State Water Resources Control Board
Environmental Laboratory Accreditation Program

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MICROBIOLOGY CHECKLIST FOR LABORATORY ON-SITE INSPECTION

LABORATORY INFORMATION

Laboratory Name _____ _____

ELAP Reference No Certificate No Expiration Date

Telephone No FAX No

Laboratory Director

Contact Person and Title

DEPARTMENT INFORMATION

Inspector/s Name

Signature of Inspector/s _____ Date

Inspection Type:
( ) Pre-certification Inspection
( ) Initial Certification Inspection
( ) Renewal Certification Inspection
( ) Post-Certification Inspection
( ) Follow-up Inspection
( ) Other (specify) _____

LABORATORY CONFIRMATION OF INSPECTION

This is to confirm that an on-site inspection was conducted on this date at the laboratory. Signature does not necessarily constitute agreement with the findings of the inspector.

_____ _____
Signature of Contact Person Date

INSTRUCTIONS FOR FILLING OUT ELAP MICROBIOLOGY LABORATORY EVALUATION CHECKLIST

A. DESCRIPTION OF COLUMNS:

1. **"Lab's Eval."** – Laboratory uses this column for self-evaluation and to indicate whether or not it is in compliance with the requirement under "ITEM."

2. **"ITEM"** describes the requirement.

3. **"ELAP Eval."** – ELAP uses this column for its evaluation.

4. **"REFERENCES and COMMENTS"** column lists the citation(s) for the requirements. The space for **"COMMENTS"** is reserved for ELAP auditors.

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B. INSTRUCTIONS FOR THE LABORATORY:

1. The laboratory records its self-evaluation in the "**Lab's Eval**." column. This is done prior to ELAP's site visit. **The completed checklist is returned to the ELAP auditor.**

2. Under "**ITEMS**," the laboratory "checks" applicable items or fills in information where *instructed in italics*.

C. MARKING THE CHECKLIST:

1. Use **"Checkmark**" to indicate that laboratory is in compliance.
2. Use **"X"** to indicate that laboratory is not in compliance.
3. Use **"NA"** to indicate that the requirement is not applicable to the laboratory.

D. SECTIONS TO COMPLETE:

Page 4 - 5: Laboratory Equipment
Page 6: Reagent Grade Water, Microbiological Water Suitability Test, Air)
Page 7-17: Quality Assurance/Quality Control and General Micro Methods (where applicable)

Microbiological Methods – Complete only those microbiological methods for which lab is certified or seeking certification.

E. 2.0 Plate Count Methods (HPC, SIMPlate)
E. 3.0 Fermentation Methods for Total Coliforms (Multiple Tube Fermentation, Presence-Absence)
E. 4.0 Fermentation Methods for Fecal Coliforms (EC, A-1)
E. 5.0 Enzyme Substrate Methods for Total Coliforms/Ecoli (EC+MUG, Colilert, Colilert 18, Colisure, E*Colite, Readycult, Colitag)
E. 6.0 Membrane Filtration (mEndo/mEndo LES, mFC, NA+MUG)
E. 7.0 Membrane Filtration (m-Coliblue24, MI, Chromocult, Coliscan, Modified mTEC)
E. 8.0 Membrane Filtration for Enterococcal Methods (m-Enterococcus, mE, mEI)
E. 9.0 Most Probable Numbers for Enterococcal Methods (Fecal Strep MTF Azide dextrose, Enterolert)

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E. REFERENCE LIST AND TOTAL COLIFORM RULE:

Page 3 lists the references cited in the Checklist
Page 4 describes the requirements of the Total Coliform Rule.

REFERENCES for CITATIONS:

1) Standard Methods for the Analysis of Water and Wastewater, 18th, 19th, 20th, 21st , 22nd, online APHA, AWWA, http://standardmethods.org/

2) Manual for the Certification of Laboratories Analyzing Drinking Water, 5th edition, January 2005. http://www.epa.gov/ogwdw/methods/pdfs/manual_labcertification.pdf

3) Code of Federal Regulations, Vol. 40, Part 141; http://www.law.cornell.edu/cfr/text/40/part-141 and Part 131
   a. Total Coliform Rule (TCR) – 40 CFR 141.21(f)(3)
   b. Surface Water Treatment Rule (SWTR) – 40 CFR 141.74(a)(1)
   c. Long Term 2 Enhanced SWTR (LT2) – 40 CFR 141.704(b)
   d. Ground Water Rule (GWR) – 40 CFR 131.402(c)(2)

4) Code of Federal Regulations, Vol. 40, Volume 77 2012, http://www.gpo.gov/fdsys/pkg/FR-2012-05-18/html/2012-10210.htm

5) AB 411 – Recommended Methods for the Analysis of Recreational Marine Water http://www.cdph.ca.gov/HealthInfo/environhealth/water/Pages/Beaches.aspx

6) Product Inserts

   a. SIMPlate, Colilert®, Colilert® 18, Colisure, Enterolert™ , http://www.idexx.com/view/xhtml/en_us/water/water-testing-solutions.jsf IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092

   b. m-ColiBlue24®, http://www.hach.com/quick.search-quick.search.jsa?keywords=mcoliblue Hach Company,100 Dayton Avenue, Ames, IA 50010

   c. E*Colite®, Charm Sciences, Inc., 36 Franklin Street, Malden, MA 02148-4120

   d. Readycult, ‘‘Readycult Coliforms 100 Presence/Absence Test for Detection and Identification of Coliform Bacteria and Escherichia coli in Finished Waters’’, November 2000, Version 1.0, available from EM Science

   e. Chromocult, ‘‘Chromocult Coliform Agar Presence/Absence Membrane Filter Test Method for Detection and Identification of Coliform Bacteria and Escherichia coli in Finished Waters’’, November 2000, Version 1.0, available from EM Science.

   f. Modified Colitag; CPI International http://media.wix.com/ugd/5b350b_77da24c5f051cd9df056b6254a0961f3.pdf

7) EPA Membrane Filter Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Drinking Water, EPA 600-R-00-013, February 2000.

8) California Code of Regulations, Title 22, Division 4, Chapter 19 http://www.cdph.ca.gov/certlic/labs/Documents/ELAPRegulations.pdf

9) Microbiological Methods for Monitoring the Environment Water and Wastes EPA/600/8-78/017, 1978, US EPA

10) Recycled Water Regulations 01-2009 Title 22, Section 60321(a).

TOTAL COLIFORM RULE (TCR) for DRINKING WATER	REFERENCES
Requirements , Action, Response	**(2) (3) CFR 141.21**
1. Only analytical methods specified in Total Coliform Rule (4CFR 141.21(f) and Surface Water Treatment Rule (40CFR 141.74(a) used for compliance purposes with drinking water.	(2) V.5.1.1
2. Laboratory is certified for all analytical methods it uses for compliance purposes.	(2) V.5.1.2
3. All total coliform positive drinking water samples tested for presence of either fecal coliforms or *E. coli*.	(2) V.9.1
4. Total Coliform positive results are based on confirmed phase for MTF technique and P-A Broth coliform tests.	(2) V.9.2
5. Total Coliform positive result for MF technique based on verification test.	(2) V.9.2
6. Laboratory promptly notifies proper authority of positive total coliform, fecal coliform, or *E. col*i results. Notification record kept.	(2) V.9.2
7. Invalidation of Total Coliform – Positive Sample ✓ Applies only if conditions listed in CFR 141.21(c)(1)(i),(ii), or (iii) are met.	(3) CFR 141.21(c)(1)
8. Invalidation of Total Coliform – Negative Sample ✓ Applies to Fermentation broths (LTB and P-A) that are turbid without gas or acid ✓ or membrane filters that show confluent growth or are TNTC with no coliforms detected. ✓ Notify supplier promptly within 24 hours ✓ Replacement sample within 24 hours.	(2) V.9.3; (3) CFR 141.21(c)(2)

Laboratories are to complete this page before site visit:

LABORATORY EQUIPMENT			
EQUIPMENT	**MANUFACTURER**	**MODEL**	**DATE LAST MAINTENANCE**
pH Meter			
Balance(s)			
Conductivity Meter			
Incubator(s)			
Water Bath(s)			
Refrigerator(s)			
Autoclave(s)			
Hot Air Oven			
Colony Counter			
Ultraviolet Light 254 nm			

Ultraviolet Light 365-366 nm			
10-15x Stereo Microscope (MF)			
Light Microscope			
Quanti Tray Sealer			

REFERENCE THERMOMETERS * (*re-calibration every 5 years*)		
Manufacturer and Identification No.	**Range and Calibration Points**	**Date of Last Certification**

Verify accuracy at least every 5 years. Specify 0°C, 35°C, 44.5°C (plus other essential temperature calibration points) when recalibrating the Reference thermometer.

NOTES: 1**.** The checklists pages 7 – 17 outlines the 22nd edition of the Standard Methods (SM) 9020 Quality Assurance/Quality Control (QA/QC) approved 2005 requirements.

2. These QA/QC requirements are applicable to SM 9221 (MTF) and SM 9223 (Colilert and Colisure) particularly for wastewater testing.

3. These QA/QC requirements can be applied to drinking water samples if the laboratory is using the 22nd edition of the SM for drinking water methods.

4. SM 18th, 19th, 20th, 21st, 22nd and online are acceptable references for drinking water regulatory testing.

5. The QA/QC for SM 9222 D – 1997 (MF) are available in SM online or in SM 20th and 21st editions for waste water regulatory testing.

Specify Standard Methods edition used ____. Check specific revision of SM used for QA/QC requirements.

REAGENT GRADE WATER	**ELAP Eval.**	**SM 9020B (2) V.4.3**
Source of reagent grade water: Lab prepared:_____ Commercially prepared (Manufacture): _____		
QC **Quality of reagent water is tested and meets the following criteria. (lab or commercially prepared)** **Tested each use:** ☐ Conductivity……<2micromhos/cm (micro Siemens/cm) at 25°C**.** **Tested monthly:** ❑ Total Chlorine residual ……<0.1 mg/L (DPD method) ❑ Heterotrophic plate count**……** <500 CFU/ml (pour or SIM plate method) ☐ Total Organic carbon……<1.0 mg/L **Tested annually***:* ❑ Pb, Cd, Cr, Cu, Ni, Zn…….. …<0.05 mg/L per contaminant ❑ Heavy Metals, total…….. …… <0.10 mg/L ❑ *Water quality test……...ratio 0.8 to 3.0 Tested by (lab): _____ Analyst: _____ Date Tested:_____ Results:_____ ❑ *****Test not required for ASTM Type 2 water or better– SM 1080C		Table 9020:II Type ___ SiO_2 (mg/L) _____
***Microbiological Water Suitability (Use Test) annually, when source is changed or problems**		9020B.5.f
Air in Workspace	**ELAP Eval**	**SM 9020B**
Monitor bacterial density **monthly**		Table 9020:I

Lab's Eval.	ITEM	ELAP Eval.	REFERENCES COMMENTS
A. LABORATORY RECORDS			**SM 9020, (7) CCR Title 22, Div.4, cha 19**
	1. Quality Assurance (QA) Plan		(7) Art.8, 64815, 9020B.12.a
	a. Written QA Plan prepared, followed, and available for inspection.		
	2. Records and Data Reporting		
	a. Sample Collection Information ❑ Identification (number) ❑ Source (location collected) ❑ Name of water system ❑ Date collected ❑ Time collected ❑ Name of collector (initial) ❑ Chlorine residual (if any) ❑ Sample type – DW, WW, surface, process, routine, repeat, etc.		
	b. Sample Transport and Condition ❑ Name of person transporting sample (if not sampler) ❑ Name of person receiving sample ❑ Date received ❑ Time Received ❑ Temperature of samples upon receipt checked/documented ❑ Holding time met (collection to testing) **30 hrs. drinking water** **8 hrs. surface water and HPC (<10°C for surface water)** **8 hrs. waste water, recycled and recreational water** ❑ Deviations noted and recorded.		9020B.12
	c. Analytical Records ❑ Sample identification ❑ Analytical method used ❑ Date and time testing started for each step ❑ Date and time each step is read ❑ Name of analyst or initials for each step ❑ Analytical results recorded ❑ Date reported		9020B.12.b
	d. Maintenance of Records: ❑ Records of microbiological analyses kept by or accessible to laboratory for at least 5 years. ❑ Client water system notified before disposal of records. ❑ Preventive maintenance and repair records kept for 5 years.		(7) Art. 7, 64813 9020B.12.c
	e. All data recorded in ink with changes lined through such that original entry visible. Changes initialed and dated.		9020B.12.c

Lab's Eval.	ITEM	ELAP Eval.	REFERENCES COMMENTS
B. FACILITIES AND WORK AREA			**(7) CCR Title 22, Div.4, Ch 19**
	1. Facilities ❑ Clean ❑ Controlled temperature and humidity ❑ Adequate lighting and ventilation ❑ Bench top is non-porous, easily cleaned and disinfected		9020B.3 (7) Art. 7, 64813
	2. Work Area ❑ Adequate bench space for peak work periods (2 meters/analyst) ❑ Sufficient space – equipment, sample processing, media prep, sterilization, glassware washing, and storage of media and supplies		9020B.3.c
	3. Provisions are made for disposal of microbiological wastes.		1090D.
C. LABORATORY EQUIPMENT			
	1. pH METER		**9020B.4.c, 9030B.6**
	a. Scale graduations within ± 0.1unit with temperature compensation		
	b. Electrodes maintained as per manufacturer's instructions.		
	c. Standardized **each day** of use with at least 2 buffers (start with a 7, then 4 or 10) plus a check that bracket pH measured. Check buffer should be within ± 0.1 units. **Date, calibration results, check buffer and analyst initials recorded**.		
	d. Commercial buffer solutions **labeled** with **date of receipt** and **date opened**. Solutions discarded when expired.		
	e. Buffer aliquots must be discarded after each use.		
	f. ***Record** pH meter **slope monthly,** after calibration (95 – 105%)		
	g. Check pH of solid, agar media with a flat head probe		
	2. BALANCE (top loader or pan)		**9020B.4.b; 9030B.7**
	a. Balance has readability of 0.1 g.		
	b. Balances should detect 100 mg (0.1g) with 150 g load.		
	c. Balance checked zero and **checked with each use** using ASTM type 1 or NIST Class S (with certificate) minimum 2 weights that bracket laboratory's weighing needs and the **data recorded**.		
	d. Non-reference weights (if used) **checked monthly** using reference weights, and the **data recorded**.		
	e. **Reference weights re-certified every five years**. If damaged or corroded should be replaced.		
	f. Balance serviced and recalibrated **annually** at minimum.		9020B.4.b

Lab's Eval.	ITEM	ELAP Evaluation	REFERENCES COMMENTS
	3. CONDUCTIVITY METER		**9020B.4.q; 2510B**
	a. Calibrate meter **monthly** using a certified low level standard at 25°C or determine **cell constant** using a method indicated in SM 2510B.		
	b. Correct reading to 25°C if no auto temperature compensation using formula in SM 2510B.5b. Documentation maintained.		
	c. If an in-line unit cannot be calibrated, it should not be used to check reagent grade water.		
	4. TEMPERATURE MEASURING DEVICES		**9020B.4.a**
	a. Temperature monitoring devices graduated in increments appropriate for use and immersed in glycerol (except for electronic thermometers) ❑ 0.1°C graduation for tests at 44.5 ± 0.2°C (**total immersion short range and length**) ❑ ≤ 0.5°C graduations for tests incubated at 35.0 ± 0.5°C ❑ ≤ 1.0°C for refrigerators		
	b. No separation in the fluid column of thermometer.		
	c. Dial thermometers that cannot be adjusted are not used.		
	d. Thermometers are calibrated against certified NIST thermometer or one traceable and conforming to NIST. **Results recorded.** ❑ **Glass (annually) preferably semi-annually** ❑ **Electronic (annually) preferably semi-annually** ❑ Continuous recording devices preferred (**annually**) ❑ Dial Thermometers (Quarterly) ❑ If a thermometer differs by more than 1°C from the reference thermometer, it should be discarded.		
	e. Thermometer is calibrated at the use temperatures.		
	f. Verify accuracy of the reference certified thermometer every 5 years. Apply correction factor (if any) to working thermometers.		9020B.4.a
	g. **Thermometers in incubators and refrigerators labeled** with **date of calibration** and **applicable correction factor**.		
	h. SOP for Thermometer Calibration and Temperature Monitoring		
	5. INCUBATOR(S)		**SM 9020B.4.o; 9230B.1**
	a. Sufficient size for workload. Do not overload. Place in room maintained between 16 – 27°C (60 -80°F).		
	b. Bring all cold samples in media to room temperature before insertion.		
	c. Thermometer is on shelves in use. Large incubators have thermometers placed on top and bottom shelf.		

Lab's Eval	ITEM	ELAP Eval	REFERENCES COMMENTS
	d. Corrected temperature recorded twice each day of use, with readings at least 4 hours apart.		
	e. Calibrate heat block incubators and determine their stability before initial use; calibrate the built-in thermostat annually thereafter with a NIST-traceable thermometer.		**SM 9030**
	6. WATER BATH		**9020B.4.n; 9230B.1**
	a. Sufficient size for workload. Fill and maintain level with **reagent quality water**.		
	b. Equipped with water circulator and a gabled cover.		
	c. Water bath has an internal temperature monitoring device and maintains temperature at 44.5 ± 0.2°C or 35 ± 0.5°C, if used must be calibrated annually.		
	d. Use a **total immersion thermometer** graduated 0.1°C.		
	e. Corrected temperature recorded twice each day of use, with readings at least 4 hours apart.		
	7. REFRIGERATOR		**9020B.4.i; 9230B.11**
	a. Refrigerator temperature maintained at 2° to 8°C		
	b. Temperature recorded at least once for days in use. (weekends excepted). Clean at least annually. Identify and date stored materials		
	8. AUTOCLAVE		**9020B.4.h; 9030B.3; 9050A.3**
	a. Size is sufficient for workload.		
	b. Pressure cooker or vertical autoclave is not used.		
	c. Autoclave has internal heat source, temperature gauge with sensor in exhaust, pressure gauge, and operational safety valve.		
	d. Maintains sterilization temperature during cycle and **completes entire cycle** within **45 minutes (except when design includes heat exchangers and solution cooling system)** when **12 to 15 minute sterilization** period is used.		9030B.3
	e. Automatic **timing device** is checked **quarterly** with stopwatch**.**		9020:I
	f. Check Stopwatch against National Time Signal **annually.**		
	g. **Maximum registering thermometer** used at least **weekly** to check that sterilization temperature is reached.		
	h. Heat indicating tape used to identify items that have been sterilized.		
	i. **Spore strips, suspension or capsules** used **monthly** as bio indicators to confirm sterilization. Placed into glassware		

	containing a liquid.		
Lab's Eval	**ITEM**	**ELAP Eval**	**REFERENCES COMMENTS**
.	j. Routine maintenance performed, including cleaning of drain screen and the door seals.		
	k. **<u>Autoclave records are maintained</u>**. ❑ date ❑ contents ❑ time in and time out ❑ total exposure/cycle time ❑ sterilization time ❑ sterilization temperature ❑ set and actual pressure readings ❑ initial of analyst or technician		9020B.4.h
	9. HOT AIR OVEN		**9020B.4.g; 9030B.2**
	a. Sterilization temperature of 160º to 180º C at least 2 – 4 hrs.		9020B.5.j.2
	b. Oven thermometer graduated in 10º C increments or less with bulb immersed in sand during use.		
	c. **Spore strip** used **monthly** to check effectiveness of the sterilization process.		
	d. Use heat-indicating tape to identify supplies and materials.		
	e. <u>Hot air oven record maintained</u>: ❑ date ❑ contents ❑ sterilization time ❑ sterilization temperature ❑ Analyst's initials		
	10. OPTICAL COUNTING EQUIPMENT		**9030B.5.a; 9222B -1997**
	a. A dark field colony counter used to count Heterotrophic Plate Count colonies.		
	b. A binocular or zoom microscope with 10 to 15X magnification using fluorescent light at angle of 60 - 80° above colonies. **Clean optics and stage, alignment quarterly**.		9222B.1.k
	c. DO NOTUSE AUTOMATIC COLONY COUNTERS		
	11. ULTRAVIOLET LAMPS		**9020B.4.l**
	CAUTION: Short and long wave UV light can damage eyes/skin a. UV Lamp – 254 nm (membrane filtration) ❑ Unit is disconnected monthly and cleaned by wiping with soft cloth moistened with ethanol or 70% methanol/water. ❑ If used for **sanitation, unit is tested quarterly** with UV light meter or by plate count method.		
	b. UV lamp replaced □ if output (meter) is <70% of initial output □ or if 2 minutes exposure of plates containing 200 to 300 CFU/ ml do not show count reduction of 99% after incubation		

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
	at 35°C for 24 & 48 H.		
	12. MEMBRANE FILTRATION (MF) EQUIPMENT- 20th, 21st and online SM 9222 1997		**9020B.3.k, 4h; 9030B; 9222B**
	a. MF units made of stainless steel, glass, or autoclavable plastic and not scratched or corroded and do not leak.		9030B.15;
	b. Membrane filtration equipment are either □ autoclaved or □ exposed to UV light for at least 2 minutes initially and before reusing units between successive filtration series. (Filtration series ends when 30 min. or longer elapse after a sample is filtered.)		9020B.2.l; 9222B.1.f;
	c. **Graduations on funnel (1/lot)**, if used to measure sample volume, are **checked for accuracy** with Class A cylinder or volumetric pipet and have tolerance of ≤2.5%. Record of calibration check retained.		9020B.4.k
	d. **Filters approved by manufacturer** for total coliform water analysis. ❑ Filters made of cellulose ester, white, grid marked, 47 mm diameter, and 0.45 µm pore size. ❑ Perform Use Test on new lots of filters ❑ Lot number and date received recorded for membrane filters.		9222B.1.g;
	e. **Absorbent pads**, if used, 48 mm diameter, of thickness 0.8 mm and able to absorb 1.8 - 2.2 mL media used.		9222B.1.h;
	f. **Membrane filters and pads** purchased: ❑ Pre-sterilized OR ❑ Autoclaved 10 minutes at 121°C before use.		9222B.1.g;
	g. **10x to 15x stereo microscope with fluorescent light source** must be used to count sheen colonies.		9222B.1.k;
	13. INOCULATING EQUIPMENT		**9030B.18**
	a. ***Check inoculating equipment used:*** ( ) Metal loops or needles of nickel alloy or platinum iridium at least 3mm in diameter. ( ) Plastic loops or applicators sterilized by autoclave stored in glass ( ) Wood applicator sticks sterilized by dry heat. ( ) Disposable plastic loops.		
	14. PIPETS, MICROPIPETS AND GRADUATED CYLINDERS		**9020B.4.s; 9030B.9**
	a. Disposable glass or plastic pipets used: ❑ Open packs resealed between use period ❑ Check for accuracy & precision **per lot** and document (1-4%)		
	b. Reusable pipets used: ❑ Stainless steel or aluminum canisters used to sterilize and maintain sterility of glass reusable pipets. ❑ Check for accuracy & precision **annually** and document (1-4%)		

	c. **Electronic Pipettors** checked for **accuracy and precision quarterly** or sooner and documented. (±2.5%)		
	d. Media-dispensing apparatus checked for **volume dispense accuracy** with **each use**. (±2.5%)		9020:I
	ITEM		9020:I
	15. PETRI DISHES	**ELAP Eval**	**REFERENCES COMMENTS**
Lab's Eval.	a. Pre-sterilized plastic dishes: Opened packages of plastic dishes re-sealed after use to maintain sterility.		V5 3.14.2
	b. Reusable glass dishes used: ❑ Glass dishes stored in stainless or aluminum canisters Wrapped in aluminum foil/char-resistant paper before sterilizing		**9030B.14**
	16. CULTURE TUBES		
	❑ Tubes made of borosilicate glass or plastic sterilized properly. Design permits conforming to medium and volume requirements for concentration of nutritive ingredients as described.		
	❑ Tube caps of stainless, aluminum or plastic slip caps or screw-caps.		**9030B.13, 17**
	Tubes used for test of gas production, enclose an inverted Durham tube or vial.		
	a. Tube and vial of such size that the vial will be filled completely with medium, at least partly submerged in the tube and large enough to make gas bubbles easily visible.		
	b. **SAMPLE CONTAINERS**		
	a. *Check type of container used*: ( ) Wide-mouthed plastic bottles; caps with non-toxic liners. ( ) Non-corrosive glass bottles; caps with non-toxic liners or non-leaking glass stoppers. Glass stoppers covered with aluminum foil or char-resistant paper for sterilization. c. ( ) Sterile leak-proof bags		
	d. Sufficient sodium thiosulfate added to sample containers before sterilization, if laboratory analyzes chlorinated water. Discard turbid solutions of 10% sodium thiosulfate.		**9020B.5.d; 9030B.18; 9060**
	17. Check **efficacy of dechlorinating agent** one per batch or lot. Record.		
	Capacity of container at least 120 ml (4 oz.) with ample air space (1 inch or 2.5 cm) to facilitate mixing.		9060A.2
	b. Check **accuracy of 100 ml mark** one bottle per lot. Record.		9060A.2
	c. At least one bottle from each batch or lot of sample containers is		

	tested for **sterility** by adding 25 ml of a sterile non-selective broth, incubating at 35 ±0.5°C for 24 and 48 hrs and checking for growth. Re-sterilize entire batch or lot if growth occurs.		
	d. If sample received is overfilled with no room to shake, transfer to larger sterile container, mix and aseptically measure 100 ml.		
	e.		
	f.		
	g.		

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
D. GENERAL LABORATORY PRACTICES			
	1. STERILIZATION TIMES AND PROCEDURES		**Table 9020:IV, 9222E.2.a**
	a. Required times for autoclaving material at 121°C being observed. ❑ Membrane filters and pads ……10 min ❑ Carbohydrate containing media ... 12 – 15 min. ❑ P-A broth…….12 min. ❑ A-1 broth …….10 min.		Table 9020:IV, 9222E.2.a, 9221D.1.a;
	b. Total autoclave exposure time being observed. ❑ Autoclaved media, filters, and pads removed immediately after completion of sterilization cycle. ❑ 45 minutes for carbohydrate containing media. ❑ 30 minutes for Presence-Absence broth.		9221D.1a; 9020B.5.j.2
	c. Minimum times for autoclaving material at 121°C. Exposure time at this temperature may require adjustment depending upon volumes, containers, and loads. ❑ Contaminated test materials ……30 min. ❑ Membrane filter assemblies …….15 min. ❑ Membrane filters & pads……10 min. ❑ Carbohydrate containing Media…….12-15 min. or manufacturer specified ❑ Sample collection bottles ……..30 min. ❑ Individual glassware ……..30 min. ❑ Dilution water blank ……15 min. ❑ Rinse water > 100 ml ……..15-30 min. depends on Vol.		Table 9020IV, 9020B.5.j.2
	2. DILUTION/RINSE WATER		**9020B; 9050C**
	a. Stock buffered water or peptone water – prepared as specified in SM 9050C.		9050C.1;
	b. Stock buffers autoclaved or filter-sterilized and the containers **labeled, dated and refrigerated free of turbidity**.		

	c. Dilution buffer if used, **check 1/batch or lot for accuracy ± 2 ml for 90 or 99ml volume**		
	d. Each batch or lot of dilution/rinse water **checked for sterility** by adding 50 ml of water to 50 ml double strength non-selective broth, incubating at 35 ± 0.5°C for 24 hrs, and checking for growth. Discard batch/lot if growth detected.		9020B.9.d
	3. GLASSWARE WASHING		**9020B, 9040**
	a. Distilled or deionized water used for final rinse.		
	b Glassware should be washed with detergent designed for lab use.		
Lab's Eval.	**ITEM**	**ELAP Eval**	**REFERENCES COMMENTS**
	c. **Each batch** of dry glassware **spot-checked for pH reaction** using bromthymol blue or other pH indicator and documented.		9020B.5.a.1) – 2)
	d. **If spot checking** is done on **each batch** of glassware and negative, □(Y) □(N), then Glassware inhibitory residue test (IRT) is performed **initial use of washing compound and whenever a new washing procedure is used**. Otherwise IRT must be done annually. Compound _____Mfg. _____ Lot _____ Tested by (lab) _____ Analyst _____ Date _____ and Results of most recent IRT: B – A / B x 100 = _____% (% difference between routinely rinsed petri dishes and controls B – C / B x 100 = _____% (% difference between un rinsed petri dishes and controls.) B – D / B x 100 = _____% (% difference between pre sterilized disposable petri dishes and controls) A – C / A x 100 = _____% (% difference between un rinsed petri dishes and routinely rinsed petri dishes)		
	4. MEDIA		**9020B.5.j, 9050A,C**
	a. Dehydrated or prepared media manufactured commercially used. *strongly recommended*		
	b. Receipt, open, and expiration dates clearly marked on dehydrated and commercially prepared media. Use by expiration date.		
	c. Store dehydrated media in cool (15 -25°C) dry location away from direct sunlight. Caked or discolored dehydrated media discarded.		
	d. Use opened bottles within 6 months		
	e. Records for laboratory prepared media include: ❑ Date of preparation ❑ Kind of medium ❑ Manufacturer and lot number ❑ Amount of medium weighed ❑ Volume of medium prepared		9020B.5.j.1), 6)

	❑ Sterilization time and ❑ temperature ❑ Final pH (if pH differs by >0.5 units, discard the batch) ❑ pH adjustments needed ❑ Technician's initial		
	f. Records for liquid media prepared commercially include: ❑ Date received ❑ expiration ❑ Kind of medium ❑ Manufacturer and lot number ❑ **pH** verification for each batch ❑ Media discarded by manufacturer's expiration date		9020B.j.7)
	g. Each **new lot of prepared commercial medium** checked before use for **sterility** and **with positive and negative control cultures**. Results recorded.		9020B.j.7)
Lab's Eval.	**ITEM**	**ELAP Eval**	**REFERENCES COMMENTS**
	h. Each **new batch of laboratory prepared media** checked for **sterility** and **with positive and negative controls**. Results recorded.		9020B.5.j.1), 6)
	i. Prepared media labeled with identification and preparation or expiration date.		9020B.5.j.1)
	j. Medium discarded if evaporation exceeds 10% of original volume.		
	k. Fermentation media, if refrigerated, is brought to room temperature before use. Reject batch with growth or false positive responses.		9020B.5.j.4)
	5. STORAGE AND HOLDING TIMES OF PREPARED MEDIA		**Table 9020:V**
	a. Agar or Broth media in loose-cap tube stored at 2 - 8°C and held no longer than **2 weeks.** Media must be at Room Temperature for use.		
	b. Agar or Broth media in tightly closed screw-cap tubes at <30°C can be kept up to **3 months**		
	c. Poured agar plates with loose fitting covers in sealed plastic bags or tightly sealed containers, refrigerated at 2 – 8°C can be held no longer than **2 weeks.**		
	d. M-Endo/M-Endo LES broth in screw-cap flasks held no longer than **96 hours** at 2 -8°C.		

MICROBIOLOGICAL MEDIA SPECIFICATIONS
Check the media used in your laboratory.

( ) Lauryl Tryptose Broth (LTB)
- ❑ 35.6 g/L (SS)
- ❑ pH = 6.8 ± 0.2

( ) Brilliant Green Lactose Bile Broth (BGLBB)
- ❑ 40.0 g/L
- ❑ pH = 7.2 ± 0.2

( ) Presence-Absence Broth (P-A)
- ❑ 91.5 g/L (3x /100 mL sample),
- ❑ pH = 6.8 ± 0.2

( ) EC Broth
- ❑ 37 g/L
- ❑ pH =6.9 ± 0.2

( ) EC + MUG Broth
- ❑ 37 g/L
- ❑ 6.9 ± 0.2

( ) A-1 Broth Store in dark 7days
- ❑ 31 g/l + 1.0 mL PEG p-isooctylphenyl ether
- ❑ pH = 6.9 ± 0.1

( ) MacConkey Agar
- ❑ 50 g/L,
- ❑ pH = 7.1 ± 0.2

( ) m-Endo Agar
- ❑ 63 g/L (Broth = 48 g/L)
- ❑ pH = 7.2 ± 0.2

( ) m-Endo LES Agar
- ❑ 51 g/L (Broth = 36 g/L)
- ❑ pH = 7.2 ± 0.2

( ) Nutrient Agar / NA + MUG
- ❑ 23 g/L + 0.1 g/L MUG
- ❑ pH = 6.8 ± 0.2

( ) m-FC Agar
- ❑ 52 g/L (Broth = 37 g/L)
- ❑ pH = 7.4 ± 0.2

( ) MI Broth or Agar
- ❑ pH = 6.95 ± 0.2
- ❑ pH = 7.02 ± 0.2

( ) m-ColiBlue24
- ❑ pH = 7.02 ± 0.2

( ) Plate Count Agar (*Tryptose glucose yeast agar)*
- ❑ 23.5 g/L
- ❑ pH = 7.0 ± 0.2

( ) Azide Dextrose Broth
- ❑ 34.7 g/L
- ❑ pH = 7.2 ± 0.2

( ) Bile Esculin Azide Agar
- ❑ pH = 7.1 ± 0.2

( ) mE Agar (enterococci)
- ❑ 70.2 g/L + Nalidixic acid and 2,3,5-triphenyl tetrazolium chloride
- ❑ pH = 7.1 ± 0.2

( ) EIA Substrate (Esculin Iron Agar)
- ❑ 16.6 g/L
- ❑ pH = 7.1 ± 0.2

( ) m-Enterococcus Agar (fecal streptococci)
- ❑ 41.5 g/L
- ❑ pH = 7.1 ± 0.2

( ) mEI agar
- ❑ pH = 7.1 ± 0.2

( ) R2A Agar
- ❑ 18.15 g/L
- ❑ pH = 7.2

SUGGESTED CONTROL CULTURES FOR MICROBIOLOGICAL TESTS
Check the control cultures used in your laboratory.

GROUP	POSITIVE CONTROL	NEGATIVE CONTROL
TOTAL COLIFORMS ferment lactose	❑ Escherichia coli ❑ Enterobacter aerogenes	❑ Staphylococcus aureus ❑ Proteus vulgaris * ❑ Pseudomonas aeruginosa
FECAL COLIFORMS thermotolerant (44.5°C)	❑ Escherichia coli ❑ Klebsiella pneumoniae (thermotolerant strain)	❑ Enterobacter aerogenes
E. COLI MUG positive**	❑ Escherichia coli (MUG positive strain)	❑ Enterobacter aerogenes ❑ Klebsiella pneumoniae (thermotolerant), MUG (-)
ENTEROCOCCI	❑ Enterococcus faecalis ❑ Enterococcus faecium	❑ Staphylococcus aureus (nalidixic acid sensitive) ❑ Escherichia coli (sodium azide sensitive) ❑ Serratia marcescens (fluorogenic compound not hydrolyzed)

* P. vulgaris uses hydrolyzed lactose indicating "overcooked" medium. ** Not all strains of E. coli are MUG (+), e.g. E.coli O157:H7.

Working stocks shall not be sequentially cultured more than 5 times and shall not be sub cultured to replace reference stocks.

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
E. MICROBIOLOGICAL METHODS			
	1. GENERAL		**CFR 141.21**
	a. For drinking water, the sample volume analyzed is 100 ± 2.5 ml.		
	b. Water samples are shaken vigorously (about 25 times) before analysis. If too full to shake, sample transferred to another, larger sterile container, shake about 25 times and measure aseptically. Document.		
	c. If greater than 102.5 but with head space, shaken vigorously (about 25 times) before lab pipets off excess with sterile pipet or pours off excess. Document.		
	d. If no total coliform positive results occur during a quarter, the laboratory spikes and analyzes a sample with a known total coliform, Fecal coliform and/or E. coli positive control organism.		
	e. Parallel testing performed between a newly approved test procedure and another EPA-approved procedure for several months and/or several seasons. (Recommended)		

E. 2.0 MICROBIOLOGICAL METHODS – PLATE COUNT METHODS			
	2.1 HETEROTROPHIC PLATE COUNT		**SM 9215A,B CFR 141.74**
	a. Pour plate method used for HPC testing of waters for compliance.		
	b. For most potable water samples, countable plates are obtained by plating 1.0 and 0.1 ml volume of undiluted sample.		9215B.2.a;
	c. At least duplicate plates per dilution are used.		9215B.2.c;
	d. For pour plate method, melted agar is tempered at 44-46°C in water bath before pouring, held no longer than 3 hours, and melted only once.		9215B.3.a; 2)
	e. 12 to 15 mL of melted agar added to sample that is aseptically poured to bottom of petri dish (100 mm x 15 mm).		
	f. Sample mixed adequately with melted medium without spillage and allowed to solidify on level surface.		9215B.3.b;
	g. Plates are inverted and incubated at 35 ± 0.5°C for 48 ± 3 hours. (Bottled water incubated 72 hours)		9215A.7;
	h. Plates are stacked no more than 4 high and arranged to allow circulation between stacks.		
	i. Agar medium sterility control included. Data rejected if control shows any contamination.		9215B.3.c;
Lab's Eval.	**ITEM**	**ELAP Eval**	**REFERENCES COMMENTS**

	j. Sterility control for dilution water (if used) is included.		9215B.3.c
	k. Room air control is included. (<15 cfu/plate for 15 min. exposure)		9215B.3.c; 9020B.1.e
	l. Colonies are counted manually using a dark field colony counter.		9215A.8;
	m. Only plates with 30 to 300 colonies counted, except for plates inoculated with 1.0 ml of undiluted sample. (drinking water and source/surface water)		9215A.8;
	n. Fully automated colony counter is not used for drinking water or source/surface water.		
	o. Colony counts rounded off to 2 significant figures.		9215A.9
	p. For systems granted a variance from Total Coliform Rule" maximum contaminant level, any method in Standard methods used with R2A medium for enumerating heterotrophic bacteria in drinking water.		
	q. Agar media in plates should not lose >15% by weight during 48 h of incubation.		
	2.2 SIMPLATE METHOD		**(2) V.5.5.10**
	a. For a <u>single sample Unit Dose</u>, a 10-mL test sample is added to a test tube containing dehydrated SIMPLATE medium and then poured onto the center of a plate containing 84 small wells.		(2)
	b. Alternatively, a 9-mL of sterile diluent is added to the test tube containing the dehydrated medium, followed by a 1-mL sample, and the medium plus sample then poured onto the center of a plate containing 84 small wells?		(2)
	c. For a <u>Multiple Dose for 10 samples of 1 mL each</u>, a 100-mL sterile diluent added to the dehydrated SIMPLATE medium and shaken to dissolve then a 1.0-mL test sample is pipetted to the center of a plate, followed by 9 mL of the reconstituted medium.		(2)
	d. For either the single or multiple dose, the mixture is distributed evenly to the 84 wells and the excess liquid is drained into the absorbent pad on the plate		(2)
	e. The plate is inverted and incubated at 35 ±0.5°C for 45-72 hours?		(2)
	f. Bacterial density is determined by counting the number of wells that fluoresce under a 365-366-nm UV light, and converting this value to a Most Probable Number/mL using the manufacturer's Unit Dose MPN table.		(2)
	g. If a 10-mL sample is used, the Unit Dose MPN/mL is read directly or, if 1-mL sample is used, the MPN/mL value is corrected by multiplying it by 10.		(2)
	h. Each batch of agar is checked for sterility by pouring a final control plate and the laboratory rejects data if the control is contaminated.		(2)

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
E. 3.0 MICROBIOLOGICAL METHODS – FERMENTATION METHODS			
	3.1 MULTIPLE TUBE FERMENTATION (MTF) **GENERAL – Total Coliforms (TC) – Applies to Drinking, Source/Surface, Waste, Recreational, Recycled Water**		**(1) SM 9221A,B,C** **(2) V.5.2** **(3) CFR40:141.21;141.74;136.3** **(5) AB411**
	a. Lauryl Tryptose Broth (LTB) used for presumptive test and Brilliant Green Lactose Bile broth (BGLB) used for confirmed test for total coliform.		(1) 9221B; (2)
	b. If lactose broth (LB) is used, system conducts at least 25 parallel tests between this medium and LTB and demonstrates false-positive rate and false-negative rate for total coliforms of less than 10%, with comparison documented and records retained.		(2);(3) 141.21(f)(3)
	c. Concentration of test medium adjusted so that sample addition does not reduce ingredient concentrations below that of standard medium. (LTB, LB)		(1) SM Table 9221:I (2)
	d. Inverted vials in sterile medium are free of bubbles and at least one half to two thirds covered after water sample is added.		(1) 9221B.1; (3) 141.21(f)(3)
	e. Inoculated LTB/LB medium is incubated at 35 ± 0.5°C and observed for gas (or acid) and growth at 24 ± 2 hrs and 48 ± 3 hrs.		(1) 9221B; (2)
	f. All 24- and 48-hour gas-positive or acid positive tubes confirmed in BGLB broth.		(1) 9221B.2; (2)
	3.1.1 MULTIPLE TUBE FERMENTATION (TC) – Drinking Water		(3) CFR 40:141.21
	g. 100 ml of drinking water tested. *Check set-up used:* ❑ 100 ml *(inverted vial replaced with acid indicator)* ❑ 10 tube x 10 ml ❑ 5 tube x 20 ml		(1) 9221A.1; (3) 141.21(f)
	h. Completed test not required for drinking water. All positives In LTB/LB inoculated into EC or ECMUG		(3) 141.21(f)(3)
	i. Negative Invalidation: All samples showing a turbid culture (i.e., heavy growth, opaque) in the absence of gas/acid production are invalidated and another sample collected from the same location within 24 H. (If lab performs confirmed test on turbid culture and confirmed test is total coliform positive, sample reported as such, but if total coliform-negative, sample is invalidated.		(2),(3)141.21(c)(2)
	j. When MTF test is used on water supplies that have a history of confluent growth or TNTC by the MF procedure, all presumptive tubes with heavy growth without gas/acid production submitted to confirmed test and fecal coliform/*E.coli* test to check for coliform suppression.		(2)

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
E. 3.0 MICROBIOLOGICAL METHODS – FERMENTATION METHODS FOR TOTAL COLIFORMS			
	3.1.2 MULTIPLE TUBE FERMENTATION (TC) Source/Surface, Waste, and Recreational, Recycled Water		**(2) (3) CFR 40:141.74; (4) 40 CFR 136 (5) AB 411**
	k. At least 3 appropriate sample dilutions of 5 tubes each are used.		(1) 9221A.2; 9221B.1 (2)
	l. Completed test not required for **source/surface water**.		(4) 141.74(a)(1)
	m. Completed test is also not required for **wastewater**.		(4) Footnote 12 Table IA
	n. **Source/surface water** samples that produce turbid growth in the absence of gas/acid production in LTB or LB are invalidated and another sample obtained, which may be tested with another method.		(2)
	o. Alternatively, a confirmed test is performed on turbid culture in the absence of gas/acid production and, if total coliform positive, most probable number reported, or if total coliform-negative, sample is invalidated and another requested. (**source/surface water**)		(2)
	p. **Source/surface water**, **wastewater**, **recreational water** and **recycled waters** results reported as MPN/100 mL using the table SM 9221.IV.		(1) 9221C
	3.2 PRESENCE-ABSENCE (CLARKS) – Total Coliforms Drinking Water		**(1) SM 9221D.1 (2) V.5.2.3 (3) CFR 141.21(c)(2)**
	a. Make P-A broth triple strength (3X) formulation when examining 100-mL sample		(1)(2)
	b. 100 ml sample inoculated into bottle of P-A medium.		(1)(2)
	c. Medium incubated at 35 $\pm$ 0.5°C and observed for yellow color (acid) and growth at 24 $\pm$ 2 and 48 $\pm$ 3 hours.		(1)(2)
	d. Yellow broths with growth are confirmed for total coliforms in BGLB broth.		(1)(2)
	e. Negative Invalidation: Non-yellow, turbid P-A medium is invalidated and another sample obtained from the same location. (If confirmed test performed and sample is total coliform-positive, sample is reported as such, but if confirmed test is negative, sample is invalidated.)		(2)(3)

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
E. 4.0 MICROBIOLOGICAL METHODS – FERMENTATION METHODS FOR FECAL COLIFORMS			
	4.1 EC MEDIUM – Fecal Coliforms in Drinking, Source/ Surface, Waste and Recreational Waters (Used with LTB/LB, P-A, m-ENDO/m-ENDO LES)		**(1) SM 9221A,B,C,E (2) V.5.2.4.1 (3) CFR 141.21, 141.74, 136.3 (4)**
	a. EC medium is used to determine presence of fecal coliforms in total coliform positive test. *(check applicable media)* ( ) LTB/LB, ( ) P-A broth, and/or ( ) m-Endo/m-Endo LES.		(3) 141.21(f)(5); 141.74(a); 136.3 (4) AB 411
	b. Inverted vials free of bubbles and at least one-half to two-thirds covered after sample added.		(2), (3) 141.21(f)(3)
	MF-DW c. Filter from m-Endo may be curled and inserted into EC medium after first removing some of the selected colonies for total coliform verification. *(not recommended)*		CFR 141.21(f)(5)
	d. EC Medium incubated at 44.5 ± 0.2°C for 24 ± 2 hrs. Incubate samples w/positive *E.coli* and negative *E.aerogenes* controls.		(1) 9221E.1; (2); (3) 141.21(f)(5)
	e. Water level in water bath above upper level of medium in culture tubes.		(1) 9221E.1; (2)
	f. Any amount of gas detected in inverted vial of tube with growth is read as a fecal coliform positive.		(1) 9221E.1; (2)
	g. Enumeration results reported using MPN table in SM 9221C.		(1) 9221C
	4.2 A-1 MEDIUM – Fecal Coliform Source/Surface and Waste Waters only		**(1) SM 9221 C,E- 2006 (2) V.5.2.4.2 (3) CFR 141.74, 136.3**
	a. Double strength medium used for 10 mL sample volumes. Sterilize at 121°C for 10 minutes. pH 6.9 ± 0.1		(1) 9221E.2
	b. Test is set up as direct fecal coliform test. Three dilutions with 5 or 10 tubes per sample dilution (volume) are used.		(2)
	c. Inverted vials free of bubbles and at least one-half to two-thirds covered after sample added.		(2)
	d. A-1 medium incubated at 35 ± 0.5°C for 3 hours and then at 44.5 ± 0.2°C for 21 ± 2 hours. Incubate samples w/positive *E.coli* and negative *E.aerogenes* control cultures.		(1) 9221E.2; (2)
	e. Water level in water bath above upper level of medium in culture tubes.		(2)
	f. Any amount of gas detected in inverted vial of tube with growth is read as a fecal coliform positive.		(1) 9221E.2; (2)
	g. Results reported using MPN table in SM 9221C.		(1) 9221C

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
E. 5.0 MICROBIOLOGICAL METHODS – ENZYME SUBSTRATE METHODS FOR COLIFORMS			
	5.1 EC + MUG MEDIUM – <u>E. coli</u> in Drinking Water (Used with LTB/LB, P-A, m-Endo/m-Endo LES)		**(1) SM 9221E (2) V.5.3.3 (3) CFR 141.21(f)(5); (f) (6)(i)**
	a. Final MUG concentration of 50 μg per ml added to EC before autoclaving, or commercially available EC + MUG used.		(2)(3)
	b. At least 10 ml of EC medium supplemented with MUG must be used.		(3)
	c. Inverted vial omitted (optional)		(2)(3)
	d. Test tubes and autoclaved media checked for auto-fluorescence with 366 nm UV light before they are used. *MUG positive and negative (uninoculated) controls may be necessary to interpret results, especially if there is weak auto-fluorescence of the tube or media.*		(2)
	MF e. Filter may be curled and inserted into EC + MUG medium, after first removing some of the selected colonies for total coliform verification. *(not recommended)*		CFR 141.21(f)(5)
	f. EC + MUG Medium incubated at 44.5°C <u>+</u> 0.2°C for 24 <u>+</u> 2 hrs.		(2)(3)
	g. Water level in water bath is above upper level of medium in culture tubes.		(2)
	h. Fluorescence is checked with 6 watt, 365-366 nm UV lamp in darkened room.		(2)(3)
	i. Bright blue fluorescence is considered a positive test.		(2)(3)
	5.2 COLILERT, COLILERT 18, COLISURE READYCULT.FLUOROCULT LMX AND COLITAG Total Coliform & E. coli **GENERAL – Applies to all of the above media/methods**		**(1) SM 9223 (2) V.5.3 (3) CFR 141.21; 141.74 (5) AB 411**
	a. Media purchased from commercially available source only.		(2)
	b. Media protected from light and stored according to manufacturer's recommendations		(2)
	c. Each lot of medium checked for auto-fluorescence before use with 6 watt, 365-366 nm UV lamp.		(2)
	d. Media exhibiting faint fluorescence discarded and another lot used.		(2)
	e. Medium exhibiting color change after sample added, but before incubation, is discarded and another batch/lot used. Color before incubation: Colilert, Colilert 18 & Colitag-------Colorless to a slight tinge of color Colisure & E Colite----------Yellow Readycult/Fluorocult----------Slightly yellow		(2)
Lab's	**ITEM**	**ELAP**	**REFERENCES**

Eval.		Eval	COMMENTS
E. 5.2 MICROBIOLOGICAL METHODS – COLILERT, COLILERT 18, COLISURE (General)			
	e. Glass bottles not used for test if they fluoresce when checked with UV light.		(2)
	QC f. Each lot of medium checked for sterility, with a MUG-positive (*E. coli)* strain, a MUG-negative coliform (*K.pneumoniae*) and a non-coliform (*P.aeruginosa*). Read and record results.		(2)
	g. Dilution water for Colilert/Colilert 18 and Colisure is sterile deionized water, or distilled water but not buffered water.		(2)V 5.3.2.1.1
	h. Quanti-Tray (**Colilert only**) sealer checked monthly by adding dye (eg. bromcresol purple) to water. If dye is observed outside of wells, there may be a problem with the sealer or with the lot of trays.		(2)
	i. Colilert/Colilert 18/Colisure Tests not used to confirm total coliforms in MTF, P-A tests or membrane filters.		(2)
	j. Samples using **Colilert®** medium incubated at 35 ± 0.5°C for 24 hours.		(1) (2)
	k. Samples using **Colilert 18** medium must **pre-warmed for 20 min** at **35°C waterbath** or **7-10min at 44.5°C water bath** and incubated for 18 hours. Times of pre-warming and temperature documented.		(3) 141.74 (a)(1) footnote 7, IDEXX instructions
	l. Samples with yellow color ≥ reference comparator indicate presence of total coliform.		(2)
	l. Samples with yellow color less than comparator incubated additional 4 hours (total of 28 hours Colilert and 22 hours for Colilert 18).		(2) (3) 141.74 (a)(1) footnote 7, IDEXX instructions
	n. Samples with yellow color less than comparator after 28 hours (22 hrs. for Colilert 18) of incubation recorded as negative.		(1)(2)
	o. Samples that are yellow and fluoresce when exposed to 365-366 nm UV lamp are positive for E.Coli.		(1)(2)
	p. Samples that produce atypical color change (greenish-black, green) should be invalidated and request another sample from same location. Another method should be used.		(2)
	q. Colilert color comparator not used beyond expiration date.		(2)
	q. If air type incubator used with cold samples, it may be necessary to determine time needed to bring sample to 35°C, to ensure specified incubation time at that temperature is followed.		(2)

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
E. 5.2 MICROBIOLOGICAL METHODS- COLILERT, COLILERT 18, COLISURE (Specific)			
	5.2.1 COLILERT/COLILERT 18 – Total Coliforms and E. coli Drinking, Source/Surface, Recreational & Recycled Water		**(2) V.5.3 (3) (4)**
	a. **Drinking Water - *Check format used***: ❑ 10 mL sample into each of 10 tubes. ❑ 100 mL sample into 1 bottle. ❑ 100 mL sample into 1 Quanti-Tray		(2); (3) 141.21(f)(3);141.74(a)(1); (4)
	r. **Source/Surface Water (enumeration only) - *Check format used***: ❑ 5 tube – 3 dilution set-up ❑ Quanti-Tray ❑ Quanti-Tray 2000		(1) (2) V.5.3.2.1.1
	s. If **Colilert 18** is used for total and "fecal coliforms" in **recreational marine water**, parallel/split sample testing with multiple tube fermentation or membrane filtration method is performed to determine that the total coliform results are comparable and that the *E. coli* results will be comparable to fecal results. Maintain records.		(4)
	t. **Recreational marine water** is diluted **1:10** with sterile water before adding **Colilert 18** medium and sealing in one Quanti-Tray or Quanti-Tray 2000 per dilution.		(3) 141.74 (a)(1) footnote7, IDEXX instructions
	5.2.2 COLISURE – Total Coliforms and E. coli - Drinking Water		**(2) V.5.3.2.2**
	u. 100 mL sample is tested in a bottle.		(2)
	v. Sample using Colisure medium is incubated at 35 ± 0.5°C for 24 to 48 hours.		(2) (3) 141.21(f)(3) footnote 9
	w. Color change from yellow to magenta indicates presence of total coliforms.		(2)
	x. Samples that fluoresces (darkened room) when exposed to 365-366 nm UV lamp are positive for *E. coli*.		(2)
	5.3 E*COLITE MEDIUM – Total Coliform and E.coli Drinking Water		**(3) CFR 141.21(f)(3) footnote 10 (2) V.5.3.2.3**
	a. Sample incubated in bag at 35°C ± 0.5°C for 28 hours.		(2)(3)
	b. Total coliforms are present if medium changes from yellow color to blue or blue-green, or a blue color in the corners of the bag.		(2)(3)
	c. E. coli is present if medium fluoresces when exposed to 365-366 nm UV lamp.		(2)(3)
	d. If no fluorescence is observed at 28 hours, medium is incubated an additional 20 hours (48 hrs.).		(2)(3)
	e. If medium becomes red, **discard sample**. Assume leakage of bactericide from third compartment of bag into medium.		(2)

Lab's Eval	ITEM	ELAP Eval	REFERENCES COMMENTS
	5.4 READYCULT COLIFORMS 100 – Total Coliform and E.coli Drinking Water		**(3) CFR 141.21(f)(3) footnote 10 (2) V.5.3.2.4**
	a. Contents of snap pack added to 100 ml sample		(2)(3)
	b. Incubate at 35°C ± 0.5°C for 24 ± 1hours		(2)(3)
	c. Coliforms present if slightly yellow color changes to blue-green.		(2)(3)
	d. E. coli is present if medium emits bright light-blue fluorescence when exposed to 365-366 nm UV light. If confirmation of E. Coli is desired, Kovac's indole reagent should be added to the broth, red ring confirms E. Coli.		(2)(3)
	e. Fluorocult LMX – dry Readycult medium		(2) V.5.3.2.5
	5.5– COLITAG - Total Coliform and E.coli in Drinking Water		**(2) V.5.3.2.6 (3) CFR 141.21(f)(3) footnote 10**
	a. Incubate at 35°C ± 0.5°C for 24 ± 2 hours		(2)(3)
	b. Coliforms present if yellow color develops		(2)(3)
	c. E. coli is present if medium fluorescence when exposed to 365-366 nm UV light.		(2)(3)

E. 6.0 MICROBIOLOGICAL METHODS – MEMBRANE FILTRATION – mEndo/mEndo LES, mFC, NA+MUG			
	6.1 GENERAL – All MEMBRANE FILTRATION (MF) METHODS This section and section C.12 (MF Equipment) applies to all Membrane Filtration Methods		**(1) SM 9222A,B,C (2) V.5.4**
	a. Sterility check conducted on each funnel in use at beginning and end of each filtration series. (Filtration series ends when 30 minutes or more elapse between sample filtrations.)		(1); (2)
	b. If sterility control indicates contamination, all data for that funnel is rejected and another sample is requested.		(2)
	c. Funnels are rinsed with two or three 20 – 30 mL portions of sterile rinse water after each sample filtration to prevent carry-over.		(1) 9222B.5.c; (2)
	d. As an alternative to "c," a 254 nm UV light for at least 2 minutes is being used to sanitize the filtration units during filtration series.		(1) 9222B.5.b; (2)
	e. Inner surface of forceps smooth and without ridges.		(1) 9222B.1.i
	f. Absorbant pads must be saturated with at least 2 ml. of broth and excess media removed by decanting the plate.		(2)

Lab's Eval	ITEM	ELAP Eval	REFERENCES COMMENTS
	6.2 M-ENDO/M-ENDO LES (broth or agar) – Total Coliforms **Applies to Drinking, Source/Surface, Waste, Recreational Waters**		**(1) SM 9222A,B,C** **(2) V.5.4.2.1.1** **(3) CFR 141.21(f)(5), 141.74, 136.3**
	a. M-Endo / LES Endo Agar used in single step or enrichment technique.		(1) SM 9222B.5; (2)
	b. Ethanol not denatured.		(1) SM 9222B.2; (2)
	c. Medium prepared in sterile flask and dissolved using boiling water bath or hot plate with stir bar. Medium not boiled.		(1) SM 9222B.2; (2)
	d. Uninoculated media discarded if growth or surface sheen observed.		(2)
	e. For total coliforms dilutions or volumes that yield 20 to 80 total coliform colonies for at least one dilution are filtered.		(1) SM 9222B.5.a
	f. Inoculated medium incubated at 35° ± 0.5°C for 22 to 24 hours in humid environment. *(60% relative humidity)*		(1) SM 9222B.1
	g. All sheen colonies (includes questionable sheen colonies and non-sheen red colonies), up to maximum of five, are verified using single strength LTB or LB and BGLBB or an EPA-approved cytochrome oxidase and beta-galactosidase rapid test procedure)		(2)
	h. For **drinking water**, entire membrane surface is being swabbed for **verification of coliform**. Order of inoculation is EC, LTB, BGLB.		(1) 9222B.6.a; (2)
	i. For **drinking water**, sample not invalidated if membrane filter contains at least one sheen colony. (drinking water)		(2)
	j. **Negative Invalidation for Drinking Water**: Samples resulting in confluent or too numerous to count (TNTC) growth are invalidated unless total coliforms are detected. TNTC is defined as >200 colonies on membrane in absence of detectable coliforms. (If verification test is performed before invalidation and test is total coliform-positive, sample is reported as such, but if test is total coliform-negative, sample is invalidated.)		(2); (3) 141.21(c)(2)
	k. For **source/surface,** initial counts are adjusted based upon verified data as according to SM 9222B. Report verified count per 100 ml.		(1) 9222B.6; (2)
	l. For **wastewater and recreational water**, verify 10 colonies from positive sample monthly, including both sheen and atypical colonies		(1) SM (20th) 9222B.5.f
	m. If two or more analysts are performing the method, each counts total coliform colonies on same membrane monthly and agree within 10%		(1) 9020B.4.d; (2)

Lab's Eval	ITEM	ELAP Eval	REFERENCES COMMENTS
	6.3 M-FC (broth or agar) – Fecal Coliforms Source/Surface, Waste, Recreational Water		**(1) SM 9222A,B,C,D (2) V.5.4.2.1.6 (3) CFR 141.74. 136.3**
	a. Brought to boiling point. Not boiled or autoclaved. pH 7.4± 0.2		(1) 9222D.1; (2)
	b. Un inoculated medium discarded if growth observed.		(2)
	c. For fecal coliforms filter dilutions or volumes that yield 20 to 60 fecal coliform colonies for at least one dilution.		(1) 9222D.1; (2)
	d. Inoculated medium is incubated at 44.5 $\pm$ 0.2°C for 24 $\pm$ 2 hrs.		(1) 9222D.2; (2)
	e. If two or more analysts are performing the method, each counts fecal coliform colonies on the same membrane monthly and the counts agree within 10%.		(1) 9020B.4.d; (2)
	6.4 NUTRIENT AGAR + MUG (MF) – *E. coli* for Drinking Water		**(2) V.5.4.3 (3) CFR 141.21**
	a. Final MUG concentration must be 100 μg/ml		(2) (3) 141.21(f)(6)(ii)
	b. Quality of lot or batch of medium evaluated by filtering or spot inoculating positive and negative control cultures onto membrane filter on m-Endo medium, incubating at 35°C for 24 hours, then transferring filter to NA+MUG and further incubating at 35°C for 4 hours. Results are read and recorded.		(2)
	c. Filter with total coliform colonies transferred to surface of Nutrient Agar + MUG.		(2)
	d. Before incubation, indicate location of each sheen colony on petri dish lid with permanent marker; dish and lid are marked to realign the lid after being separated.		(2)
	e. For the total coliform verification test, a portion of the colony is transferred with needle before or after NA + MUG incubation.		(2)
	f. As an alternative to "e," the membrane filter surface is swabbed with sterile cotton swab after 4-hour incubation and transferred to total coliform verification media.		(2)
	g. Inoculated NA + MUG medium incubated at 35 $\pm$ 0.5°C for 4 hours.		(2)
	h. Any fluorescence in halo around sheen colony when checked with 6 watt, 365-366 nm UV lamp in a darkened room is reported positive for *E. coli*.		(2)

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
E. 7.0 MICROBIOLOGICAL METHODS – MEMBRANE FILTRATION – TOTAL AND E. COLI			
	7.1 GENERAL – All MEMBRANE FILTRATION (MF) METHODS **This section and section C.12 (MF Equipment) applies to all Membrane Filtration Methods (E.7.1 is same as E.6.1)**		**(1) SM 9222A,B,C** **(2) V.5.4**
	a Sterility check conducted on each funnel in use at beginning and end of each filtration series. (Filtration series ends when 30 minute or more elapse between sample filtrations.)		(1); (2)
	b. If sterility control indicates contamination, all data for that funnel is rejected and another sample is requested.		(2)
	c. Funnels are rinsed with two or three 20 – 30 mL portions of sterile rinse water after each sample filtration to prevent carry-over.		(1) 9222B.5.c; (2)
	e. As an alternative to "c," a 254 nm UV light is being used to sanitize the filtration units during filtration series.		(1) 9222B.5.b; (2)
	f. Inner surface of forceps smooth and without ridges.		(1) 9222B.1.i
	7.2 M-ColiBlue24 MEDIUM (MF) – Total Coliforms and *E. coli* Drinking Water		**(2) V.5.4.2.1.2** **(3) CFR 141.21(f) footnote 11**
	a. Ampules inverted 2-3 times to mix contents before breaking poured evenly over adsorbent pad.		(2)
	b. Unopened refrigerated ampules may be stored in the dark until the expiration date, but discarded earlier if growth is observed.		(2)
	c. Inoculated medium incubated 35°C ± 0.5°C for 24 hours.		(3)
	d. Total coliform colonies (non *E. coli*) are red. *E. coli* colonies are blue to purple.		(3)
	7.3 MI MEDIUM (MF) – Total Coliforms and *E. coli* Drinking Water and Total Coliforms in Source/Surface Water		**(1) SM 9020:IV** **(2) V.5.4.2.1.3** **(3) CFR 141.21(f) footnote 6** **(6) EPA 600-R-00-013**
	a. Inoculated medium incubated 35 ± 0.5°C for 24 hours.		(2) (6)
	. b. Blue colonies under **normal light** are counted as ***E. coli***.		(2)(6)
	c. **Fluorescent colonies** under 366nm UV lamp counted as **total coliforms**. Blue/white fluorescent colonies under 366 nm UV light are counted as **total coliforms**. Blue/green colonies under normal light are ***E. coli***. Blue/green with fluorescent edges counted as ***E. coli***. ??? Non-fluorescent blue colonies (*E. coli*) are also counted as ***E.coli***.		(2)(6)
	d. **Source/surface water** reported as total coliform per 100 ml.		(3) (6)
Lab's	ITEM	ELAP	REFERENCES

Eval.		Eval	COMMENTS
	7.4 CHROMOCULT COLIFORM AGAR – Total Coliforms and *E. coli* Drinking Water		**(1) SM 9020:IV (2) V.5.4.2.1.4 (3) CFR 141.21(f) footnote 6 (6) EPA 600-R-00-013**
	a. Inoculated medium incubated 36 ± 1°C for 24 ± 1 hours.		(2) (6)
	. b. Salmon to red colonies are Total coliforms.		(2)(6)
	c. Dark –blue to violet colonies are E.coli. If confirmation of E. Coli is desired, add 1 drop of Kovac's reagent to each dark-blue to violet colony; the formation of cherry-red color within seconds confirms the presence of E. coli.		(2)(6)
	7.5 COLISCAN – Total Coliforms and *E. coli* in Drinking water or Total Coliform in Source/Surface Water		**(1) SM 9020:IV (2) V.5.4.2.1.5 (3) CFR 141.21(f) footnote 6 (6) EPA 600-R-00-013**
	a. Inoculated medium incubated 32 -37°C for 24 – 28 hours.		(2) (6)
	b. Pink-magenta colonies are counted as Total coliforms.		(2)(6)
	c. Purple-blue colonies are *E.coli*		(2)
	7.6 MODIFIED mTEC - E. coli in ambient waters & disinfected wastewaters (MF) thermotolerant Escherichia coli Agar		(4) EPA 1603
	a. Inoculated medium incubated 35°C ± 0.5°C for 2 ± 0.5 hours.		(4)
	b. After 2 hours transfer the plates to a Whirl-Pak bag, seal and submerge at 44.5°C ± 0.2°C for 22 ± 2 hours in a water bath. Do not overfill the bag.		(4)
	c. Red or Magenta colonies are counted with the aid of an illuminated lens with *2*-5X magnification or a stereoscopic microscope.		(4)

E. 8.0 MICROBIOLOGICAL METHODS – MEMBRANE FILTRATION – ENTEROCOCCAL METHODS			
	8.1 All MEMBRANE FILTRATION (MF) METHODS - GENERAL This section and section C.12 (MF Equipment) applies to all Membrane Filtration Methods (E.8.1 is same as E.6.1& E.7.1)		**(1) SM 9222A,B,C (2) EPA 815-B-97-001**
	QC a Sterility check conducted on each funnel in use at beginning and end of each filtration series. (Filtration series ends when 30 minutes or more elapse between sample filtrations.)		(1); (2) V.5.2.1.3; V.5.12
	QC b. If sterility control indicates contamination, all data for that funnel is rejected and another sample is requested.		(2) V.5.2.1.3
	d. Funnels are rinsed with two or three 20 – 30 mL portions of sterile rinse water after each sample filtration to prevent carry-over.		(1) 9222B.5.c; (2) V.5.2.2; V.5.12
	e. As an alternative to "c," a 254 nm UV light is being used to sanitize the filtration units during filtration series.		(1) 9222B.5.b; (2) V.4.1.4
	f. Inner surface of forceps smooth and without ridges.		(1) 9222B.1.i

	8.2 m ENTEROCOCCUS (MF) in Wastewater		**(1) SM 9230 C 2007 (2) V.5.4.4 (4) 40 CFR 136.3**
	a. Medium not autoclaved.		(1) 9230C.2; (2)
	b. Appropriate volumes filtered to yield 20 to 60 colonies on membrane.		(1)
	c. Plates stand for 30 minutes before inverting and incubating at 35 ± 0.5°C for 48 ± 4 hours.		(1)
	d. All light and dark red colonies counted as enterococci.		(1)
	e. Use low-power binocular, wide field dissecting microscope, or other optical device along with cool, white fluorescent light		(1)
	f. Densities reported as enterococci per 100 ml		(1) 9230C.4
	g. Include routine verification procedure especially if used as evidence.		(1) 9230C.5
	8.3 mE AGAR (MF) – Enterococci in Waste and Recreational Water		(1) SM 9230C (2) V.5.4.4.1.1 (4) 40 CFR 136.3
	Safety a. Proper handling of the toxic nalidixic acid (or its salt) is observed.		(2)
	b. Appropriate volumes filtered to yield 20 to 60 colonies on membrane.		(1) 9230C.3
	c. Plates inverted and incubated at 41°C ± 0.5°C for 48 ± 4 hours.		(1) 9230C.3; (2)
	d. Membrane filter transferred to Esculin Iron Agar (EIA) medium and incubated at 41°C ± 0.5°C for 20 minutes.		(1) 9230C.3; (2)
Lab's Eval.	**ITEM**	**ELAP Eval**	**REFERENCES COMMENTS**

	e. Pink to red colonies with black or reddish brown precipitate on the underside of filter counted as enterococci.		(1) 9230C.3
	f. Use low-power binocular, wide field dissecting microscope, or other optical device along with cool, white fluorescent light		(1) 9230C.3
	g. Densities reported as enterococci per 100 ml.		(1) 9230C.4
	h. Include routine verification procedure especially if used as evidence.		(1) 9230C.5
	8.4 mEI AGAR (MF) – Enterococci in Recreational Water		**(1) SM 9230C (2) V.5.4.4.1.3 (4) EPA 1600**
	Safety a. Proper handling of the toxic nalidixic acid (or its salt) is observed.		(2)
	b. Invert plates and incubate at 41 ± 0.5° C for 24 ± 2 hours.		(2) (4)
	c. Use low-power binocular, wide field dissecting microscope, or other optical device along with cool, white fluorescent light		(2) (4)
	d. Colonies with blue halo, regardless of color, counted as enterococci.		(1) (2) (4)
	e. Colonies must be greater than 0.5 mm in diameter.		(1) (4)
	f. Density reported as enterococci per 100 ml		(1)(4)

E. 9.0 MICROBIOLOGICAL METHODS – MPN – Fecal Streptococci			
	9.1 AZIDE DEXTROSE MEDIUM – Fecal Streptococci (multiple tube) Waste and Recreational Water		**(1) SM 9230B 2007, (3) cfr 136.3 (2) V.5.2.5**
	a. Multiple dilutions (e.g. 10, 1, 0.1), and 5 tubes for each dilution.		(1)
	b. Double strength broth used for 10 ml volumes.		(1)
	c. Tubes incubated at 35 ± 0.5°C and observed for growth (turbidity) at 24 ± 2 hour and at 48 ± 3 hours.		(1) (2)
	d. The confirmed test is performed on all tubes that become turbid at 24 and 48 hours.		(1) (2)
	e. Turbid broth suspensions are streaked onto Bile Esculin Azide (BEA) agar plate and incubated at 35 ± 0.5°C for 24 ± 2 hours.		(1) (2)
	f. Brownish-black colonies with brown halos (on BEA agar) read as confirmation of fecal streptococci.		(1) (2)

Lab's Eval.	ITEM	ELAP Eval	REFERENCES COMMENTS
	9.2 ENTEROLERT- Enterococci in Recreational Water		**(2) V.5.3.4 (6) Product insert - IDEXX**

	a. Media purchased from commercially available source only.		(2)
	b. Medium stored in dark at 4-30°C until use.		(2) (6)
	c. Each lot of medium checked for auto-fluorescence with 6 watt, 365-366 nm UV lamp before use.		(2)
	d. Media exhibiting faint fluorescence discarded and another lot used.		(2)
	e. **Recreational marine water** is diluted 1:10 with sterile water (e.g. 10 ml sample + 90 ml water) before powdered medium is added.		(6)
	f. Dilution water for Enterolert is sterile de chlorinated tap water, deionized water, or distilled water.		(6)
	g. Enterococci are enumerated using Quanti-Tray or Quanti-Tray 2000.		(6)
	h. Quanti-Tray sealer checked monthly by adding dye to water sealed in tray and observing for leakage.		(2)
	i. Enterolert tests incubated at 41° ± 0.5°C for 24 to 28 hours and are examined for fluorescence under UV lamp (positive).		(2) (6)
	j. Wells that are positive before 24 hours and wells that are negative after 28 hours are also valid.		(6)
	k. Reported as Enterococci per 100 ml, using MPN table provided by manufacturer.		(6)