 24 hours m-Endo plate or 22 - 26 hours m-FC plate to NA-MUG (465.360r2). _____
2. Sheen (m-Endo) or blue (m-FC) colonies marked on lid; incubated additional four hours at 35 ± 0.5°C. (465.360r2). _____
3. Fluorescent colonies reported as *E. coli* (465.360r4). _____

6. **Enzyme Substrate Methods**

a. Colilert, Colilert-18, Colisure.

RTCR (Detect)		
Total Coliform and *E. coli*	SM 21st Ed. 9223 B	
	SM 22nd Ed. 9223 B	
	SM 23rd Ed. 9223 B	
	SM online 9223 B-97, B-04	

SWTR (Count)		
Total Coliform and *E. coli*	SM 21st Ed. 9223 B	
	SM 22nd Ed. 9223 B	
	SM 23rd Ed. 9223 B	
	SM online 9223 B-97, B-04	

b. Readycult®, E*Colite®, Modified Colitag™, Tecta EC/TC.

RTCR (Detect)		
Total Coliform and *E. coli*	Readycult® Manufacturer's Instructions	
	E*Colite® Manufacturer's Instructions	
	Modified Colitag™ Manufacturer's Instructions	
	Tecta EC/TC Manufacturer's Instructions	

c. Colilert/Colilert-18 with Quanti-Tray or Quanti-Tray 2000, or MTF (5-10 tubes) used for SWTR samples; dilution water, if used, is sterile deionized water or distilled water (465.360j14). _____

d. Colilert Test: samples incubated at 35 ± 0.5°C for 24 hours, up to 28 hours; yellow, coliform positive (465.360j13). After 28 hours, negative results are still considered valid, but positive results are not. _____

e. Colilert-18 Test: samples incubated at 35°C ± 0.5°C for 18 hours, up to 22 hours; yellow, coliform positive (465.360j13). After 22 hours, negative results are still considered valid, but positive results are not. _____

f. Colisure Test: samples incubated at 35°C ± 0.5°C for 24 hours, up to 48 hours; red/magenta, coliform positive (465.360j16). _____

g. E*Colite Test: samples incubated at 35°C ± 0.5°C for 28 hours; blue/blue-green, coliform positive; add 20 hours incubation if no fluorescence; if red, sample discarded and resample requested (465.360j17). _____

h. Readycult Test: samples incubated at 35°C ± 0.5°C for 24 ± 1 hours; blue-green, coliform positive (465.360j18). _____

i. Modified Colitag Test: samples incubated at 35°C ± 0.5°C for 16 - 48 hours; yellow, coliform positive (465.360j19). _____

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) (465.360j9) (465.360j18). _____

k. Enzyme tests not used to verify/confirm coliform on membrane filters or in broth cultures (465.360j10). _____

l. Media protected from light and only from commercially available source (465.360j2) (465.360j3). _____

QA m. For Quanti-Tray, sealer checked monthly for leakage (465.360j15). _____

n. Reference comparators provided by manufacturer discarded by expiration date (465.360j12).

Lot Number: _____ Exp. Date: _____ _____

o. 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 (465.360j7). _____

3. Colilert -18 P/A: sample pre-warmed to 35 ± 0.5°C water bath 20 minutes or 44.5°C for 7-10 minutes (465.360j7). _____

4. Colilert P/A, Quanti-Tray, Readycult: 24-hour incubation time includes time to bring sample temperature to 35°C±0.5 C (465.360j7). _____

5. Modified Colitag: If results read before 22 hours, samples must be prewarmed in a 44.5°C water bath for 7-10 minutes (465.360j7). _____

6. Sample load brought to room temperature before incubation (465.360j7). _____

7. **Heterotrophic Plate Count (HPC)**

SWTR (Count)		
Heterotrophic Plate Count	Pour Plate SM 21st Ed. 9215 B	
	Pour Plate SM 22nd Ed. 9215 B	
	Pour Plate SM 23rd Ed. 9215 B	
	Pour Plate SM on-line 9215 B-00, B-04	
	SimPlate Manufacturer's Instructions	

a. Work area disinfected. _____

b. No more than 1 mL or less than 0.1 mL of sample plated (465.360s5). _____

c. Refrigerated medium stored up to three months (465.360s4). _____

d. At least two replicate plates per dilution prepared for each sample (465.360s5). _____

e. Agar tempered to 44 - 46°C before plating; melted agar used within three hours; temperature control bottle containing media used (465.360s3). _____

f. Center of media in containers not more than 2.5 cm from some surface (465.360s3). _____

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; 0.1 mL portions not touched off. _____

l. 10 -12 mL agar poured per plate (465.360s6). _____

m. Sample and agar mixed carefully (465.360s6). _____

n. Plates incubated in inverted position, no more than four high at 35 ± 0.5°C for 48 ± 3 hours (465.360s6). _____

QA 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). _____

QA p. Agar weight loss determined quarterly, less than 15% (465.360s6). _____

q. Counts reported for plates having 30-300 colonies (If 1.0 mL of undiluted sample results in fewer than 30 colonies, actual count reported) (465.360s9).

r. If more than one analyst in laboratory, each counted the colonies on same plate monthly; colony counts agreed within 10% (465.390c4) (SWTR). (*Not required for SimPlate*).

s. Simplate (Unit Dose or Multiple Dose) incubated in inverted position at 35 ± 0.5°C for 48 ± 3 hours. (465.360s7).

t. 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 (465.360s7).

u. Wells that fluoresce under UV light counted; count converted with Idexx Unit Dose MPN table (465.360s7).

v. 10 mL sample most probable number (MPN) read directly; 1 mL sample multiplied by 10 (465.360s7).

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 (465.360s8).

x. Wells that fluoresce counted under UV light; count converted with Idexx Multi-Dose MPN table; if dilution made, MPN value multiplied by dilution factor (465.360s8).

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), 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 <10°C and verified with a temperature control (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/signed by person(s) responsible for recording any or all of the data or performing the tests (465.410a).
3. A careful check shall be made to verify that each result is entered accurately from the bench sheet onto the sample report form. The sample report form shall be initialed or signed by the person who verified the entry of information from the bench sheet (465.410d).
4. Quality control records maintained for five years (465.390a).

QUALITY ASSURANCE PROGRAM

(USEPA Certification Manual Fifth Edition Chapter III, and 465.390)

1. Written QA plan maintained and available to analysts in area where analytical work takes place (465.390a). _____

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.

      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. _____

      5. 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 for membrane filtration and multiple tube fermentation methods (465.430b). _____

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 (465.430b). _____

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

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

References:

Ill. 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 (RTCR)
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)

QA = Records must be maintained

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