Chapter V
Critical Elements for Microbiology

Note 1: This chapter uses the term "must" to refer to certification criteria that are required by the National Primary Drinking Water Regulations. The term "should" is used for procedures that, while not specifically required by the regulations, are considered good laboratory practices. To assure the validity of the data, it is critical that laboratories observe both the regulatory and non-regulatory criteria. Certification Officers have the prerogative to refuse certification if the quality control data are judged unsatisfactory or insufficient.

Note 2: Quality control items, designated by a "QC," necessitate written records. Each record should include analyst's initials and date(s).

Note 3: References to *Standard Methods for the Examination of Water and Wastewater* are to the 18th, 19th, and 20th editions (except where specifically noted).

1. Personnel

1.1 Supervisor/Consultant

The supervisor of the microbiology laboratory should have a bachelor's degree in microbiology, biology, or equivalent. Supervisors who have a degree in a subject other than microbiology should have had at least one college-level microbiology laboratory course in which environmental microbiology was covered. In addition, the supervisor should have a minimum of two weeks training at a Federal agency, State agency, or academic institution in microbiological analysis of drinking water or 80 hours of on-the-job training in water microbiology at a certified laboratory, or other training acceptable to the State or EPA. If a supervisor is not available (and a waiver not granted per paragraph 1.3), a consultant having the same qualifications may be substituted, as long as the laboratory can document that the consultant is acceptable to the State and is present on-site frequently enough to satisfactorily perform a supervisor's duties.

The laboratory supervisor has the responsibility to ensure that all laboratory personnel have demonstrated their ability to satisfactorily perform the analyses to which they are assigned and that all data reported by the laboratory meet the required quality assurance and regulatory criteria.

1.2 Analyst (or equivalent job title)

The analyst should perform microbiological tests with minimal supervision and have at least a high school education. In addition, the analyst should have a minimum of at least three months of bench experience in water, milk, or food microbiology. The analyst should also have training acceptable to the State (or EPA for non-primacy States) in microbiological analysis of drinking water and a minimum of 30 days of on-the-job training in drinking water microbiology under an experienced analyst. Analysts should take advantage of workshops and training programs that may be available from State regulatory agencies, professional societies, and manufacturers. Before analyzing compliance samples, the analyst should demonstrate acceptable results on unknown samples.

1.3 Waiver of Academic Training

The certification authority may waive the need for the above specified academic training, on a case-by-case basis, for highly experienced analysts. The certification authority may also waive the need for the above specified training, on a case-by-case basis, for supervisors of laboratories associated with drinking water systems that only analyze samples from that system. If such a waiver for supervisor training is granted, the certification authority will prepare a written and signed justification for such a waiver and have it available for inspection. Laboratories should also keep a copy of the waiver.

1.4 Personnel Records

Personnel records that include academic background, specialized training courses completed, and types of microbiological analyses conducted should be maintained on laboratory analysts.

2. Laboratory Facilities

Laboratory facilities should be clean, temperature and humidity controlled, and have adequate lighting at bench tops. The

laboratory should maintain effective separation between areas where activities are incompatible, minimize traffic flow and ensure that contamination does not adversely affect data quality. Bench tops and floors should be of a material that is easily cleaned and disinfected. Laboratory facilities should have sufficient bench-top area for processing samples; storage space for media, glassware, and portable equipment; floor space for stationary equipment (incubators, water baths, refrigerators, etc.); and associated area(s) for cleaning glassware and sterilizing materials. They should also have provisions for disposal of microbiological waste.

3. Laboratory Equipment and Supplies

The laboratory must have the equipment and supplies needed to perform the approved methods for which certification has been requested.

3.1 pH Meter

3.1.1 Accuracy and scale graduations should be within ±0.1 units.

3.1.2 pH buffer aliquots should be used only once.

3.1.3 Electrodes should be maintained according to the manufacturer's recommendations.

QC 3.1.4 pH meters should be standardized before each use period with pH 7.0 and either pH 4.0 or 10.0 standard buffers, whichever range covers the desired pH of the media or reagent. The date and buffers used should be recorded in a logbook, along with analyst's initials.

QC 3.1.5 Record pH meter slope monthly, after calibration.

3.1.5.1 If the pH meter does not have a feature to automatically calculate the slope, but can provide the pH in millivolts (mV), use the following formula to calculate the slope.

Slope (as %) = |mV at pH 7 - mV at pH 4| x 100/177

3.1.5.2 If the slope is below 95% or above 105%, the electrode or meter may need maintenance. Follow manufacturer's instructions for electrode maintenance and general cleaning.

QC 3.1.6 Commercial buffer solution containers should be dated upon receipt and when opened. Buffers should be discarded by the expiration date.

3.2 *Balance (top loader or pan)*

3.2.1 Balances should have readability of 0.1 g.

3.2.2 Balances should provide a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less.

QC 3.2.3 Balances should be calibrated monthly using ASTM Class 1, 2, or 3 weights (minimum of three traceable weights which bracket laboratory weighing needs, with a readability of 0.1 g.)(ASTM, 1916 Race St., Philadelphia, PA 19103). Non-reference weights should be calibrated every six months with reference weights. Record calibrations in a logbook with the initials of the individual performing the calibration. Correction values should be on file and used. A reference weight should be re-certified every five years. Damaged or corroded weights should be replaced.

QC 3.2.4 Service contracts or internal maintenance protocols and maintenance records should be available. Maintenance, calibration, and cleaning should be conducted at least annually by a qualified independent technician. In cases where a laboratory is geographically isolated such that an annual visit from a technician is impractical, the certification officer may modify or waive the need for a technician.

3.3 Temperature Monitoring Device

3.3.1 Glass, dial, or electronic thermometers must be graduated in 0.5°C increments (0.2°C increments for tests which are incubated at 44.5°C) or less, except as noted for hot air ovens (3.6.1) and refrigerators (3.9.1). The fluid column in glass thermometers should not be separated. Dial thermometers that cannot be calibrated should not be used.

QC **3.3.2** The calibration of glass and electronic thermometers should be checked annually, and dial thermometers quarterly, at the temperature used, against a National Institute of Standards and Technology (NIST)-traceable reference thermometer or one that meets the requirements of NBS Monograph SP 250-23. The calibration factor and date of calibration should be indicated on the thermometer. In addition, the laboratory should record in a QC record book the following information:

- serial number of laboratory thermometer
- serial number of NIST-traceable thermometer (or other reference thermometer)
- temperature of laboratory thermometer
- temperature of NIST-traceable thermometer (or other reference thermometer)
- correction (or calibration) factor
- date of check
- analyst's initials

QC **3.3.3** If a thermometer differs by more than 1°C from the reference thermometer, it should be discarded. Reference thermometers should be recalibrated at least every five years. Reference thermometer calibration documentation should be maintained.

QC **3.3.4** Continuous recording devices that are used to monitor incubator temperature should be recalibrated at least annually. A reference thermometer that meets the specifications described in paragraph 3.3.2 should be used for calibration.

3.4 Incubator Unit

3.4.1 Incubator units must have an internal temperature monitoring device and maintain the temperature specified by the method used, usually 35°±0.5°C and 44.5°±0.2°C. For non-portable incubators, thermometers should be placed on the top and bottom shelves of the use area and immersed in liquid as directed by the manufacturer (except for electronic thermometers). If an aluminum block incubator is used, culture dishes and tubes should fit snugly. Laboratories which use the enzyme substrate tests with air-type incubators should note the product incubation details indicated in paragraph 5.3.1.5.

QC **3.4.2** Calibration-corrected temperature should be recorded for each thermometer being used at least twice per day during each day the incubator is in use, with readings separated by at least 4 hours. Documentation should include the date and time of reading, temperature, and technician's initials.

3.4.3 An incubation temperature of 44.5°±0.2°C can best be maintained with a circulating water bath equipped with a gable cover.

3.5 Autoclave

3.5.1 The autoclave should have an internal heat source, a temperature gauge with a sensor on the exhaust, a pressure gauge, and an operational safety valve. The autoclave should maintain a sterilization temperature during the sterilizing cycle and complete an entire cycle (i.e., time between starting autoclave and removing items from autoclave) within 45 minutes when a 12-15 minute sterilization period is used. The autoclave should depressurize slowly enough to ensure that media will not boil over and bubbles will not form in inverted tubes.

3.5.2 Because of safety concerns and difficulties with operational control, pressure cookers should not be used.

QC **3.5.3** The date, contents, sterilization time and temperature, total time in autoclave, and analyst's initials should be recorded each time the autoclave is used. Copies of the service contract or internal maintenance protocol and maintenance records should be kept. Maintenance should be conducted at least annually. A record of the most recent service performed should be on file, available for inspection.

QC **3.5.4** A maximum-temperature-registering thermometer, electronic temperature readout device, or continuous recording device should be used during each autoclave cycle to ensure that the proper temperature was reached, and the temperature recorded. Overcrowding should be avoided. Spore strips or spore ampules should be used monthly as bioindicators to confirm sterilization. (Since chemical indicators will respond to a wide range of times and temperatures, i.e., a longer time at a lower temperature, as well as a shorter time at a higher temperature, a positive result with the indicator does not necessarily show that sterilization has occurred.)

QC **3.5.5** Automatic timing mechanisms should be checked quarterly with a stopwatch or other accurate timepiece or time signal, and the results recorded and initialed.

3.5.6 Autoclave door seals should be clean and free of caramelized media. Also, autoclave drain screens should be cleaned frequently and debris removed.

3.6 Hot Air Oven

3.6.1 The oven should maintain a stable sterilization temperature of 170°-180°C for at least two hours. Overcrowding should be avoided. The oven thermometer should be graduated in 10°C increments or less, with the bulb placed in sand during use.

QC **3.6.2** The date, contents, sterilization time and temperature, and analyst's initials should be recorded.

QC **3.6.3** Spore strips should be used monthly to confirm sterilization. Ampules are not recommended for hot air ovens because they may explode or melt.

3.7 Colony Counter

A dark field colony counter should be used to count Heterotrophic Plate Count colonies.

3.8 Conductivity Meter

3.8.1 Meters should be suitable for checking laboratory reagent-grade water and readable in units of either micromhos/cm or microsiemens/cm.

QC **3.8.2** Calibrate the meter at least monthly, following the manufacturer's recommendations and using an appropriate certified and traceable low-level standard. If the meter cannot be calibrated with a commercial standard, the cell constant should be determined at monthly intervals, using a method in Section 2510, "Conductivity," in *Standard Methods*.

3.8.3 If an in-line unit cannot be calibrated, it should not be used to check reagent-grade water.

3.9 Refrigerator

3.9.1 Refrigerators should maintain a temperature of 1°-5°C. Calibrated thermometers should be graduated in at least 1°C increments and the thermometer bulb immersed in liquid.

QC **3.9.2** On days the refrigerator is in use, and the laboratory is staffed, the calibrated-corrected temperature should be recorded at least once per day.

3.10 Inoculating Equipment

Sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipet tips should be used. If wood applicator sticks are used, they should be sterilized by dry heat. The metal inoculating loops and/or needles should be made of nickel alloy or platinum. (When performing an oxidase test, do not use nickel alloy loops because they may interfere with the test).

3.11 Membrane Filtration Equipment (if MF procedure is used)

3.11.1 MF units must be stainless steel, glass, porcelain, or autoclavable plastic, not scratched or corroded, and must not leak.

QC **3.11.2** If graduation marks on clear glass or plastic funnels are used to measure sample volume, their accuracy should be checked with a Class B graduated cylinder or better (or other Class B glassware), and a record of this calibration check retained.

3.11.3 A 10X to 15X stereo microscope with a fluorescent light source must be used to count the target colonies (e.g., sheen colonies on M-Endo or Endo LES media).

3.11.4 Membrane filters must be approved by the manufacturer for total coliform water analysis. Approval is based on data from tests for toxicity, recovery, retention, and absence of growth-promoting substances. Filters must be gridmarked, 47 mm diameter, and 0.45 µm pore size, or alternate pore sizes if the manufacturer provides performance data equal to or better than the 0.45 µm pore size. They should also be white, and of celluose ester. Membrane filters and pads must be purchased presterilized or autoclaved for 10 minutes at 121°C before use.

QC **3.11.5** The lot number for membrane filters and the date received should be recorded. Ensure that membrane filters are not brittle or distorted, and that manufacturer's specification/certification sheet is available.

3.11.6 Forceps used should be blunt and smooth-tipped without corrugations on the inner sides of tips.

3.12 Culture Dishes (loose or tight lids)

3.12.1 Presterilized plastic or sterilizable glass culture dishes should be used. To maintain sterility of glass culture dishes, use stainless steel or aluminum canisters, or a wrap of heavy aluminum foil or char-resistant paper.

3.12.2 Loose-lid petri dishes should be incubated in a tight-fitting container, e.g., plastic vegetable crisper containing a moistened paper towel to prevent dehydration of membrane filter and medium.

3.12.3 Opened packs of disposable culture dishes should be resealed between use periods.

3.12.4 For membrane filter methods, culture dishes should be of an appropriate size to allow for the transfer of a single membrane per plate.

3.13 Pipets

3.13.1 To sterilize and maintain sterility of glass pipets, stainless steel or aluminum canisters should be used, or individual pipets should be wrapped in char-resistant paper or aluminum foil.

3.13.2 Pipets should have legible markings and should not be chipped or etched.

3.13.3 Opened packs of disposable sterile pipets should be resealed between use periods.

3.13.4 Pipets delivering volumes of 10 mL or less must be accurate to within a 2.5% tolerance.

QC **3.13.5** Calibrated micropipetters may be used if tips are sterile. Micropipetters should be calibrated annually and adjusted or replaced if the precision or accuracy is greater than 2.5%.

3.14 Glassware and Plasticware

3.14.1 Glassware should be borosilicate glass or other corrosion-resistant glass and free of chips and cracks. Markings on graduated cylinders and pipets must be legible. Plastic items should be clear and non-toxic to microorganisms.

QC **3.14.2** Graduated cylinders for measurement of sample volumes must be accurate to within a 2.5% tolerance. In lieu of graduated cylinders, precalibrated containers that have clearly marked volumes accurate to within a 2.5% tolerance may be used.

3.14.3 Culture tubes and containers containing fermentation medium should be of sufficient size to contain medium plus sample without being more than three quarters full.

3.14.4 Tube closures should be stainless steel, plastic, aluminum, or screw caps with non-toxic liners. Cotton plugs and foam plugs should not be used.

3.15 Sample Containers

3.15.1 Sample containers should be wide-mouth plastic or non-corrosive glass bottles with non-leaking ground glass stoppers or caps with non-toxic liners that should withstand repeated sterilization, or sterile plastic bags containing sodium thiosulfate. Other appropriate sample containers may be used. The capacity of sample containers should be at least 120 mL (4 oz.) to allow at least a 1-inch head space.

3.15.2 Glass stoppers must be covered with aluminum foil or char-resistant paper for sterilization.

3.15.3 Glass and plastic bottles that have not been presterilized should be sterilized by autoclaving. Glass bottles may also be sterilized by dry heat. Empty containers should be moistened with several drops of water before autoclaving to prevent an "air lock" sterilization failure.

3.15.4 If chlorinated water is to be analyzed, sufficient sodium thiosulfate ($Na_2S_2O_3$) must be added to the sample bottle before sterilization to neutralize any residual chlorine in the water sample. Dechlorination is addressed in Section 9060A of *Standard Methods*.

3.16 Ultraviolet lamp (if used)

3.16.1 A germicidal unit (254-nm) should be disconnected monthly and the lamp cleaned by wiping with a soft cloth moistened with ethanol. A longwave unit (365-366-nm), used for fluorometric tests, should also be kept clean.

QC **3.16.2** A germicidal unit should be tested quarterly with a UV light meter or agar spread plate. The lamp should be replaced if it emits less than 70% of its initial output or if an agar spread plate containing 200 to 250 microorganisms, exposed to the UV light for two minutes, does not show a count reduction of 99%. Other methods may be used to test a lamp if data demonstrate that they are as effective as the two suggested methods. (UV protective eye wear should be used when checking the operation of a 254-nm lamp.)

3.17 Spectrophotometer or colorimeter (if used)

3.17.1 Wavelengths should be in the visible range—Spectronic 20 (Thermo Spectronic), or equivalent, with cell holder for ½" diameter cuvettes (Model # 4015) or 13 mm × 100 mm cuvettes.

QC **3.17.2** A calibration standard and a method-specific blank should be analyzed every day the instrument is used, prior to sample analysis. The calibration standard should give a reading in the desired absorbance range and should be obtained from an outside source.

4. General Laboratory Practices

Although safety criteria are not covered in the laboratory certification program, laboratory personnel should be aware of general and customary safety practices for laboratories. Each laboratory is encouraged to have a safety plan available. Also, each laboratory should keep a copy, and follow the personal protection guidelines, of any material safety data sheet accompanying the receipt of a toxic material.

4.1 Sterilization Procedures

4.1.1 Autoclaving times at 121°C are listed below. Except for membrane filters and pads and carbohydrate-containing media, indicated times are minimum times and may necessitate adjustment depending upon volumes, containers, and loads. Carbohydrate-based media should not be over-sterilized.

Item	Time (min)
Membrane filters & pads	10
Carbohydrate containing media	12-15[1]
Contaminated test materials	30[2]
Membrane filter assemblies	15
Sample collection bottles	15
Individual glassware	15
Dilution water blank	15
Rinse water (0.5 - 1 L)	15-30[2]

[1] except when otherwise specified by the manufacturer
[2] time depends upon water volume per container and autoclave load

4.1.2 Autoclaved membrane filters and pads and all media should be removed immediately after completion of the sterilization cycle.

4.1.3 Membrane filter equipment must be autoclaved before the beginning of a filtration series. A filtration series ends when 30 minutes or longer elapses after a sample is filtered.

4.1.4 Ultraviolet light (254 nm) may be used to sanitize equipment (after initial autoclaving for sterilization), if all supplies are presterilized. Ultraviolet light may be used to reduce bacterial carry-over between samples during a filtration series.

4.2 Sample Containers

QC At least one sample container should be selected at random from each batch of sterile sample bottles or other containers (or lot of commercially available sample containers), and the sterility confirmed by adding approximately 25 mL of a sterile non-selective broth (e.g., tryptic soy, trypticase soy, or tryptone broth). The broth should be incubated at 35°±0.5°C, and checked after 24 and 48 hours for growth. Record results. Resterilize entire batch if growth is detected.

4.3 Reagent-Grade Water

4.3.1 Only satisfactorily tested reagent water from stills or deionization units may be used to prepare media, reagents, and dilution/rinse water for performing microbial analyses.

QC **4.3.2** The quality of the reagent water should be tested and should meet the following criteria:

Parameter	Limits	Frequency
Conductivity	>0.5 megohms resistance or <2 micromhos/cm (microsiemens/cm) at 25°C	Monthly[4]
Pb, Cd, Cr, Cu, Ni, Zn	Not greater than 0.05 mg/L per contaminant. Collectively, no greater than 0.1 mg/L	Annually
Total Chlorine Residual[1]	<0.1 mg/L	Monthly
Heterotrophic Plate Count[2]	< 500 CFU/mL[5]	Monthly

Parameter	Limits	Frequency
Bacteriological Quality of Reagent Water[3]	Ratio of growth rate 0.8 to 3.0	Annually

[1] DPD Method should be used. Not required if source water is not chlorinated.
[2] Pour Plate Method. See *Standard Methods* 9215B.
[3] See *Standard Methods* (18th or 19th eds.), Section 9020B, under *Laboratory Supplies*. This bacteriological quality test is not needed for Type II water or better, as defined in *Standard Methods* (18th and 19th eds), Section 1080C, or Medium quality water or better, as defined in *Standard Methods* (20th ed.), Section 1080C. If Type II or Medium quality water or better is not available, and a glass still is used for reagent water, a silicon test that meets the specifications of *Standard Methods,* Section 1080C (20th ed.) should also be accomplished.
[4] Monthly, if meter is in-line or has a resistivity indicator light; otherwise, with each new batch of reagent water.
[5] CFU means colony-forming units (same as colonies, but is a more precise term).

4.4 Dilution/Rinse Water

4.4.1 Stock buffer solution or peptone water should be prepared, as specified in *Standard Methods*, Section 9050C.

4.4.2 Stock buffers should be autoclaved or filter-sterilized, and containers should be labeled and dated. Stock buffers should be refrigerated. Stored stock buffers should be free from turbidity.

QC **4.4.3** Each batch (or lot, if commercially prepared) of dilution/rinse water should be checked for sterility by adding 50 mL of water to 50 mL of a double strength non-selective broth (e.g., tryptic soy, trypticase soy or tryptose broth). Incubate at 35°±0.5°C, and check for growth after 24 and 48 hours. Record results. Discard batch if growth is detected.

4.5 Glassware Washing

4.5.1 Distilled or deionized water should be used for final rinse.

4.5.2 Laboratory glassware should be washed with a detergent designed for laboratory use.

QC **4.5.3** A glassware inhibitory residue test (*Standard Methods*, Section 9020B, under *Laboratory Supplies*) should be performed before the initial use of a washing compound and whenever a different formulation of washing compound, or washing procedure, is used. Record results. This test will ensure that glassware is free of toxic residue.

QC **4.5.4** Each batch of dry glassware used for microbial analysis should be checked for pH reaction, especially if glassware is soaked in alkali or acid (*Standard Methods*, Section 9020B, under *Laboratory Supplies*). Use 0.04% bromthymol blue (or equivalent pH indicator) and observe color reaction. Clean glassware without an alkali or acid residual should have a neutral color reaction (blue-green for bromthymol blue). Record results. This test will ensure that glassware is at a neutral pH.

5. Analytical Methodology

5.1 General

5.1.1 For compliance samples, laboratories must use only the analytical methodology specified in the Total Coliform Rule (40 CFR 141.21(f)), the Surface Water Treatment Rule (SWTR) (40 CFR 141.74(a)), and the Groundwater Rule (TBD). For convenience, these regulations are reproduced in Appendix G.

5.1.2 A laboratory must be certified for all analytical methods that it uses for compliance purposes. At a minimum, the laboratory must be certified for one total coliform method and one fecal coliform or *E. coli* method. A laboratory should also be certified for a second total coliform method if one method cannot be used for some drinking waters (e.g., where the water usually produces confluent growth on a plate). In addition, for laboratories that may enumerate heterotrophic bacteria (as measured by the Heterotrophic Plate Count, HPC) for compliance with the Surface Water Treatment Rule, the laboratory must be certified either for the Pour Plate Method or the SimPlate method for heterotrophic bacteria.

5.1.3 Water samples should be shaken vigorously at least 25 times before analyzing.

QC **5.1.4** If dilution buffer is used, check the accuracy of the buffer volume in one dilution bottle in each batch or lot. For a 90-mL or 99-mL volume, the tolerance should be ±2 mL.

5.1.5 Sample volume analyzed for total coliforms in drinking water must be 100 mL. To assure accuracy and consistency within methods and between methods it is important that the laboratory obtain precise measurement of the volume of sample to be analyzed. To ensure that the required volume of 100 mL is analyzed, no matter which of the approved methods the laboratory will be employing for analysis, good laboratory practice dictates that a sterile, calibrated measuring vessel be used for measurement of the sample volume. It is inappropriate for a portion of the sample to be poured to waste in order to meet the required sample volume, as this practice could easily result in laboratory error which could then require the sample to be invalidated.

5.1.6 Media (or defined substrate)

5.1.6.1 The use of dehydrated or prepared media manufactured commercially is strongly recommended due to concern about quality control. Dehydrated media should be stored in a cool, dry location, and discarded by manufacturer's expiration date. Caked or discolored dehydrated media should be discarded.

QC **5.1.6.2** For media prepared in the laboratory, the date of preparation, type of medium, lot number, sterilization time and temperature, final pH (after sterilization), and the technician's initials should be recorded.

QC **5.1.6.3** For media prepared commercially, the date received, type of medium, lot number, and (if identified by the manufacturer or method) pH verification for each lot should be recorded. Media should be discarded by manufacturer's expiration date.

QC **5.1.6.4** Each new lot of dehydrated or prepared commercial medium and each batch of laboratory-prepared medium should be checked before use for sterility and with positive and negative culture controls. Those laboratories using commercially prepared media with manufacturer shelf-lives of greater than 90 days should run positive and negative controls each quarter, in addition to running these controls and sterility checks on each new lot of media. Laboratories are encouraged to perform positive and negative control tests on a more frequent basis. Control organisms (total coliforms, fecal coliforms, and/or *E. coli*, as appropriate) can be stock cultures (periodically checked for purity) or commercially available disks impregnated with the organism. Results should be recorded. The following Table identifies a few positive and negative culture controls that laboratories might consider, although other culture controls are also acceptable.

Control Cultures for Microbiological Tests

Group	Positive Culture Control[9]	Negative Culture Control[9]
Total coliforms	*Escherichia coli* *Enterobacter aerogenes*	*Staphylococcus aureus*[1] *Proteus vulgaris*[2] *Pseudomonas aeruginosa*[1]
Fecal coliforms	*Escherichia coli* *Klebsiella pneumoniae* (thermotolerant)	*Enterobacter aerogenes*[3]
E. coli	*Escherichia coli* (MUG-positive strain)	*Enterobacter aerogenes* *Klebsiella pneumoniae*[4] (thermotolerant)
Enterococci[5]	*Enterococcus faecalis* *Enterococcus faecium*	*Staphylococcus aureus*[6] *E. coli*[7] *Serratia marcesens*[8]

[1] *S. aureus, P. aeruginosa* - not lactose fermenter

[2] *P. vulgaris* - not lactose fermenter; uses hydrolyzed lactose, indicating "overcooked" medium

[3] *E. aerogenes* - ferments lactose, but is not typically thermotolerant

[4] *K. pneumoniae* - ferments lactose, but does not hydrolyze MUG

[5] Do not use closely related strains from genus *Streptococcus* as a positive control

[6] *S. aureus* - sensitive to nalidixic acid in medium

[7] *E. coli* - sensitive to sodium azide in medium

[8] *S. marcescens* - will not hydrolyze fluorogenic compound in medium

[9] Examples of appropriate ATCC strains include the following:

Enterococcus faecalis ATCC 11700
Enterobacter aerogenes ATCC 13048
Klebsiella pneumoniae (thermotolerant) ATCC 13883
Pseudomonas aeruginosa ATCC 27853
Staphylococcus aureus ATCC 6538
Enterococcus faecium ATCC 6057
Escherichia coli ATCC 8739 or 25922
Proteus vulgaris ATCC 13315
Serratia marcesens ATCC 14756

5.1.6.5 If prepared medium is stored after sterilization, it should be maintained in the dark, avoiding moisture loss, per the following Table. Prepared plates may be stored in sealed plastic bags or containers. For either broth or agar media, each bag or container should include the date prepared or an expiration date. If the medium is stored in a refrigerator, it should be warmed to room temperature before use; tubes or plates that show growth and/or bubbles should be discarded. Liquid media should be discarded if evaporation exceeds 10% of the original volume.

Maximum Holding Times and Temperatures for Prepared Media

Container	Max storage temp.	Max. storage time
Poured agar plates	1-5°C	2 weeks
Broth in tubes, bottles, or flasks with loose-fitting closures	1-30°C	2 weeks
Broth in tightly closed screw-cap tubes, bottles, or flasks	1-30°C	3 months

QC **5.1.7** Laboratories are encouraged to perform parallel testing between a newly approved test and another EPA-approved procedure for enumerating total coliforms for at least several months and/or over several seasons to assess the effectiveness of the new test for the wide variety of water types submitted for analysis. During this testing, spiking the samples occasionally with sewage or a pure culture may be necessary to ensure that some of the tests are positive.

5.1.8 A list of approved analytical methods (or proposed methods, where noted), applicable regulations, and section identifiers for each method is provided in the Table below.

Approved Methods	Part	Media	Method Citation[1]	TCR[2] (Detect)	SWTR[2] (Count)	GWR[2] (Detect)
Total Coliforms						
Fermentation broth method	5.2.2	LTB⇒BGLB Broth	SM 9221B,C	X	X	
	5.2.3	P-A Broth ⇒ BGLB Broth	SM 9221D	X		
Enzyme substrate method	5.3.2	Colilert®, Colilert-18®	SM 9223	X	X	
	5.3.2	Colisure®	SM 9223	X		
	5.3.2	Readycult® or Fluorocult LMX®		X		
	5.3.2	E*Colite®		X		
	5.3.2	Colitag®		X		
Membrane filter method	5.4.2	M-Endo or LES-Endo ⇒ LTB, BGLB Broth	SM 9222B,C	X	X	
	5.4.2	MI Medium	SM 9222	X	X	
	5.4.2	m-ColiBlue 24®		X		
	5.4.2	Chromocult®		X		
	5.4.2	Coliscan®		X	X	
Fecal Coliforms						
Fermentation broth method	5.2.4	LTB or P/A broth ⇒ EC broth	(SM 9221B,D) SM 9221E	X	X	
	5.2.4	A-1 broth	SM 9221E		X	
Membrane filter method	5.2.4	M-Endo medium ⇒ EC broth	(SM 9222B) SM 9221E	X	X	
	5.4.2	mFC	SM 9222D		X	

Approved Methods	Part	Media	Method Citation[1]	TCR[2] (Detect)	SWTR[2] (Count)	GWR[2] (Detect)
Escherichia coli						
Enzyme substrate method	5.3.2	Colilert® or Colilert-18®	SM 9223	X		X
	5.3.2	Colisure®	SM 9223	X		X
	5.3.2	E*Colite®		X		X
	5.3.2	Readycult® or Fluorocult LMX®		X		
	5.3.3	LTB, P/A broth, M-Endo ⇒ EC-MUG	(SM 9221B,D; SM 9222B) SM 9221F	X		X
	5.3.2	Colitag®		X		
Membrane filter method	5.4.2	MI Medium	SM 9222	X		X
	5.4.2	m-ColiBlue24®		X		X
	5.4.2	Chromocult®		X		
	5.4.2	Coliscan®		X		
	5.4.3	M-Endo or LES Endo ⇒ NA-MUG	(SM 9222B) → SM 9222G	X		X
Enterococci[3]						
Enzyme substrate method	5.3.4	Enterolert	ASTM D6503-99			X
Fermentation broth method	5.2.5	Azide Dextrose ⇒ BEA/BHI	SM 9230B			X
Membrane filter method	5.4.4	mE ⇒EIA m-Enterococcus	SM 9230C			X
	5.4.4	mEI	EPA 1600			X
Heterotrophic Bacteria						
Pour plate method	5.5	Plate count agar	SM 9215B		X	
Multiple enzyme substrate	5.5	SimPlate®			X	
Pour plate, spread plate, or membrane filter methods	5.5	R2A		X[4]		

Approved Methods	Part	Media	Method Citation[1]	TCR[2] (Detect)	SWTR[2] (Count)	GWR[2] (Detect)
Male-Specific and Somatic Coliphage[3]						
Agar plate method	5.6.2	Two-Step Enrichment	EPA 1601			X
	5.6.3	Single Agar Layer	EPA 1602			X

[1] SM = *Standard Methods for the Examination of Water and Wastewater*, 18th, 19th or 20th edition.
[2] TCR=Total Coliform Rule (40 CFR 141.21 (f)), SWTR=Surface Water Treatment Rule (40 CFR 141.74 (a)). For convenience, analytical methods approved for the TCR and SWTR are reproduced in Appendix G.
[3] GWR = Based on proposed Groundwater Rule (65 FR 30194, dated 5/10/2000). Until the GWR is promulgated, laboratories will not be certified for enterococci or coliphage methods.
[4] For possible use if system operates under a variance to the TCR.

5.2 Fermentation broth methods

5.2.1 General

5.2.1.1 The water level of the water bath should be above the upper level of the medium in the culture tubes.

5.2.1.2 A Dri-bath incubator is acceptable if the specified temperature requirement can be maintained in all tube locations used.

5.2.2 Multiple Tube Fermentation Technique (for detecting total coliforms in drinking water and enumerating total coliforms in source water)

5.2.2.1 For drinking water samples: Various testing configurations can be used (CFR141.21(f)(3), see Appendix G), as long as a total sample volume of 100 mL is examined for each test.

5.2.2.2 For source water samples: Laboratories must use at least 3 series of 5 tubes each with appropriate sample dilutions of source water (e.g., 0.1 mL, 0.01 mL, 0.001 mL).

5.2.2.3 Media

5.2.2.3.1 Lauryl tryptose broth (LTB) (also known as lauryl sulfate broth) must be used in the presumptive test and 2% brilliant green lactose bile broth (BGLBB) in the confirmed test. Lactose broth (LB) may be used in lieu of LTB (40 CFR 141.21(f)(3)) if the laboratory conducts at least 25 parallel tests between this medium and LTB using the waters normally tested and this comparison demonstrates that the false-positive rate and false-negative rate for total coliforms, using LB, is less than 10%. This comparison should be documented and the records retained. The final pH must be 6.8±0.2 for LTB, and 7.2±0.2 for 2% BGLBB.

5.2.2.3.2 The test medium concentration must be adjusted to compensate for the sample volume so that the resulting medium after sample addition is single strength. Optionally, if a single 100-mL sample volume is used, the inverted vial should be replaced with an acid indicator (bromcresol purple) to prevent problems associated with gas bubbles in large inverted tubes. The media must be autoclaved at 121°C for 12-15 minutes.

5.2.2.3.3 Sterile medium in tubes must be examined to ensure that the inverted vials, if used, are free of air bubbles and are at least one-half to two-thirds covered after the water sample is added.

5.2.2.4 After the medium is inoculated, it must be incubated at 35°±0.5°C for 24±2 hours. If no gas or acid is detected, it must be incubated for another 24 hours (total incubation time 48±3 hours).

5.2.2.5 Each 24- and 48-hour tube that contains growth, acid, or gas must be confirmed using 2% BGLBB. A completed test is not required.

5.2.2.6 For drinking water samples: Test each total coliform-positive sample for the presence of either fecal coliforms or *E. coli*.

5.2.2.7 Invalidation of total coliform-negative samples

5.2.2.7.1 For drinking water samples: All samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, must be invalidated. The laboratory must collect, or request that the system collect, another sample within 24 hours from the same location as the original invalidated sample. (Before invalidation, the laboratory may perform a confirmed test and/or a fecal coliform/*E. coli* test on the total coliform-negative culture to check for coliform suppression. If the confirmed test is total coliform-positive or if fecal coliforms/*E. coli* are detected, the sample must be reported as such. A fecal coliform/*E. coli*- positive result is considered a total coliform-positive, fecal coliform/*E. coli*-positive sample, even if the presumptive or confirmed total coliform test is negative. If the follow-up test(s) is negative, the sample must be invalidated because high levels of non-coliform bacteria in the presumptive tubes may have injured, killed, or suppressed the growth of any coliforms in the sample.)

5.2.2.7.2 For source water samples: All samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, should be invalidated. The laboratory should collect, or request that the system collect, another sample from the same location as the original invalidated sample. (Before invalidation, the laboratory may perform a confirmed test on the total coliform-negative culture. If the confirmed test is total coliform-positive, the MPN should be reported. If the test is total coliform-negative, the sample should be invalidated.)

5.2.3 Presence-Absence (P-A) Coliform Test (for detecting total coliforms in drinking water)

5.2.3.1 Medium

5.2.3.1.1 Six-times formulation strength may be used. If the 6-times formulation is used, it must be filter-sterilized rather than autoclaved.

5.2.3.1.2 The medium must be autoclaved for 12 minutes at 121°C. Total time in the autoclave should be less than 30 minutes. Space should be allowed between bottles. The final pH must be 6.8±0.2.

5.2.3.1.3 If prepared medium is stored, it should be maintained in a culture bottle at 1°-30°C in the dark for no longer than three months. If evaporation exceeds 10% of original volume, the medium should be discarded.

5.2.3.2 A 100-mL sample must be inoculated into a P-A culture bottle.

5.2.3.3 Medium must be incubated at 35°±0.5°C and observed for a yellow color (acid) after 24 and 48 hours.

5.2.3.4 Yellow cultures must be confirmed in BGLBB and a fecal coliform/*E. coli* test conducted.

5.2.3.5 All samples which produce a non-yellow turbid culture in P-A medium must be invalidated. The laboratory must collect, or request that the system collect, another sample from the same location as the original invalidated sample. (Before invalidation, the laboratory may perform a confirmed test on the total coliform negative culture and/or a fecal coliform/*E. coli* test. If the confirmed test is total coliform-positive, the sample must be reported as such. If the confirmed test is negative, the sample must be invalidated. A fecal coliform/*E. coli* positive result is considered a total coliform-positive, fecal coliform/*E. coli* positive sample, even if the presumptive and/or confirmed total coliform test is negative.)

5.2.4 Fecal Coliform Test (using EC Medium for fecal coliforms in drinking water or source water, or A-1 Medium for fecal coliforms in source water only)

5.2.4.1 EC Medium

5.2.4.1.1 Use EC medium to test a total coliform-positive culture for fecal coliforms under the Total Coliform Rule. The laboratory must transfer each total coliform-positive culture from a presumptive tube/bottle, or each presumptive total coliform-positive colony unless a cotton swab is used, to at least one tube containing EC Medium with an inverted vial, as specified by §141.21(f)(5)(See Appendix G).

5.2.4.1.2 EC Medium may be used to enumerate fecal coliforms in source water, in accordance with the Surface Water Treatment Rule. Initially, conduct a MTF test (presumptive phase). Three sample volumes of source water (e.g., 10, 1 and 0.1 mL), 5 or 10 tubes/sample volume, should be used. A culture from each total coliform-positive tube should be transferred to a tube containing EC Medium with an inverted vial.

5.2.4.1.3 Autoclave EC Medium for 12-15 minutes at 121°C. The final pH should be 6.9±0.2.

5.2.4.1.4 Inverted vials should be examined to ensure that they are free of air bubbles. The inverted vial must be at least one-half to two-thirds covered after the sample is added.

5.2.4.1.5 EC Medium must be incubated at 44.5°±0.2°C for 24±2 hours.

5.2.4.1.6 Any amount of gas detected in the inverted vial of a tube that has turbid growth must be considered a fecal coliform-positive test, regardless of the result of any subsequent test on that culture.

5.2.4.2 A-1 Medium

5.2.4.2.1 A-1 medium may be used as an alternative to EC Medium to enumerate fecal coliforms in source water, in accordance with the Surface Water Treatment Rule. A-1 Medium must not be used for drinking water samples. Three sample volumes of source water (e.g., 10, 1 and 0.1 mL), 5 or 10 tubes/sample volume, should be used. Unlike EC Medium, A-1 Medium may be used for the direct isolation of fecal coliforms from water.

5.2.4.2.2 A-1 Medium must be sterilized by autoclaving at 121°C for 10 minutes. The final pH must be 6.9±0.1.

5.2.4.2.3 Inverted vials should be examined to ensure that they are free of air bubbles.

5.2.4.2.4 A-1 Medium must be incubated at 35°±0.5°C for three hours, then at 44.5°±0.2°C for 21±2 hours.

5.2.4.2.5 Loose-cap tubes should be stored in dark at room temperature not more than two weeks. A-1 Medium must not be held more than three months in tightly closed screw-cap tubes in the dark at 4°C.

5.2.4.3 Any amount of gas detected in the inverted vial of a tube that has turbid growth must be considered a fecal coliform-positive test, regardless of the result of any subsequent test on that culture.

5.2.5 Azide dextrose medium (for detecting fecal streptococci in ground water)

5.2.5.1 For testing 100-mL samples, prepare triple strength (3X) formulation in a culture bottle and autoclave at 121°C for 15 minutes. Final pH should be 7.2±0.2.

5.2.5.2 Add a 100-mL water sample to the sterilized medium, and incubate at 35°±0.5°C.

5.2.5.3 Check culture for turbidity after 24±2 hours. If turbidity is not observed, reincubate and check again after a total incubation period of 48±3 hours.

5.2.5.4 A turbid culture may be confirmed as fecal streptococci by streaking a portion of the broth onto bile esculin agar (BEA) or bile esculin azide agar (BEAA). (The confirmation medium in *Standard Methods*, PSE Medium, is no longer commercially available.)

5.2.5.5 Before streaking, BEA and BEAA must be sterilized by autoclaving at 121°C for 15 minutes. Final pH should be 6.6±0.2 for BEA and 7.1±0.2 for BEAA.

5.2.5.6 After streaking, BEA and BEAA plates must be incubated at 35°±0.5°C for 48 hours.

5.2.5.7 Brownish-black colonies on BEA or BEAA with brown halos confirm the presence of fecal streptococci. If required, an enterococci test can be performed on one or more fecal streptococci colonies by transferring them to brain heart infusion broth supplemented with 6.5% NaCl, and incubating the culture at 35°±0.5°C for 48 hours. Growth indicates the presence of enterococci.

5.3 Enzyme (Chromogenic/fluorogenic) substrate tests

5.3.1 General

5.3.1.1 For detecting total coliforms and *E. coli* in drinking water samples, a laboratory may use the MMO-MUG test (Colilert), Colisure test, E*Colite test, Readycult Coliforms 100 Presence/Absence Test (or Fluorocult LMX Broth test), or Colitag test. These tests may be available in various configurations. For enumerating total coliforms in source waters, a laboratory may use the Colilert test. If a laboratory uses a fermentation method to detect total coliforms in drinking water, and the sample is total coliform-positive, the laboratory may transfer the positive culture to the EC+MUG test to detect *E. coli*, but not to any other enzyme substrate test medium in this section.

5.3.1.2 Media

5.3.1.2.1 Media must not be prepared from basic ingredients, but rather purchased from a commercially available source.

5.3.1.2.2. The media must be protected from light.

5.3.1.2.3 Some lots of enzyme substrate media have been known to fluoresce. Therefore, each lot of medium should be checked before use with a 365-366-nm ultraviolet light with a 6-watt bulb. For checking Colilert, Colilert-18, Colisure, Readycult/Fluorocult LMX, and Colitag media, a packet of medium should be dissolved in sterile water in a non-fluorescing vessel. If the medium exhibits faint fluorescence, the laboratory should use another lot that does not fluoresce.

5.3.1.2.4 If the samples plus a medium exhibit an inappropriate color change before incubation, it should be discarded and another lot of medium used. The laboratory should notify the medium vendor and request another water sample from the water system. Before incubation, Colilert, Colilert-18, and Colitag should appear colorless to a slight tinge of color, while Colisure and E*Colite are yellow and Readycult/Fluorocult is slightly yellow.

5.3.1.3 Glass and plastic bottles and test tubes should be tested before use with a 365-366-nm ultraviolet light source with a 6-watt bulb to ensure they do not fluoresce. If they fluoresce, use another lot of containers that do not fluoresce.

5.3.1.4 If a Whirl-Pak® bag is used to incubate the Colilert or Colitag medium or any other medium which changes to a yellow color to indicate a positive result, use a type that has a barrier (e.g., B01417) to prevent gaseous emissions to other Whirl-Pak® bags during incubation.

QC **5.3.1.5** Incubators, especially small, low wattage air-type incubators, may not bring a cold 100-mL water sample(s) to the specified incubation temperature for several hours. The problem may cause false-negative results with the enzyme substrate tests and possibly other tests as well. Therefore, laboratories with air-type incubators should observe the following instructions for chromogenic/fluorogenic substrate tests:

Test	Pre-incubation sample instructions[1,2]
Colilert (Presence/Absence)	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°C[1]
Colilert Quanti-Tray	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°C
Colilert-18 (Presence/Absence)	Prewarm sample in 35°C water bath for 20 minutes or 44.5°C for 7-10 minutes
Colilert-18 Quanti-Tray	Allow sample to equilibrate to room temperature (20-30°C) before beginning 18-hour incubation time
Colisure	Allow sample to equilibrate to room temperature (20-30°C) before beginning 24-hour incubation time
Readycult Coliforms 100 Presence/Absence Test and Fluorocult LMX Broth	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°±0.5°C
Colitag	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°±0.5°C

[1] If the laboratory plans to put a large load into a small incubator, samples should be brought to room temperature before incubation.
[2] Information based on manufacturer's instructions.

5.3.1.6 If a water bath is used, the water level should be above the upper level of the medium.

5.3.1.7 For *E. coli* testing, the laboratory must place all total coliform-positive samples under an ultraviolet lamp (365-366 nm, 6-watt) in a darkened area. If *E. coli* is present, the medium will emit a blue fluorescence.

5.3.1.8 The enzyme substrate tests should not be used to confirm a presumptive total coliform-positive culture in fermentation broth (e.g., LTB, LB, P-A coliform test) or on a membrane filter. The high densities of non-coliforms or turbidity in the inoculum may either suppress coliforms or overload the enzyme substrate test suppressant reagent system and cause false-positive results.

5.3.1.9 Any sample that produces an atypical color change (e.g., greenish-black or black) in the absence of a yellow color should be invalidated. The laboratory must collect, or request that the system collect, another sample from the same location as the original invalidated sample. The laboratory should use another method to test the second sample. According to the manufacturer of Colilert, water with high iron or manganese levels in the presence of hydrogen sulfide may cause a greenish-black or black color. This greenish-black color does not occur when using Readycult, Colisure, or Colitag, according to their manufacturers.

5.3.1.10 Any reference comparator provided by the manufacturer should be discarded by the manufacturer's expiration date.

5.3.2 Criteria for specific media

5.3.2.1 For the Colilert test, samples must be incubated at 35°±0.5°C for 24 hours. A yellow color in the medium equal to or greater than the reference comparator indicates that the sample is total coliform-positive. If the sample is yellow, but lighter than the comparator, it must be incubated for another four hours (do not incubate more than 28 hours total). If the color is still lighter than the reference comparator at 28 hours, the sample should be reported as negative. A coliform-positive sample that fluoresces under a UV light indicates the presence of *E. coli*. Laboratories that use the Colilert-18 test must incubate samples for 18 hours (up to 22 hours if sample after 18 hours is yellow, but is lighter than the comparator).

5.3.2.1.1 For enumerating total coliforms in source water with the Colilert test, a 5- or 10-tube configuration, Quanti-Tray, or Quanti-Tray 2000 may be used for each sample dilution tested. Dilution water (if used) may be sterile deionized or sterile distilled water, but not buffered water.

QC 5.3.2.1.2 If the Quanti-Tray or Quanti-Tray 2000 test is used, the sealer should be checked monthly by adding a dye (e.g., bromcresol purple) to the water. If dye is observed outside the wells, either perform maintenance or use another sealer.

5.3.2.2 For the Colisure test, samples must be incubated at 35°±0.5°C for 24 hours. If an examination of the results at 24 hours is not convenient, then results may be examined at any time up to 48 hours. If the medium changes from a yellow color to a red/magenta color, the sample is total coliform-positive. A coliform-positive sample that fluoresces under a UV light indicates the presence of *E. coli*.

5.3.2.3 For the E*Colite test, samples must be incubated at 35°±0.5°C for 28 hours. If total coliforms are present, the medium changes from a yellow color to a blue or blue-green color, or a blue color in the corners of the bag. If *E. coli* is present, medium will fluoresce under a UV light. If no fluorescence is observed, re-incubate for an additional 20 hours (for a total incubation time of 48 hours) and again check for fluorescence. If medium becomes red in color, assume that a faulty seal has allowed the bactericide (in the third compartment of the bag) to leak into the compartment containing the medium. In this case, discard the sample, and request another sample.

5.3.2.4 For the Readycult Coliforms 100 Presence-Absence test, the contents of a snap pack should be added to a 100-mL water sample, followed by incubation at 35°±05°C for 24±1 hours. If coliforms are present, the medium changes color from a slightly yellow color to blue-green. In addition, if *E. coli* is present, the medium will emit a bright light-blue fluorescence when subjected to a long wave (365-366 nm) ultraviolet (UV) light. If confirmation of *E. coli* is desired, Kovac's indole reagent should be added to the broth; the immediate formation of a red ring confirms the presence of *E. coli*.

5.3.2.5 Fluorocult LMX broth is identical to Readycult, except that it is a dehydrated culture medium in granulated form packed primarily in a 500 g plastic bottle. For testing a 100-mL water sample, suspend 34 g of Fluorocult LMX in 1L purified water and boil to dissolve completely. Transfer 100-mL aliquots to 250-mL bottles and autoclave for 15 min at 121°C. Cool to room temperature, add the 100-mL water sample, and incubate. Do not add *E. coli*/Coliform Supplement to the medium.

5.3.2.6 For the Colitag test, samples must be incubated at 35°±0.5°C for 24±2 hours. During incubation, trimethylamine-N-oxide in the Colitag medium causes the pH of the medium to increase from 6.2 to 6.8-7.2. A yellow color in the medium indicates the presence of total coliforms. A coliform-positive sample that fluoresces under a UV light indicates the presence of *E. coli*.

5.3.3 EC Medium + MUG Test (for detection of *E. coli*)

5.3.3.1 If EC medium + MUG is used, a total coliform-positive culture must be transferred from a presumptive tube/bottle or colony to EC medium + MUG, as specified by §141.21(f)(5)(See Appendix G).

5.3.3.2 MUG may be added to EC Medium before autoclaving. EC Medium+MUG is also available commercially. The final MUG concentration must be 50 µg/mL. The final pH should be 6.9±0.2.

5.3.3.3 The inverted vial may be omitted, because gas production is not relevant to the *E. coli* test.

5.3.3.4 The medium must be incubated at 44.5°±0.2°C for 24±2 hours, and tested for fluorescence.

5.3.4 Enterolert test (for detection of enterococci in ground water)

5.3.4.1 Medium should be stored in the dark at 4-30°C until use.

5.3.4.2 Add Enterolert reagent to 100-mL water sample, and incubate at 41°± 0.5°C for 24-28 hours. Fluorescence under a UV lamp indicates the presence of enterococci.

5.3.4.3 The development of fluorescence after 28 hours is not a valid test for enterococci.

5.4 Membrane Filter (MF) methods

5.4.1 General

5.4.1.1 For source water samples (SWTR): To optimize counting, appropriate sample dilutions must be used to yield 20 to 80 total coliform colonies or 20-60 fecal coliform colonies for at least one dilution or volume.

QC **5.4.1.2** At least one membrane filter and filtration unit sterility check should be conducted at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter and testing for growth. If the control indicates contamination, all data from affected samples must be rejected and an immediate resampling should be requested. A filtration series ends when 30 minutes or more elapse between sample filtrations.

5.4.1.3 Each filtration funnel must be rinsed after each sample filtration with two or three 20-30 mL portions of sterile rinse water to ensure that entire sample is rinsed off the funnel before the filter is removed. After the filter is removed, the funnel may be rinsed again with two or three 20-30 mL portions of sterile rinse water or exposed to UV light with a 254-nm wavelength for at least two minutes to prevent carry-over between samples, especially for surface water samples..

5.4.1.4 Absorbent pads must be saturated with a liquid medium (at least 2 mL of broth) and excess medium removed by "decanting" the plate.

5.4.2 MF method for detecting total coliforms and *E. coli* in drinking water, enumerating total coliforms or fecal coliforms in source water, and detecting *E. coli* in ground water

5.4.2.1 Media for total coliforms, fecal coliforms, and *E. coli*

5.4.2.1.1 M-Endo Medium agar or broth (also known as M-Endo broth MF and M-Coliform Broth) or LES Endo agar (also known as M-Endo Agar LES) for detecting total coliforms in drinking water or enumerating total coliforms in source water. Medium may be used in the single step or enrichment techniques. Ensure that ethanol used in the rehydration procedure is not denatured. Medium should be prepared in a sterile flask and brought just to the boiling point with a boiling water bath or, if constantly attended, a hot plate with a stir bar. The medium must not be boiled. Final pH should be 7.2±0.2 for M-Endo Agar LES and 7.2±0.1 for M-Endo medium.

5.4.2.1.2 m-ColiBlue24 medium for detecting total coliforms and *E. coli* in drinking water. Ampules

of broth should be inverted 2-3 times to mix contents before breaking. Then contents should be poured evenly over absorbent pad. Unopened refrigerated ampules may be stored in the dark until the expiration date, but should be discarded earlier if growth is observed. The final pH of medium should be 7.0±0.2.

5.4.2.1.3 MI Medium (with or without agar) for detecting total coliforms and E. coli in drinking water or enumerating total coliforms in source water. Do not autoclave commercially made, presterilized bottled MI agar or broth. Melt bottled agar in a boiling water bath (or by other processes recommended by the manufacturer). As soon as complete melting has occurred, cool slightly and pour immediately into sterile plates. Care should be taken to prevent overheating the agar, as excessive heat destroys the effectiveness of the antibiotic, cefsulodin. If dehydrated culture medium is used, it should be prepared and autoclaved according to the manufacturer's instructions. Cool the agar, add freshly prepared, filter-sterilized cefsulodin, and pour immediately into sterile plates. The final pH of MI agar should be 6.95±0.20; the final pH of MI broth should be 7.05±0.20. The preparation and use of MI agar and MI broth is described in the article, "New medium for the simultaneous detection of total coliform and *Escherichia coli* in water" by Brenner, K.P., *et al.*, 1993, *Applied and Environmental Microbiology* 59:3534-3544. EPA Method 1604, which can be found online at www.epa.gov/microbes, is identical.

5.4.2.1.4 Chromocult® Coliform Agar for detecting total coliforms and *E. coli* in drinking water. Do not autoclave or overheat. The final pH should be 6.8±0.2. If a heavy background of heterotrophic bacteria is expected (especially *Pseudomonas* and *Aeromonas* spp.), add cefsulodin solution to the cooled (45°-50°C) medium (dissolve 10 mg cefsulodin in 2 mL deionized or distilled water, and add solution to 1L of medium). Check with the manufacturer, EMD Chemicals, Inc., at www.emdchemicals.com, or call (800) 222-0342 for additional information on the performance of this test with various filter types.

5.4.2.1.5 Coliscan® for detecting total coliforms and *E. coli* in drinking water or enumerating total coliforms in source water. Coliscan is available as a dry powder agar mix or as a presterilized bottled agar. For reconstitution and antibiotic addition, follow the protocol of the manufacture (Micrology Laboratories, LLC). Do not overheat the antibiotic, cefsulodin. The final pH of Coliscan agar should be 7.00±0.20.

5.4.2.1.6 m-FC broth (with or without agar) for enumerating fecal coliforms in source water. Do not autoclave. Bring medium just to the boiling point. The final pH should be 7.4±0.2.

5.4.2.1.7 When stored, prepared medium should be refrigerated. Petri dishes containing medium should be stored in a plastic bag or tightly closed container, and used within two weeks. Before use, refrigerated sterilized medium should be brought to room temperature. Plates with laboratory prepared broth medium must be discarded after 96 hours, poured MF agar plates discarded after two weeks, and ampuled M-Endo broth and other prepared media discarded in accordance with the manufacturer's expiration date. Broth, plates, or ampules should be discarded earlier if growth or (for M-Endo agar) surface sheen is observed. Record date and time prepared.

5.4.2.2 Incubation conditions and colony color of inoculated medium

Medium	Incubation	Total coliforms[1]	*E. coli*
M-Endo medium or M-Endo agar LES	35°±0.5°C for 22-24 hrs	Metallic (golden) sheen colonies (presumptive)	N/A
m-ColiBlue24	35°±0.5°C for 24 hrs	Red colonies	Blue to purple colonies
MI	35°±0.5°C for 24±2 hrs	Fluorescent colonies under UV light	Blue colonies under normal light

Medium	Incubation	Total coliforms[1]	*E. coli*
Chromocult	36°±1°C for 24±1 hrs	Salmon to red colonies	Dark-blue to violet colonies[2]
Coliscan	32°-37°C for 24-28 hrs	Pink-magenta colonies	Purple-blue colonies
m-FC	44.5°±0.2°C for 24±2 hrs	N/A	Blue colonies (fecal coliforms)

[1] Without the presence of *E. coli*. If an *E. coli* colony is present, as indicated by the last column, it should be counted as a total coliform-positive colony.

[2] If confirmation of *E. coli* is desired, add one drop of Kovac's reagent to each dark-blue to violet colony; the formation of a cherry-red color within seconds confirms the presence of *E. coli*.

5.4.2.3 Invalidation of a total coliform-negative drinking water sample: All samples resulting in confluent or TNTC (too numerous to count) growth must be invalidated unless total coliforms are detected. If no total coliforms are detected, record as "confluent growth" or "TNTC" and request an additional sample from the same sampling site. Confluent growth is defined as a continuous bacterial growth covering the entire membrane filter without evidence of total coliform-type colonies. TNTC is defined as greater than 200 colonies on the membrane filter in the absence of detectable coliforms. Laboratories must not invalidate samples when the membrane filter contains at least one coliform-type colony (i.e., sheen colony for M-Endo medium, red or blue colony for m-ColiBlue 24 agar, fluorescent or blue colony for MI agar, salmon to red or dark-blue to violet colonies for Chromocult Coliform agar, pink/magenta or blue/purple colony for Coliscan). (Before invalidation, the laboratory may perform a verification test on the total coliform-negative culture, i.e., on confluent or TNTC growth, and a fecal coliform/*E. coli* test. If the verification test is total coliform-positive, the sample must be reported as total coliform-positive. If the test is total coliform-negative, the sample must be invalidated. A fecal coliform/*E. coli*-positive result is considered a total coliform-positive, fecal coliform/*E. coli*-positive sample, even if the initial and/or verification total coliform test is negative.)

5.4.2.4 Invalidation of source water samples (SWTR): Laboratories must invalidate any sample which results in confluent growth or TNTC, even when total coliform or fecal coliform colonies are present, because coliform density must be determined.

5.4.2.5 For drinking water samples (to verify colonies on Endo-type medium): At least five typical sheen colonies and five nontypical colonies must be verified using either single strength lactose broth (LB) or lauryl tryptose broth (LTB) and then single strength 2% brilliant green lactose bile broth (BGLBB). Alternatively, sheen colonies may be verified using a cytochrome oxidase and ß-galactosidase procedure. Individual colonies can be transferred with a sterile needle or loop, or applicator stick. If no sheen colonies are observed, verify up to five red questionable sheen colonies and/or red non-sheen colonies representing different morphological types. Alternatively, wipe the entire surface of the membrane filter with a sterile cotton swab, and inoculate the verification media (LTB, then BGLBB).

5.4.2.6 For drinking water samples: Total coliform-positive colonies must be tested for *E. coli* or fecal coliforms. The membrane filter tests approved by EPA to date do not require additional media for such a test, except for those using Endo-type medium (M-Endo medium or M-Endo agar LES). EPA has approved several options for testing a total coliform-positive colony on Endo-type medium for *E. coli* or fecal coliforms. When EC Medium (for fecal coliforms) or EC Medium + MUG (for *E. coli*) is used, the colonies must be transferred by employing one of the options specified by the Total Coliform Rule at 40 CFR 141.21(f)(5)(See Appendix G). For the swab technique, a single swab can be used to inoculate a presumptive total coliform-positive culture into up to three different media (e.g., EC or EC-MUG Medium, LTB, and BGLBB, in that order). If Nutrient Agar + MUG is used, refer to paragraph 5.4.3.

5.4.2.7 For source water samples: Initial total coliform counts must be adjusted based upon verified data, as in *Standard Methods*, Section 9222B(5).

QC **5.4.2.8** For source water samples (SWTR): If two or more analysts are available, each analyst should count total coliforms or fecal coliform colonies on the same membrane monthly. Colony counts should agree within 10%.

5.4.3 Nutrient Agar + MUG Test (for detection of *E. coli* in drinking water or ground water)

5.4.3.1 Medium must be autoclaved at 121°C for 15 minutes. MUG may be added to Nutrient Agar before autoclaving. Nutrient Agar + MUG is also available commercially. The final MUG concentration must be 100 µg/mL. The final pH should be 6.8±0.2.

QC **5.4.3.2** Positive and negative controls should be tested as stated in paragraph 5.1.6.4. Filter or spot-inoculate control cultures onto a membrane filter on M-Endo agar LES or M-Endo broth or agar, and incubate at 35°±0.5° C for 24 hours. Then transfer the filter to Nutrient Agar + MUG and incubate at 35°C for another four hours. The results should be read and recorded.

5.4.3.3 The membrane filter containing coliform colony(ies) must be transferred from the total coliform medium to the surface of Nutrient Agar + MUG medium. Each sheen colony should be marked with a permanent marker on the lid. Also, the lid and the base should be marked with a line to realign the lid should it be removed. (A portion of the colony may be transferred with a needle to the total coliform verification test before transfer to Nutrient Agar + MUG or after the 4-hour incubation time. Another method is to swab the entire membrane filter surface with a sterile cotton swab after the 4-hour incubation time on Nutrient Agar + MUG medium, and transfer to a total coliform verification test.)

5.4.3.4 Inoculated medium must be incubated at 35°±0.5C° for four hours.

5.4.3.5 Check the fluorescence using an ultraviolet lamp (365-366 nm) with a 6-watt bulb in a darkened area. Any amount of fluorescence in a halo around a sheen colony should be considered positive for *E. coli*.

5.4.4 MF method for detecting enterococci/fecal streptococci in ground water

5.4.4.1 Media

5.4.4.1.1 For mE agar (SM 9230C) for the detection of enterococci: Prepare basal mE agar. Then autoclave and cool in a 44-46°C water bath. Dissolve 0.48 g nalidixic acid and 0.4 mL 10 N NaOH into 10 mL of reagent-grade distilled water and mix. Filter-sterilize the solution, and add 5.2 mL per liter of basal mE agar. For triphenyl tetrazolium chloride (TTC), add 0.25 g of TTC to 25 mL of reagent-grade water, and warm to dissolve. Filter-sterilize the solution, and add 15 mL per liter of basal mE agar. Final pH should be 7.1± 0.2.

5.4.4.1.2 For m-Enterococcus agar (SM 9230C) for the detection of fecal streptococci (not enterococci): Heat to dissolve ingredients, but do not autoclave. Dispense into sterile petri plates (9 X 50 mm) (about 4 mL), and allow to solidify. Final pH should be 7.2±0.2.

5.4.4.1.3 For mEI agar (EPA Method 1600) for the detection of enterococci: Add 0.75 g indoxyl-β-D-glucoside to 1L of basal mE agar, and proceed according to paragraph 5.4.4.1.1, except that the preparation of TTC is as follows: Add 0.1 g of TTC to 10 mL of reagent-grade distilled water, and warm to dissolve. Filter-sterilize the solution, and add 2 mL per liter of medium. Final pH should be 7.1±0.2.

5.4.4.2 After filtering a 100-mL sample, place membrane in a petri dish on one of the agar media listed above. Serial dilutions should not normally be necessary for detecting enterococci in ground water.

5.4.4.3 *If* m-Enterococcus agar is used, incubate inverted plate at 35°±0.5°C for 48 hours and, using magnification and a fluorescent lamp, count all light and dark red colonies as fecal streptococci.

5.4.4.4 If mE agar is used, incubate inverted plate for 48 hours at 41°±0.5°C, and then transfer filter to EIA medium. Incubate at 41°± 0.5°C for 20-30 minutes and, using magnification and a fluorescent lamp, examine the colonies. Pink to red colonies on mE agar with a black or reddish brown precipitate on the underside of filter on EIA indicates the presence of enterococci.

5.4.4.5 If mEI agar is used, incubate inverted plate for 24 hours at 41°±0.5°C. Using magnification and small fluorescent lamp, examine both the top and bottom of the plate for colonies with a blue halo. A colony with a blue halo, regardless of colony color, indicates presence of enterococci.

5.5 Heterotrophic Plate Count (for enumerating heterotrophic bacteria in drinking water)

5.5.1 The Pour Plate Method (*Standard Methods* 9215B) or the SimPlate Method must be used for determining compliance with 40 CFR 141.74(a)(1) (also listed in Appendix G) and should also be used for testing reagent grade water. For systems that have been granted a variance from the Total Coliform Rule's maximum contaminant level (see variance criteria in the preamble of FR 56:1556-1557, January 15, 1991), any method in *Standard Methods*, Section 9215, *Heterotrophic Plate Count*, may be used with R2A medium, for enumerating heterotrophic bacteria in drinking water.

5.5.2 Media

Method	Medium	Final pH
Pour Plate	Plate count agar, also known as tryptone glucose yeast agar	7.0 ± 0.2
Pour Plate	R2A agar	7.2 ± 0.2
Spread Plate	R2A agar	7.2 ± 0.2
Membrane Filter	R2A agar	7.2 ± 0.2
SimPlate	Multiple enzyme substrate	7.2 ± 0.2

5.5.3 (For Pour Plate Method) Melted agar must be tempered at 44°-46°C in waterbath before pouring. Melted agar should be held no longer than three hours. Sterile agar medium should not be melted more than once.

5.5.4 (For Spread Plate Method) 15 mL of R2A agar medium (or other medium) should be poured into a petri dish (100 x 15 mm or 90 x 15 mm) and allowed to solidify.

5.5.5 Refrigerated medium may be stored in bottles or in screw-capped tubes for up to six months, or in petri dishes for up to two weeks. Prepared petri dishes with R2A medium may be stored for up to one week.

5.5.6 For most potable water samples, countable plates can be obtained by plating 1.0 mL and/or 0.1 mL volumes of the undiluted sample (dilutions may not be necessary for SimPlate, which has a counting range up to 738/mL). At least duplicate plates per dilution should be used.

5.5.7 (For Pour Plate Method) The sample must be aseptically pipetted onto the bottom of a sterile petri dish. Then at least 10-12 mL mL of tempered melted (44°-46°C) agar must be added to each petri dish. The sample and melted agar must be mixed carefully to avoid spillage. After agar plates have solidified on a level surface, the plates must be inverted and incubated at 35°±0.5°C for 48±3 hours. Plates should be stacked no more than four high and arranged in the incubator to allow proper air circulation and to maintain uniform incubation temperature. Avoid excessive humidity in the incubator to reduce the possibility of spreader formation on the agar medium. Also avoid excessive drying of the agar medium; agar medium in plates should not lose more than 15% by weight during 48 hours of incubation.

5.5.8 (For Spread Plate Method) 0.1 or 0.5 mL of the sample (or dilution) should be pipetted onto the surface of the predried agar in the plate, and then spread over the entire surface of the agar using a sterile bent glass rod. The inoculum should be absorbed completely by the agar before the plate is inverted and incubated. The plate should be incubated at 20°-28°C for 5-7 days.

5.5.9 (For Membrane Filter Technique) The volume to be filtered should yield between 20-200 colonies. The filter is transferred to a petri dish containing 5 mL of solidified R2A medium, and incubated at 20°-28°C for 5-7 days. If plates with loose-fitting lids are used, plates should be placed in a plastic box with a close fitting lid containing moistened paper towels. Paper towels should be rewetted as necessary to maintain moisture. Colonies should be counted using a stereoscopic microscope at 10-15X amplification.

5.5.10 (For SimPlate Method)

5.5.10.1 Unit Dose (for a single sample). A10-mL volume of test sample is added to a test tube containing dehydrated SimPlate medium. Then the dissolved medium should be poured onto the center of a plate containing 84 small wells (provided by the manufacturer, IDEXX Laboratories, Inc.). Alternatively, 9 mL of sterile diluent (D.I. water, distilled water, or buffered water [*Standard Methods*, 9050 C,1a]) can be added to the tube, followed by 1-mL sample. Then follow the procedure as indicated above for the 10-mL sample. The mixture should be distributed evenly to the 84 wells on the plate, and the excess liquid drained into an absorbent pad on the plate. The plate should then be inverted (the fluid in each well is held in place by surface tension), and incubated for 45-72 hours at 35°±0.5°C. 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 using the Unit Dose MPN table provided by the manufacturer. If 10-mL sample is used, read the Unit Dose MPN/mL directly. If a 1-mL sample is used, then correct the MPN/mL value by multiplying it by 10.

5.5.10.2 Multiple Dose (for 10 samples of 1 mL each): A 100-mL sterile diluent should be added to the dehydrated SimPlate medium to reconstitute, and shaken to dissolve. Then a 1.0-mL test sample should be pipetted to the center of a plate containing 84 small wells, followed by 9.0 mL of the reconstituted medium. Gently swirl plate to mix the sample and medium, and distribute the mixture evenly to the 84 wells on the plate. Then continue with the procedure indicated in paragraph 5.5.10.1 above, except that the Multi-Dose table supplied by the manufacturer should be used to determine the MPN/mL. If a dilution is made during sample preparation, then multiply the MPN/mL value by the dilution factor.

5.5.11 (For Pour Plate and Spread Plate Techniques) Colonies should be counted manually using a dark-field colony counter. In determining sample count, laboratories must only count plates having 30 to 300 colonies, except for plates inoculated with 1.0 mL of undiluted sample. Counts less than 30 for such plates are acceptable. (Fully automatic colony counters are not suitable because of the size and small number of colonies observed when potable water is analyzed for heterotrophic bacteria.)

QC 5.5.12 Each batch or flask of agar should be checked for sterility by pouring a final control plate. Data should be rejected if control is contaminated.

5.6 Coliphage (Draft Method 1601 and 1602, proposed Ground Water Rule)

Note: EPA Method 1601 and 1602 are performance-based methods for detecting the presence of male-specific (F^+) and somatic coliphage in ground water and other waters. **(Performance-based method:** In recognition of the variety of situations to which some methods may be applied, and in recognition of continuing technological advances, some methods are performance-based. A performance-based method permits laboratories to modify or omit steps or procedures, provided that all performance requirements set forth in the validated methods are met. Any steps that may not be modified or omitted must be specified in the method.)

5.6.1 EPA Method 1601: Male-specific (F^+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure

Method Summary: A 100-mL (or 1-L water sample) is supplemented with magnesium chloride, log-phase host

bacteria (*E. coli* F_{amp} for male-specific coliphage and *E. coli* CN-13 for somatic coliphage), and Tryptic Soy Broth (TSB) as an enrichment step for coliphage. After an overnight incubation, samples are "spotted" onto a lawn of host bacteria specific for each type of coliphage, incubated, and examined for circular lysis zones, which indicate the presence of coliphage.

5.6.1.1 Media

5.6.1.1.1 Antibiotic stocks— Antibiotics must always be added to medium ***after*** the medium has been autoclaved. Store frozen at -20°C for up to one year. Thaw at room temperature or rapidly in a water bath up to 37°C and mix well prior to use. ***Please note: Antibiotics may be toxic. Wear suitable protective clothing, gloves, and eye/face protection and use in a chemical fume hood.***

5.6.1.1.2 10X Tryptic Soy Broth (TSB)—Store at 1°-5°C until use.

5.6.1.1.3 1.5% Tryptic Soy Agar (TSA)—If not used immediately after adding antibiotic and letting the plated medium solidify, store the plates inverted at 1°-5°C for up to 2 weeks.

5.6.1.1.4 0.7% TSA top agar tubes with appropriate antibiotics—Dispense 5 mL per sterile 10-mL tube, label, and keep at 45°- 48°C until use. Tubes must be used the day they are prepared.

5.6.1.1.5 Spot plates—Condensation may accumulate at the edges of stored spot plates and may drip over agar surface if tilted, ruining the spot pattern. If the stored spot plates have condensation, incubate plates for approximately 10 minutes to reduce condensation prior to inoculation. Spot plates may be used that day or stored at 1°-5°C for up to four days.

5.6.1.2 Coliphage stock

5.6.1.2.1 MS2 (ATCC#15597-B1, male-specific) and phi-X 174 stock coliphage (ATCC#13706-B1, somatic)—May be stored at 2-8°C for up to 5 years. Refer to http://www.atcc.org for initial preparation of pure coliphage stock.

5.6.1.2.2 Analysis of raw sewage filtrate should begin within 24 hours of collection.

5.6.1.2.3 Allow the raw sewage to settle at 1°-5°C for 1 to 3 hours. This will make the filtration process easier.

5.6.1.2.4 Hold the assembly over a 15-mL polypropylene tube with screw-cap or snap-cap, insert the plunger into the syringe barrel, and push the sewage through the filter into the sterile tube. If filter clogs, change it as necessary and continue to filter sewage until at least 10 mL of filtered sewage is obtained in the 15-mL polypropylene tube (filtration may require use of numerous filters).

5.6.1.2.5 If filtrate is stored more than 24 hours, it must be re-titered before use.

5.6.1.3 Host bacteria stock cultures

5.6.1.3.1 Frozen host bacteria stock cultures—After preparation, freeze host bacteria stock cultures at -70°C, if possible. Cultures can be frozen at -20°C if the laboratory does not have the capability to freeze samples at -70°C. Host bacteria stored at -70°C may be retained for up to one year. If stored at -20°C, the host bacteria may be retained for up to two months.

5.6.1.3.2 Overnight host bacteria stock cultures—After preparation, chill on wet ice or at 1°-5°C until ready for use.

5.6.1.3.3 Log-phase host bacteria stock cultures—After preparation, chill on wet ice or at 1°-5°C to slow replication until ready for use. The suspension may be stored up to 48 hours. However, the best results occur when cultures are used immediately (within 6 hours). Store remaining bacterial host culture at 1°-5°C overnight to inoculate flasks for the preparation of new log-phase bacterial hosts.

5.6.1.4 General QC

5.6.1.4.1 Initial demonstration of capability (IDC). *The laboratory must demonstrate the ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples.* The IDC test consists of ten reagent water samples spiked with enumerated sewage or equivalent at 1-2 PFU per sample for each coliphage type used, according to the IDC Table below. A minimum number of samples must be positive, depending on coliphage type used (see IDC Table). Spike samples in "bulk" at concentrations in the Table. Tests must be accompanied by a method blank for each coliphage type.

5.6.1.4.2 Method blanks. *The laboratory must analyze method blanks (reagent water sample containing no coliphage) to demonstrate freedom from contamination.* For each coliphage type used, prepare and analyze a sterile reagent water sample containing no coliphage using the same procedure used for analysis of the field or QC samples. At a minimum, the laboratory must analyze one method blank for each spot plate used for field samples. In an effort to determine if cross-contamination is an issue, the sterile method blank should be spotted onto the lawn of host bacteria immediately following the positive control spot.

5.6.1.4.3 Positive controls. *The laboratory must analyze positive controls to ensure that stock coliphage suspensions, host bacterial cultures, and growth media are performing properly.* For each coliphage type used, a 100-mL reagent water sample must be spiked with 20 PFU from sewage filtrate or 60 PFU from a pure coliphage stock culture. The laboratory must inoculate one positive control spot for each spot plate used for field samples. If multiple spot plates are inoculated with samples on the same day, a single enriched positive control sample may be used to inoculate multiple spot plates on that day.

5.6.1.4.4 Matrix spikes (MS). *To assess method performance in a given source water matrix, the laboratory must analyze one set of MS samples for each coliphage type when samples are first received from a ground water source for which the laboratory has never before analyzed samples.* For each coliphage type analyzed, three field samples are spiked with 1-2 PFU. At a minimum, one out of the three MS samples for each coliphage type must be positive for method performance to be considered acceptable for that ground water source. If the MS results are unacceptable, and the ODC sample and positive control sample results associated with this batch of samples are acceptable, a matrix interference may be causing the poor results. In addition, the laboratory must analyze one set of MS samples on an ongoing basis after every 20th field sample for each ground water source. (For example, when a laboratory receives the first sample from a source, the laboratory must obtain additional aliquots of the field samples to be used for the MS test. When the laboratory receives the 20th field sample from this site, additional aliquots of this sample must be collected and spiked.) MS samples should be collected at the same time as routine field samples. Spike samples in "bulk" at the concentrations indicated in the MS and ODC Table below.

5.6.1.4.5 Ongoing demonstration of capability (ODC). *The laboratory must demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples.* For each coliphage type used, three reagent water samples are spiked with 1-2 PFU. The ODC test samples are analyzed exactly like field samples, and at a minimum, one out of three ODC test samples must be positive for each coliphage type used. If not, method performance is unacceptable, and analysis of field samples must be stopped. Identify and correct the problem and demonstrate acceptable performance through analysis of another ODC test before continuing with the analysis of field samples. The laboratory must analyze one set of ODC samples after every 20 field and MS samples or one per week, whichever occurs more frequently. Spike samples in "bulk" at the concentrations indicated in the MS and ODC Table below.

5.6.1.4.6 Performance studies. The laboratory should periodically analyze an external QC sample, such as a performance testing sample, when available. The laboratory should also participate in available interlaboratory performance studies conducted by local, State, and federal agencies or commercial organizations. The laboratory should review results, correct unsatisfactory performance, and record corrective actions.

Initial demonstration of laboratory capability (IDC) for Method 1601

Coliphage type	Sample size[1]	Target spike concentration (PFU per sample)	"Bulk" volume to be spiked	Bulk spike concentration (PFU per bulk volume)	Minimum number of positive samples out of 10
F^+	100-mL	1.3	1000 mL	13	5
Somatic	100-mL	1.5	1000 mL	15	5

[1] A 100-mL sample is required under the Ground Water Rule. However, for other purposes, this test may be used with a 1-L sample volume. Because IDC samples should be analyzed just like field samples, including sample volumes, the IDC analyses should be performed at the 1-L sample volume when the laboratory is evaluating 1-L samples. (The IDC procedure for 1-L samples is provided in the protocol to Method 1601, Table 1.)

MS and ODC sample spiking requirements for ongoing evaluation of Method 1601 performance

Coliphage type	Sample size[1]	Target spike concentration (PFU per sample)	Number of samples that must be spiked (≥1 must be positive)	"Bulk" volume to be spiked	Bulk spike concentration (PFU per bulk volume)
F^+	100-mL	1.3	3	300-mL	3.9
Somatic	100-mL	1.5	3	300-mL	4.5

[1] A 100-mL sample is required under the Ground Water Rule. However, for other purposes, this test may be used with a 1-L sample volume. Because ODC and MS samples should be analyzed just like field samples, including sample volumes, the ODC and MS analyses should be performed at the 1-L sample volume when the laboratory is evaluating 1-L samples. (The MS and ODC procedure for 1-L samples is provided in the protocol to Method 1601, Tables 2.)

5.6.2 EPA Method 1602: Male-specific (F^+) and Somatic Coliphage in Water by Single Layer Agar (SAL) Procedure

Method Summary: Method 1602 is a performance-based method for detecting or enumerating male-specific (F^+) and somatic coliphage in ground water and other waters. A 100-mL ground water sample is assayed by adding magnesium chloride and host bacteria (*E. coli* F_{amp} for F^+ coliphage and *E. coli* CN-13 for somatic coliphage), and then adding the sample/host bacteria mixture to 100 mL of double-strength molten Tryptic Soy Agar containing the appropriate antibiotic. The sample is thoroughly mixed and the total volume is poured into 5 to 10 plates (dependent on plate size). After an overnight incubation, any circular lysis zones (plaques) indicate the presence of coliphage.

5.6.2.1. Media--Please refer to Section 5.6.1 for antibiotic stocks, 10X Tryptic Soy Broth (TSB), 1.5% Tryptic Soy Agar (TSA), 0.7% TSA top agar tubes with appropriate antibiotics, and spot plates.

5.6.2.1.1 Double Strength Tryptic Soy Agar (2X TSA)—Medium may become darker after autoclaving but this should not affect media performance.

5.6.2.1.2 2X TSA with appropriate antibiotics—Keep molten at 45°-48°C in water bath until use. Agar must be used the day of preparation.

5.6.2.2 Coliphage stock—Please refer to Section 5.6.1.2 for coliphage stock.

5.6.2.3 Host bacteria stock cultures — Please refer to Section 5.6.1.3 for host bacteria stock cultures.

5.6.2.4 General QC

5.6.2.4.1 Initial precision and recovery (IPR). *The laboratory must demonstrate the ability to perform this method acceptably by performing an IPR test before analyzing any field samples.* Four reagent water

samples for each coliphage type are required for the IPR test. IPR samples must be spiked in bulk to yield a target spike concentration of 80 PFU per sample. IPR samples must be spiked with enumerated sewage filtrate or equivalent. The relative standard deviation of the recovery (RSD_r) and the average percent recovery ($\bar{x}$) based on all four sample results for each coliphage type should meet the acceptance criteria in the QC acceptance table below.

5.6.2.4.2 Method blanks. *The laboratory must analyze method blanks (reagent water sample containing no coliphage) to demonstrate freedom from contamination.* The laboratory must analyze one method blank with each analytical batch. For each coliphage type used, prepare and analyze a sterile reagent water sample containing no coliphage using the same procedure as used for analysis of the field or QC samples. An analytical batch is defined as all samples analyzed during a single day, up to a maximum of 20 samples (field samples and matrix spike samples) per coliphage type.

5.6.2.4.3 Matrix spikes (MS). *To assess method performance in a given matrix, the laboratory must analyze one set of MS samples for each coliphage type when samples are first received from a ground water source for which the laboratory has never before analyzed samples.* The MS analysis is performed on an additional (second) sample aliquot collected from the ground water source at the same time as the routine field sample. If the laboratory routinely analyzes samples from one or more ground water sources, one MS analysis must be performed per 20 field samples. For example, when a laboratory receives the first sample from a source, the laboratory must obtain a second aliquot of this sample to be used for the MS. When the laboratory receives the 20th sample from this site, a separate aliquot of this 20th sample must be collected and spiked. Compare the coliphage recovery with the corresponding limits in the QC Table below. If the recovery for coliphage falls outside its limit, method performance is unacceptable for that sample. If the results for the OPR sample associated with this batch of samples are within their respective control limits, a matrix interference may be causing poor recovery. If the results for the OPR are not within their control limits, method performance is unacceptable (see Section 5.6.2.4.4). The problem should be identified and corrected, and the matrix spike and associated field sample(s) should be qualified. The recovery should be maintained on a control chart and updated on a regular basis.

5.6.2.4.4 Ongoing precision and recovery (OPR). *The laboratory must, on an ongoing basis, demonstrate acceptable performance through analysis of an OPR sample.* For each coliphage type used, a reagent water sample is spiked with approximately 80 PFU. The OPR is analyzed exactly like a field sample. The laboratory must analyze one OPR sample for each analytical batch. An analytical batch is defined as all samples analyzed during a single day, up to a maximum of 20 samples (field samples and matrix spike samples) per coliphage type used. ***Please note: the OPR serves as the positive control for Method 1602.*** Compare the OPR percent recovery (R) with the corresponding limits for ongoing precision and recovery in the QC Table below. If R meets the acceptance criteria, system performance is acceptable and analysis of samples may continue. If R falls outside the range for recovery, method performance is unacceptable, and analysis of field samples must be stopped. Identify and correct the problem and demonstrate acceptable performance through successful analysis of another OPR test before continuing with the analysis of field samples.

5.6.2.4.5 Performance studies. The laboratory should periodically analyze an external QC sample, such as a performance testing sample, when available. The laboratory also should participate in available interlaboratory performance studies conducted by local, state, and federal agencies or commercial organizations. The laboratory should review results, correct unsatisfactory performance, and record corrective actions.

Quality control acceptance criteria for Method 1602

Performance test	Male-specific acceptance criteria	Somatic acceptance criteria
Initial precision and recovery (IPR)		
• Mean percent recovery	9% - 130%	86% - 177%
• Precision (as maximum relative standard deviation)	46%	23%
Ongoing precision and recovery (OPR) as percent recovery	4% - 135%	79% - 183%
Matrix spike (MS)		
• MS percent recovery	Detect - 120%	48% - 291%
Matrix spike, matrix spike duplicate (MS/MSD)		
• Mean percent recovery for MS/MSD	Detect - 120%	48% - 291%
• Precision (as maximum relative percent difference of MS/MSD)	57%	28%

6. Sample Collection, Handling, and Preservation

Paragraphs 6.1-6.5 are applicable to those laboratories that collect samples. However, all laboratories should make an effort to ensure proper sample collection; all laboratories are responsible for paragraph 6.6.

6.1 Sample Collector

The sample collector should be trained in aseptic sampling procedures and, if required, approved by the appropriate regulatory authority or its designated representative.

6.2 Sampling

6.2.1 (For TCR) Samples must be representative of the water distribution system. Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. Cold water taps should be used. The service line must be cleared before sampling by maintaining a steady water flow for at least two minutes (until a steady water temperature is achieved). At least 100 mL of sample must be collected, allowing at least a 1-inch air space to facilitate mixing of the sample by shaking. Immediately after collection, a sample information form should be completed (see paragraph 6.5). See Section 3.15.4 regarding sample dechlorination. If a sample bottle is filled too full to allow for proper mixing, do not pour off and discard a portion of the sample. Rather, pour the entire sample into a larger sterile container, mix properly, and proceed with the analysis.

6.2.2 (For SWTR) Source water samples must be representative of the source of supply, collected not too far from the point of intake, but at a reasonable distance from the bank or shore. The sample volume should be sufficient to perform all the tests required.

6.2.3 (For coliphage analysis under GWR) A 100-mL sample volume is required for the assay. Collection of an additional 100-mL water sample would allow for sample re-analysis, if necessary (e.g., if the positive or negative controls fail). To ensure sufficient sample volume, an additional 50-mL water sample should be collected.

6.2.4 (For *E. coli* and enterococci under GWR) A 100-mL sample volume is required for the assay.

6.3 Sample Icing

6.3.1 (Bacterial samples) Samplers are encouraged, but not required, to hold drinking water samples at <10°C during transit to the laboratory. Source water samples required by the Surface Water Treatment Rule (SWTR) must be held

at <10°C during transit (see *Standard Methods*, Section 9060B). Laboratories should reject samples that have been frozen.

6.3.2 (For coliphage analysis under GWR) Ship samples at <10°C using wet ice, Blue Ice®, or similar products to maintain temperature, and store at 1°-5°C. Samples should not be frozen.

QC **6.3.3** For SWTR samples and coliphage samples, sample temperature upon receipt should be recorded. A sample that has a temperature upon receipt of >10°C , whether iced or not, should be flagged unless the time since sample collection has been less than two hours.

6.4 Sample Holding/Travel Time

6.4.1 For the analysis of total coliforms in drinking water, the time between sample collection and the placement of sample in the incubator must not exceed 30 hours (per regulation at 40 CFR 141.21(f)(3)). All samples received in the laboratory should be analyzed on the day of receipt. If the laboratory receives the sample late in the day, the sample may be refrigerated overnight as long as analysis begins within 30 hours of sample collection.

6.4.2 The time from sample collection to placement of the sample in the incubator for total coliforms and fecal coliforms in surface water sources, and heterotrophic bacteria in drinking water, must not exceed eight hours (per regulation at 40 CFR 141.74(a)(1)).

6.4.3 (For coliphage analysis) The time between sample collection and the placement of sample in the incubator must not exceed 48 hours. The time from sewage sample collection to analysis of QC spiking suspensions may not exceed 24 hours, unless re-titered and titer has not decreased by more than 50%. If titer has not decreased by more than 50%, the sample can be stored for up to 72 hours.

6.4.4 (For *E. coli* and enterococci under GWR) The time between sample collection and the placement of sample in the incubator must not exceed 30 hours.

6.5 Sample Information Form

After collection, the sampler should enter on a sample information form, in indelible ink, the following information:

- Name of system (public water system site identification number, if available)
- Sample identification (if any)
- Sample site location
- Sample type (e.g., routine distribution system sample, repeat sample, raw or process water, other special purpose sample)
- Date and time of collection
- Analysis requested
- Disinfectant residual
- Name of sampler
- Any remarks

6.6 Chain-of-Custody

Sample collectors and laboratories must follow applicable State regulations pertaining to chain-of-custody. An example of such a plan is provided in Appendix A.

7. Quality Assurance

7.1 A written QA plan should be prepared and followed (see Chapter III). The QA plan should be available for inspection by the certification officer. As specified by the QA plan, a laboratory that performs its own calibration of equipment or supplies (e.g., thermometers) should have a Standard Operating Procedure available for review. If a laboratory wishes to perform additional QA beyond those in this manual, the laboratory may refer to *Standard Methods*, Section 9020, *Quality Assurance (Quality Assurance/Quality Control*, in 20th ed.).

7.2 States are encouraged to establish proficiency testing (PT) as part of their drinking water certification program for microbiology. A laboratory should successfully analyze at least one set of PT samples once every 12 months, for each method for which it is certified.

For methods used to test the presence or absence of an organism in a sample, each PT set should contain ten samples, all shipped at the same time in either a lyophilized, dehydrated, or aqueous state. The set should include samples, in various combinations, that contain total coliforms, fecal coliforms, *E. coli*, non-coliforms, and at least one blank. Each set should be used only with a single analytical method. To be acceptable, a laboratory should correctly analyze a minimum of nine of the ten samples, with no false-negative result (i.e., a single false-positive result may be acceptable).

Because even methods based upon the same principle (e.g., membrane filtration) may be quite dissimilar, a Region or State should consider certifying a laboratory only for those specific methods for which the laboratory has successfully analyzed a set of PT samples. The Table below reflects this approach, and identifies the few methods that may be sufficiently similar to allow a laboratory to be certified for more than one method upon successful completion of a single set of PT samples.

Method Category	Specific Method[1]
Fermentation broth method	LTB or P-A broth, followed by BGLB and either EC or EC-MUG
Fermentation broth method	A-1 broth (fecal coliform, SWTR only)
Enzyme substrate method	Colilert or Colilert 18
Enzyme substrate method	Colisure
Enzyme substrate method	Readycult or Fluorocult LMX
Enzyme substrate method	E*Colite
Enzyme substrate method	Colitag
Membrane filter method	M-Endo or LES Endo, followed by BGLB and either EC, EC-MUG, or NA-MUG
Membrane filter method	MI Medium
Membrane filter method	Coliscan
Membrane filter method	m-ColiBlue24
Membrane filter method	Chromocult
Membrane filter method	mFC agar (fecal coliform, SWTR only)
HPC method	PCA
HPC method	SimPlate

[1] Separate set of proficiency test samples recommended for each cell. A single set of PT samples would cover every method within the same cell.

8. Records and Data Reporting

8.1 Legal Defensibility: Compliance monitoring data should be made legally defensible by keeping thorough and accurate records. The QA plan and/or SOPs should describe the policies and procedures used by the facility for record retention and storage. If samples are expected to become part of a legal action, chain-of-custody procedures should be used (See Appendix A).

8.2 Maintenance of Records: Public water systems are required to maintain records of microbiological analyses of compliance samples for five years (40 CFR 141.33). The laboratory should maintain easily accessible records for five years or until the next certification data audit is complete, whichever is longer. A change in ownership, merger, or closure of a laboratory does not cancel this requirement. The client water system should be notified before disposing of records so they may request copies if needed. This includes all raw data, calculations, and quality control data. These data files may be either hard copy, microfiche or electronic. Electronic data should always be backed up by protected tape or disk or hard

copy. If the laboratory changes its computer hardware or software, it should make provisions for transferring old data to the new system so that it remains retrievable within the time frames specified above. Data which is expected to become part of a legal action will probably need to be maintained for a longer period of time. Check with your legal counsel. See *Good Automated Laboratory Practices*, EPA 2185, Office of Information Management, Research Triangle Park, NC 27711, 8/10/95.

8.3 Sampling Records: Data should be recorded in ink with any changes lined through such that original entry is visible. Changes should be initialed and dated. The following information should be readily available in a summary or other record(s):

8.3.1 Sample information form, from 6.5 above
8.3.2 Date and time of sample receipt by the laboratory
8.3.3 Name of laboratory person receiving the sample
8.3.4 Any deficiency in the condition of the sample. A sample should be invalidated for the following reasons:
- Time between sample collection and receipt by laboratory has been exceeded
- Presence of disinfectant in sample is noticed (e.g., odor)
- Evidence of freezing
- Use of a container not approved by the laboratory for the purpose intended
- Insufficient sample volume (e.g., <100 mL)
- Presence of interfering contaminant, if noticed (e.g., hydrocarbons, cleansers, heavy metals, etc.)
- Sample temperature exceeds the maximum allowable

8.4 *Analytical Records*: Data should be recorded in ink with any changes lined through such that original entry is visible. Changes should be initialed and dated. The following information should be readily available in a summary or other record(s):

8.4.1 Laboratory sample identification
8.4.2 Date and time analysis begins
8.4.3 Laboratory and a signature or initials of person(s) performing analysis
8.4.4 Analytical technique or method used
8.4.5 All items marked QC
8.4.6 Results of analyses

8.5 Preventive Maintenance

Laboratories should maintain preventive maintenance and repair activities records for all instruments and equipment (including pH meters, analytical balances, incubators, refrigerators, autoclaves, and water baths). Records should be kept for five years in a manner that allows for easy inspection.

9. Action Response to Laboratory Results

9.1 Testing Total Coliform-Positive Cultures

For the Total Coliform Rule, laboratories must test all total coliform-positive cultures for the presence of either fecal coliforms or *E. coli*.

9.2 Notification of Positive Results

9.2.1 For the Total Coliform Rule, laboratories must promptly notify the proper authority of a positive total coliform, fecal coliform, or *E. coli* result, so that appropriate follow-up actions (e.g., collection of repeat samples) can be conducted (see 40 CFR 141.21(b) and (e), and 141.31, etc.).

9.2.2 If any sample is fecal coliform- or *E. coli*-positive, "the system must notify the State by the end of the day when the system is notified of the test result, unless the system is notified of the result after the State office is closed, in which case the system must notify the State before the end of the next business day." (40 CFR 141.21(e)(1)).

9.2.3 A total coliform-positive result is based on the confirmed phase if the Multiple Tube Fermentation Technique or Presence-Absence (P-A) Coliform Test is used, or the verified test for the Membrane Filter Technique if M-Endo medium or LES Endo agar is used. No requirement exists to confirm a total coliform-positive result using Colilert, Colisure, MI agar, E*Colite, MI agar, m-ColiBlue24, Chromocult, Readycult/Fluorocult, Coliscan, or Colitag test. Also, no requirement exists to confirm a positive fecal coliform or *E. coli* test. In those rare cases where a presumptive total coliform-positive culture does not confirm/verify as such, but is found to be fecal coliform or *E. coli*-positive, the sample is considered total coliform-positive and fecal coliform/*E. coli*-positive.

9.3 Notification of Total Coliform Interference

For the Total Coliform Rule, the laboratory must promptly notify the proper authority (usually the water system) when results indicate that non-coliforms may have interfered with the total coliform analysis, as described in 40 CFR 141.21(c)(2).

Example Checklists for On-site Evaluation of Laboratories Analyzing Drinking Water

MICRO CHECKLISTS

General Audit Information

Laboratory:
Mailing Address (mailing address of owner if different):
Street
City, State, Zip code
Audit Location (if different):
Telephone:
Fax:
E-mail:
Other:
Audit Organization:
Auditors/Signatures:
Audit Date(s):

Laboratory Personnel

Position/Title	Name	Education Level Degree/Major	Specialized Training	Present Specialty	Experience, including # yrs at current position
Laboratory Supervisor					
Laboratory Consultant					
Primary Analyst					
Analyst 2					
Analyst 3					
Analyst 4					
Others					

Element	Number	Yes	No	NA	Comments
1. PERSONNEL					
Supervisor/Consultant	**1.1**				
Does the supervisor of the microbiology laboratory have a bachelor's degree in microbiology, biology, or equivalent?					
Has a supervisor with a degree in a subject other than those listed above had at least one college-level microbiology laboratory course in which environmental microbiology was covered?					
In addition, has the supervisor had a minimum of two weeks training at a Federal or State agency or academic institution in microbiological analysis of drinking water or 80 hours of on-the-job training in water microbiology at a certified laboratory, or other training acceptable to the State or EPA?					
If a supervisor is not available, and a waiver has not been granted as per Section 1.3, is a consultant with the same qualifications substituted?					
Can the laboratory document that the consultant is acceptable to the State, and present on-site frequently enough to satisfactorily perform a supervisor's duties?					
Can the laboratory supervisor demonstrate that all laboratory personnel have the ability to satisfactorily perform the analyses to which they are assigned?					
Can the laboratory supervisor demonstrate that all data reported by the laboratory meets the required quality assurance and regulatory criteria?					
Analyst (or equivalent job title)	**1.2**				
Does the analyst have at least a high school education, a minimum of three months bench experience in water, milk or food microbiology, training in microbiological analysis of drinking water acceptable to the State (or EPA), and a minimum of 30 days on-the-job training under an experienced analyst?					
Has the analyst demonstrated acceptable results on unknown samples before analyzing compliance samples?					
Waiver of Academic Training	**1.3**				
Has the certification authority waived the need for the above specified academic training for highly experienced analysts in this laboratory?					
Has the certification authority waived the need for the above specified training for supervisors of laboratories associated with drinking water systems that only analyze samples from that system?					

Element	Number	Yes	No	NA	Comments
If yes to either of the above, does the laboratory have a copy of that written and signed waiver available for inspection?					
Personnel Records	**1.4**				
Does the laboratory maintain personnel records on laboratory analysts that include academic background, specialized training courses completed, and types of microbiological analyses conducted?					
2. LABORATORY FACILITIES					
Does the laboratory have facilities that are clean and temperature and humidity controlled, and with adequate lighting at the bench tops?					
Does the laboratory maintain effective separation of incompatible testing areas?					
Does the laboratory control access where appropriate, and minimize traffic flow through the work areas?					
Does the laboratory ensure that contamination does not adversely affect data quality?					
Does the laboratory have bench tops and floors that are easily cleaned and disinfected?					
Does the laboratory have sufficient space for processing samples; storage space for media, glassware, and portable equipment; floor space for stationary equipment; and areas for cleaning glassware and sterilizing materials?					
Does the laboratory have provisions for disposal of microbiological wastes?					
3. LABORATORY EQUIPMENT AND SUPPLIES					
Does the laboratory have the equipment and supplies needed to perform the approved methods for which certification has been requested?					
pH meter	**3.1**				
Are accuracy and scale graduations within ±0.1 units?	**3.1.1**				
Are pH buffer aliquots used only once?	**3.1.2**				
Are electrodes maintained according to the manufacturer's recommendations?	**3.1.3**				
QC Are pH meters standardized before each use period with pH 7.0 and either 4.0 or 10.0 standard buffers, whichever covers the desired pH of the media or reagent?	**3.1.4**				

Element	Number	Yes	No	NA	Comments
QC Are both the date and buffers used recorded in a logbook along with the analyst's initials?					
QC Is the pH slope recorded monthly, after calibration?	3.1.5				
QC If the pH meter does not have a feature to automatically calculate the slope, but can provide in the pH in millivolts, is the formula in Section 3.1.5.1 used to calculate the slope?	3.1.5.1				
QC If the slope is below 95% or above 105%, are the manufacturer's instructions followed for meter or electrode maintenance and general cleaning?	3.1.5.2				
QC Are commercial pH buffer solutions dated when received and when opened?	3.1.6				
QC Are pH buffer solutions discarded by the expiration date?					
Balance (top loader or pan)	3.2				
Does the balance have a readability of 0.1 g?	3.2.1				
Does the balance have a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less?	3.2.2				
QC Are the balances calibrated monthly using ASTM Class 1, 2, or 3 weights (minimum 3 traceable weights which bracket laboratory weighing needs, with a readability of 0.1 g)?	3.2.3				
QC Are non-reference weights calibrated every six months with reference weights?					
QC Are calibrations recorded in a logbook with the initials of the individual performing the calibration?					
QC Are correction values on file and used?					
QC Are reference weights re-certified every five years?					
QC Are damaged or corroded weights replaced?					
QC Are service contracts or internal maintenance protocols and maintenance records available?	3.2.4				
QC Is maintenance, calibration, and cleaning conducted at least annually by a qualified independent technician, unless the need is modified or waived by the certification officer?					
Temperature Monitoring Device	3.3				
Are glass, dial, or electronic thermometers graduated in 0.5°C increments (0.2°C increments for tests which are incubated at 44.5°C) or less, except as noted for hot air ovens (Section 3.6.1) and refrigerators (Section 3.9.1)?	3.3.1				

Element	Number	Yes	No	NA	Comments
Does observation of glass thermometers indicate no separation in fluid columns?					
Are only dial thermometers which can be adjusted used?					
QC Are glass and electronic thermometers calibrated annually and dial thermometers quarterly at the temperature used, against a NIST-traceable reference thermometer or one that meets the requirements of NBS Monograph SP 250-23?	3.3.2				
QC Are both the calibration factor and calibration date indicated on the thermometer?					
QC Is the following calibration information recorded in a QC record book? – Serial number of the laboratory thermometer – Serial number of the NIST-traceable thermometer (or other reference thermometer) – Temperature of the laboratory thermometer – Temperature of the NIST-traceable thermometer (or other reference thermometer) – Correction (or calibration) factor – Date of check – Analyst's initials					
QC Is the thermometer discarded if it differs by more than 1 °C from the reference thermometer?	3.3.3				
QC Are reference thermometers recalibrated at least every five years?					
QC Is reference thermometer calibration documentation maintained?					
QC Are continuous recording devices used to monitor incubator temperature recalibrated at least annually, using a reference thermometer that meets the specifications noted in Section 3.3.2?	3.3.4				
Incubator Unit	3.4				
Do incubator units have an internal temperature monitoring device and maintain a temperature specified by the method used, usually 35°±0.5°C and 44.5°±0.2°C?	3.4.1				
For non-portable incubators, are thermometers placed on top and bottom shelves of the use area and immersed in liquid as directed by the manufacturer (except for electronic thermometers)?					
When aluminum block incubators are used, do culture dishes and tubes fit snugly?					

Element	Number	Yes	No	NA	Comments
QC Is the calibration-corrected temperature recorded for each thermometer being used at least twice per day during each day the incubator is in use?	3.4.2				
QC Are these readings separated by at least four hours?					
QC Does the documentation include the date and time of reading, temperature, and technician's initials?					
If a circulating water bath is used, is it equipped with a gable cover to ensure an incubation temperature of 44.5°±0.2°C?					
Autoclave	3.5				
Does the autoclave have an internal heat source, a temperature gauge with a sensor on the exhaust, a pressure gauge, and an operational safety valve?	3.5.1				
Can the autoclave maintain a sterilization temperature during the sterilizing cycle and complete an entire cycle within 45 minutes when a 12-15 minute sterilization period is used?					
Does the autoclave depressurize slowly enough to ensure that media will not boil over and bubbles will not form in inverted tubes?					
QC Is the following information recorded each time the autoclave is used? – Date – Contents – Sterilization time and temperature – Total time in the autoclave – Analyst's initials	3.5.3				
QC Are copies of the service contracts or internal maintenance protocols and maintenance records kept?					
QC Is maintenance conducted at least annually?					
QC Is a record of the most recent service performed on file and available for inspection?					
QC Is a maximum-temperature-registering thermometer, electronic temperature readout device, or continuous recording device used each autoclave cycle to ensure that the proper temperature was reached?	3.5.4				
QC Is the temperature recorded?					
QC Is overcrowding avoided?					
QC Are spore strips or spore ampules used monthly as bioindicators to confirm sterilization?					

Element	Number	Yes	No	NA	Comments
QC Are automatic timing mechanisms checked quarterly with a stopwatch or other accurate timepiece or time signal, and the results recorded and initialed?	3.5.5				
Are autoclave door seals clean and free of caramelized media?	3.5.6				
Are autoclave drain screens cleaned frequently and debris removed?					
Hot Air Oven	3.6				
Does the oven maintain a stable sterilization temperature of 170°-180°C for at least two hours?	3.6.1				
Is overcrowding avoided?					
Is the oven thermometer graduated in 10°C increments or less, with the bulb placed in sand during use?					
QC Is the following information recorded for each cycle? – Date – Contents – Sterilization time and temperature – Analyst's initials	3.6.2				
QC Are spore strips used monthly to confirm sterilization?	3.6.3				
Colony Counter	3.7				
Is a dark field colony counter used to count Heterotrophic Plate Count colonies?					
Conductivity Meter	3.8				
Are meters suitable for checking laboratory reagent-grade water and readable in units of either micromhos/cm or microsiemens/cm?	3.8.1				
QC Is the meter calibrated at least monthly, following the manufacturer's recommendations and using an appropriate certified and traceable low-level standard?	3.8.2				
QC If the meter cannot be calibrated as noted above, is the cell constant determined at monthly intervals using a method in *Standard Methods*, Section 2510?					
Is an in-line unit that cannot be calibrated used to check reagent-grade water?	3.8.3				
Refrigerator	3.9				
Does the refrigerator maintain a temperature of 1°-5°C?	3.9.1				
Is the refrigerator thermometer graduated in at least 1°C increments and the thermometer bulb immersed in liquid?					

Element	Number	Yes	No	NA	Comments
QC On days the refrigerator is in use, and the laboratory is staffed, is the calibrated-corrected temperature recorded at least once per day?	3.9.2				
Inoculating Equipment	3.10				
Are sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipet tips used?					
Are wood applicator sticks, if used, sterilized by dry heat?					
Are metal inoculating loops and/or needles made of nickel alloy or platinum?					
Membrane Filtration (MF) Equipment	3.11				
Are MF units made of stainless steel, glass, porcelain, or autoclavable plastic?	3.11.1				
Are they scratched, corroded, or leaking?					
QC If graduations on clear or plastic funnels are used to measure sample volume, is their accuracy checked with a Class B graduated cylinder or better (or other Class B glassware) and a record of this calibration check retained?	3.11.2				
Is a 10x to 15x stereo microscope with a fluorescent light source used to count sheen colonies?	3.11.3				
Are the membrane filters approved by the manufacturer for total coliform water analysis?	3.11.4				
Are membrane filters to be used cellulose ester, white, gridmarked, 47 mm diameter, and 0.45 µm pore size?					
If alternate pore sizes are used, does the manufacturer provide performance data equal to or better than the 0.45 µm pore size?					
Are membrane filters and pads purchased presterilized or autoclaved for 10 minutes at 121°C before use?					
QC Is the lot number for membrane filters and the date received recorded?	3.11.5				
QC Are the membranes checked to see that they are not brittle or distorted?					
QC Are the manufacturer's specification/certification sheets available?					
Are the forceps blunt and smooth-tipped without corrugations on the inner sides of the tips?	3.11.6				
Culture Dishes (loose or tight lids)	3.12				

Element	Number	Yes	No	NA	Comments
Are presterilized plastic or sterilizable glass culture dishes used?	3.12.1				
Is the sterility of the glass culture dishes maintained by placement in stainless steel or aluminum canisters or a wrap of heavy aluminum foil or char-resistant paper?					
Are loose-lid petri dishes incubated in a tight-fitting container with a moistened paper towel?	3.12.2				
Are opened packs of disposable culture dishes resealed between use periods?	3.12.3				
For membrane filter methods, are culture dishes of an appropriate size to allow the transfer of a single membrane per plate?	3.12.4				
Pipets	3.13				
Are glass pipets sterilized and maintained in stainless steel or aluminum canisters or wrapped individually in char-resistant paper or aluminum foil?	3.13.1				
Do pipets have legible markings and are they not chipped or etched?	3.13.2				
Are opened packs of disposable sterile pipets resealed between use periods?	3.13.3				
Are pipets delivering volumes of 10 mL or less accurate to within a 2.5% tolerance?	3.13.4				
Are calibrated micropipetters used with sterile tips?	3.13.5				
Are micropipetters calibrated annually and adjusted or replaced if the precision or accuracy is greater than 2.5%?					
Glassware and Plasticware	3.14				
Is the glassware made of borosilicate glass, or other corrosion-resistant glass, and free of chips and cracks?	3.14.1				
Are markings on graduated cylinders and pipets legible?					
Are plastic items clear and nontoxic to microorganisms?					
QC Are the graduated cylinders used for measurement of sample volumes, or other precalibrated containers that have clearly marked volumes used in lieu of graduated cylinders, accurate to within a 2.5% tolerance?	3.14.2				
Are culture tubes and containers containing fermentation medium of sufficient size to contain medium plus sample without being more than three quarters full?	3.14.3				
Are tube closures made of stainless steel, plastic, aluminum, or screw caps with nontoxic liners?	3.14.4				

Element	Number	Yes	No	NA	Comments
Are cotton or foam plugs used?					
Sample Containers	**3.15**				
Are sample containers wide-mouth plastic or non-corrosive glass bottles with non-leaking ground glass stoppers or caps with nontoxic liners, sterile plastic bags containing sodium thiosulfate, or other appropriate sample containers?	**3.15.1**				
Is sample container capacity at least 120 mL (4 oz) to allow at least a 1-inch head space?					
Are glass stoppers covered with aluminum foil or char-resistant paper for sterilization?	**3.15.2**				
Are unsterilized glass and plastic bottles sterilized by autoclaving or, alternatively, by dry oven for glass bottles?	**3.15.3**				
Are empty containers moistened with several drops of water before autoclaving to prevent an "air lock" sterilization failure?					
If chlorinated water is to be analyzed, is sufficient sodium thiosulfate added to the sample bottles before sterilization to neutralize any residual chlorine in the water sample?	**3.15.4**				
Ultraviolet Lamp (if used)	**3.16**				
Is the germicidal unit disconnected monthly and the lamp cleaned by wiping with soft cloth moistened with ethanol?	**3.16.1**				
Is the longwave unit used for fluorometric tests kept clean?					
QC Is the germicidal unit tested quarterly with a UV light meter or agar spread plate?	**3.16.2**				
QC Is the lamp replaced if it emits less than 70% of its initial output or if an agar spread plate containing 200 to 250 microorganisms, exposed to the UV light for two minutes, does not show a count reduction of 99%?					
Spectrophotometer or colorimeter (if used)	**3.17**				
Are wavelengths in the visible range?	**3.17.1**				
QC Is a calibration standard and a method-specific blank analyzed every day the instrument is used, prior to sample analysis?	**3.17.2**				
QC Is this calibration standard obtained from an outside source?					
QC Does the calibration standard give a reading in the desired absorbance range?					

Element	Number	Yes	No	NA	Comments
4. GENERAL LABORATORY PRACTICES					
Are laboratory personnel aware of general and customary safety practices for laboratories?					
Does the laboratory have a safety plan available?					
Does the laboratory keep a copy, and follow the personal protection guidelines, of any material safety data sheet accompanying the receipt of a toxic material?					
Sterilization Procedures	**4.1**				
Does the laboratory follow the minimum times for autoclaving the materials listed below at 121°C? – Membrane filters and pads 10 min – Carbohydrate containing media 12-15 min[1] – Contaminated test materials 30 min[2] – Membrane filter assemblies 15 min – Sample collection containers 15 min – Individual glassware 15 min – Dilution water blank 15 min – Rinse water (0.5 - 1 L) 15-30 min[2] [1] except where otherwise specified by the manufacturer [2] time depends upon water volume per container and autoclave load	**4.1.1**				
Are autoclaved membrane filters and pads and all media removed immediately after completion of the sterilization cycle?	**4.1.2**				
Is membrane filter equipment autoclaved before the beginning of a filtration series?	**4.1.3**				
If a UV light (254 nm) is used to sanitize equipment after initial autoclaving for sterilization, are all supplies presterilized?	**4.1.4**				
Sample Containers	**4.2**				
QC Is at least one sample container selected at random from each batch of sterile sample bottles, or other containers (or lot of commercially available sample containers), and the sterility confirmed by adding 25 mL of a sterile non-selective broth, incubating at 35°±0.5°C, and checking for growth after 24 and 48 hours?					
QC Are these results recorded?					
QC If growth is detected, is the entire batch resterilized?					
Reagent-Grade Water	**4.3**				

Element	Number	Yes	No	NA	Comments
Does the laboratory only use satisfactorily tested reagent water from stills or deionization units to prepare media, reagents, and dilution/rinse water for performing microbial analyses?	4.3.1				
QC Is the quality of reagent water tested and does it meet the following criteria? Conductivity >0.5 megohms resistance or <2 micromhos/cm (microsiemens/cm) at 25°C Monthly* Pb, Cd, Cr, Cu, Ni, Zn Not greater than 0.05 mg/L per contaminant. Collectively no greater than 0.1 mg/L Annually Total chlorine residual* <0.1 mg/L Monthly Heterotrophic plate count* <500/mL CFU/mL* Monthly Bacteriological quality of reagent water* Ratio of growth rate 0.8 to 3.0 Annually *See Section 4.3.2 for footnotes	4.3.2				
Dilution/Rinse Water	4.4				
Is stock buffer solution or peptone water prepared as specified in *Standard Methods*, Section 9050C?	4.4.1				
Are stock buffers autoclaved or filter-sterilized?	4.4.2				
Are these containers labeled, dated, and refrigerated?					
Are stored stock buffers free from turbidity?					
QC Is each batch (or lot, if commercially prepared) 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, and checking for growth after 24 hours and 48 hours?	4.4.3				
QC Are these results recorded?					
QC Is the batch/lot discarded if growth is detected?					
Glassware Washing	4.5				
Is distilled or deionized water used for the final rinse?	4.5.1				
Is laboratory glassware washed with a detergent designed for laboratory use?	4.5.2				

Element	Number	Yes	No	NA	Comments
QC Is the glassware inhibitory residue test performed before the initial use of a washing compound and whenever a different formulation, or washing procedure is used?	4.5.3				
QC Are these results recorded?					
QC Is each batch of dry glassware used for microbial analysis spot-checked for pH reaction using 0.04% bromthymol blue (or equivalent pH indicator) and the color reaction recorded?	4.5.4				
5. ANALYTICAL METHODOLOGY					
General	5.1				
For compliance samples, does the laboratory use only the analytical methodologies specified in the Total Coliform Rule (TCR), the Surface Water Treatment Rule (SWTR), and the Groundwater Rule (GWR)?	5.1.1				
Is the laboratory certified for all analytical methods it uses for compliance purposes?	5.1.2				
At a minimum, is the laboratory certified for one total coliform method and one fecal coliform or *E. coli* method?					
Is the laboratory certified for a second total coliform method if one method cannot be used for some drinking waters?					
For a laboratory that enumerates heterotrophic bacteria for compliance with the SWTR, is the laboratory certified for either the Pour Plate Method or the SimPlate method for heterotrophic bacteria?					
Are water samples shaken vigorously at least 25 times before analyzing?	5.1.3				
QC If dilution buffer is used, does the laboratory check the buffer volume in one dilution bottle of each batch or lot?	5.1.4				
QC For a 90-mL or 99-mL volume, is the tolerance ±2 mL?					
Does the laboratory analyze a 100-mL sample volume for total coliforms in drinking water?	5.1.5				
Media (or defined substrate)	5.1.6				
Are dehydrated media stored in a cool dry location and discarded by the manufacturer's expiration date?	5.1.6.1				
Is caked or discolored dehydrated media discarded?					

Element	Number	Yes	No	NA	Comments
QC For media prepared in the laboratory is the following information recorded? – Date of preparation – Type of medium – Lot number – Sterilization time and temperature – Final pH (after sterilization) – Technician's initials	5.1.6.2				
QC For media prepared commercially is the following recorded for each lot? – Date received – Type of medium – Lot number – pH verification	5.1.6.3				
QC Are media prepared commercially discarded by manufacturer's expiration date?					
QC Is each new lot of dehydrated or prepared commercial medium and each batch of laboratory-prepared medium checked before use for sterility and with positive and negative culture controls?	5.1.6.4				
QC Are these results recorded?					
QC For laboratories using commercially prepared media with manufacturer shelf-lives of greater than 90 days, are positive and negative controls run each quarter, in addition to that noted above?					
QC Are these results recorded?					
QC For control organisms, are stock cultures periodically checked for purity and the results recorded, or are commercially available disks impregnated with the organism used?					
If prepared medium is stored after sterilization, is it maintained in the dark as follows? – poured plates 1°-5°C 2 weeks – broth in containers with loose-fitting closures 1°-30°C 2 weeks – broth in tightly closed containers 1°-30°C 3 months	5.1.6.5				
QC Does the laboratory perform parallel testing between a newly approved test and another EPA-approved procedure for enumerating total coliforms for at least several months and/or several seasons to assess the effectiveness of the new test for the wide variety of water types submitted for analysis? Recommended.	5.1.7				
Does the laboratory perform the approved methods listed in this section for the TCR, SWTR, and/or GWR?	5.1.8				

Element	Number	Yes	No	NA	Comments
Fermentation broth methods	**5.2**				
General	**5.2.1**				
Is the water level of the water bath above the upper level of the medium in the culture tubes?	**5.2.1.1**				
If a Dri-bath incubator is used, is the specified temperature requirement maintained in all tube locations used?	**5.2.1.2**				
Multiple Tube Fermentation Technique (for detecting total coliforms in drinking water and enumerating total coliforms in source water)	**5.2.2**				
For drinking water samples, is the total sample volume of 100 mL used for each test?	**5.2.2.1**				
For source water samples, are at least 3 series of five tubes each with appropriate sample dilutions used?	**5.2.2.2**				
Media	**5.2.2.3**				
Is lauryl tryptose broth (LTB) used in the presumptive test and 2% brilliant green lactose bile broth (BGLBB) in the confirmed test?	**5.2.2.3.1**				
If lactose broth (LB) is used in lieu of LTB, has the laboratory conducted at least 25 parallel tests between this medium and LTB using the waters normally tested?					
Has this comparison demonstrated that the false-positive rate and false-negative rate for total coliforms, using LB, is less than 10%?					
Is this comparison information documented and the records retained?					
Is the final pH of LTB medium 6.8 ± 0.2?					
Is the final pH of 2% BGLBB 7.2 ± 0.2?					
Is the test medium concentration adjusted to compensate for the sample volume so that the resulting medium after sample addition is single strength?	**5.2.2.3.2**				
If a single 100-mL sample volume is used, is the inverted vial replaced with an acid indicator (bromcresol purple)?					
Is the medium autoclaved at 121°C for 12-15 minutes?					
Is the sterile medium in tubes examined to ensure that the inverted vials, if used, are free of air bubbles and are at least one-half to two-thirds covered after the water sample is added?	**5.2.2.3.3**				
Is the inoculated medium incubated at 35°±0.5°C for 24±2 hours?	**5.2.2.4**				
If no gas or acid detected, is the inoculated medium incubated for another 24 hours for a total incubation time of 48±3 hours?					

Element	Number	Yes	No	NA	Comments
Is each 24- and 48-hour tube that has growth or is gas-positive or acid-positive confirmed using 2% BGLBB?	5.2.2.5				
For drinking water samples, is each total coliform-positive sample tested for the presence of either fecal coliforms or *E. coli*?	5.2.2.6				
Invalidation of total coliform-negative samples	5.2.2.7				
For drinking water samples, are all samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, invalidated?	5.2.2.7.1				
Does the laboratory then collect, or request that the system collect, another sample within 24 hours from the same location as the original invalidated sample?					
Although not required before invalidation, does the laboratory perform a confirmed test and/or a fecal coliform/*E. coli* test on the total coliform-negative culture to check for coliform suppression?					
And if the confirmed test is total coliform-positive or fecal coliform/*E. coli*-positive, does the laboratory report the sample as such?					
If the follow-up test is total coliform-negative, does the laboratory invalidate the sample?					
For source water samples, are all samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production in LTB or LB invalidated?	5.2.2.7.2				
Does the laboratory collect, or request that the system collect, another sample from the same location as the original invalidated sample?					
Although not required before invalidation, does the laboratory perform a confirmed test on the total coliform-negative culture and, if the confirmed test is total coliform-positive, is the MPN reported?					
If the confirmed test is total coliform-negative, is the sample invalidated?					
Presence-Absence (P-A) Coliform Test (for detecting total coliforms in drinking water)	5.2.3				
Medium	5.2.3.1				
When six-times formulation strength medium is used, is it filter-sterilized rather than autoclaved?	5.2.3.1.1				
Is the medium autoclaved for 12 minutes at 121°C?	5.2.3.1.2				
Is the total time in the autoclave less than 30 minutes?					

Element	Number	Yes	No	NA	Comments
Are the bottles placed in the autoclave with space between them?					
Is the final pH of the medium 6.8±0.2?					
If the prepared medium is stored, is it maintained in a culture bottle at 1°-30°C in the dark for no more than 3 months?	5.2.3.1.3				
Is the stored medium discarded if evaporation exceeds 10% of original volume?					
Is a 100-mL sample inoculated into a P-A culture bottle?	5.2.3.2				
Is the sample/medium incubated at 35°±0.5°C and observed for yellow color (acid) after 24 and 48 hours?	5.2.3.3				
Are yellow cultures confirmed in BGLBB and a fecal coliform/*E. coli* test conducted?	5.2.3.4				
Are all samples which produce a non-yellow turbid culture in P-A medium invalidated?	5.2.3.5				
Does the laboratory collect, or request that the system collect, another sample from the same location as the original invalidated sample?					
Although not required before invalidation, does the laboratory perform a confirmed test on the total coliform-negative culture and/or a fecal coliform/*E. coli* test and, if the confirmed test is total coliform-positive, is the sample reported as such?					
If the confirmed test is total coliform-negative, is the sample invalidated?					
Fecal Coliform Test (using EC Medium for fecal coliforms in drinking or source water, or A-1 Medium for fecal coliforms in source water only)	5.2.4				
EC Medium	5.2.4.1				
Is EC medium used to test a total coliform-positive culture for fecal coliforms under the Total Coliform Rule?	5.2.4.1.1				
Is each total coliform-positive culture transferred from a presumptive tube/bottle, or each presumptive total coliform-positive colony (unless a cotton swab is used), to at least one tube containing EC Medium with an inverted vial?					
Is EC medium used to enumerate fecal coliforms in source water, in accordance with the SWTR?	5.2.4.1.2				
When conducting a MTF test, are three sample volumes of source water with five or ten tubes/sample volume used?					

Element	Number	Yes	No	NA	Comments
Is a culture from each total coliform-positive tube transferred to a tube containing EC Medium with an inverted vial?					
Is EC Medium autoclaved at 121°C for 12-15 minutes?	5.2.4.1.3				
Is the final pH of EC medium 6.9±0.2?					
Are the inverted vials examined to ensure that they are free of air bubbles and at least one-half to two-thirds covered after the sample is added?	5.2.4.1.4				
Is EC Medium incubated at 44.5°±0.2°C for 24±2 hours?	5.2.4.1.5				
Is any amount of gas detected in the inverted vial of a tube that has turbid growth considered a fecal coliform-positive test, regardless of the result of any subsequent test on that culture?	5.2.4.1.6				
A-1 Medium	5.2.4.2				
If A-1 Medium is used, is it used to enumerate only fecal coliforms in source water, in accordance with SWTR, and not for drinking water samples?	5.2.4.2.1				
Are three sample volumes of source water used in a five- or ten-tube/sample volume format?					
Is A-1 Medium autoclaved at 121°C for 10 minutes?	5.2.4.2.2				
For A-1 Medium, is the final pH 6.9±0.1?					
Are inverted tubes examined to ensure that they are free of air bubbles?	5.2.4.2.3				
Is A-1 Medium incubated at 35°±0.5°C for three hours, and then incubated at 44.5°±0.2 °C for 21±2 hours?	5.2.4.2.4				
Are loose-cap tubes stored in the dark at room temperature for no longer than two weeks, or in tightly closed screw-cap tubes in the dark at <30°C for no longer than three months?	5.2.4.2.5				
Is any amount of gas detected in the inverted vial of a tube with turbid growth considered a fecal coliform-positive test?	5.2.4.3				
Azide dextrose medium (for detecting fecal streptococci in ground water)	5.2.5				
For testing 100-mL samples, is triple strength (3X) formulation in a culture bottle prepared and then autoclaved at 121°C for 15 minutes?	5.2.5.1				
Is medium final pH 7.2±0.2?					
Is a 100-mL water sample added to the sterilized medium and incubated at 35°±0.5°C?	5.2.5.2				

Element	Number	Yes	No	NA	Comments
Is the culture checked for turbidity after 24±2 hours?	5.2.5.3				
If turbidity is not observed, is the culture reincubated and checked again after a total incubation period of 48±3 hours?					
Are turbid cultures confirmed as fecal streptococci by streaking a portion of the broth onto bile esculin agar (BEA) or bile esculin azide agar (BEAA)?	5.2.5.4				
Are BEA and BEAA autoclaved at 121°C for 15 minutes?	5.2.5.5				
Is the final pH 6.6±0.2 for BEA and 7.1±0.2 for BEAA?					
After streaking, are plates incubated at 35°±0.5°C for 48 hours?	5.2.5.6				
Are the brownish-black colonies with brown halos on BEA or BEAA used as confirming the presence of fecal streptococci?	5.2.5.7				
If required, does the laboratory perform an enterococci test by transferring one or more fecal streptococci colonies to brain heart infusion broth supplemented with 6.5% NaCl and incubating the culture at 35°±0.5C for 48 hrs?					
Enzyme (chromogenic/fluorogenic) substrate tests	5.3				
General	5.3.1				
For detecting total coliforms and *E. coli* in drinking water by an enzyme substrate test, does the laboratory use one of the following: MMO-MUG test (Colilert), Colisure test, E*Colite test, Readycult Coliforms 100 Presence/Absence Test, Fluorocult LMX test, or Colitag test?	5.3.1.1				
For enumerating total coliforms in source waters by an enzyme substrate test, does the laboratory use the Colilert test?					
If a laboratory uses a fermentation method to detect total coliforms in drinking water, and the sample is total coliform-positive, does the laboratory transfer the positive culture to the EC+MUG test to detect *E. coli*, but not to any other enzyme substrate test medium in Section 5.3?					
Media	5.3.1.2				
Does the laboratory purchase media from a commercially available source only, and not prepare media from basic ingredients?	5.3.1.2.1				
Are media kept protected from light?	5.3.1.2.2				
Is each lot of medium checked for fluorescence before use with a 365-366-nm ultraviolet light with a six watt bulb?	5.3.1.2.3				

Element	Number	Yes	No	NA	Comments
If medium exhibits faint fluorescence, is another lot used that does not fluoresce?					
If samples plus medium exhibit color changes before incubation, is the medium discarded and another lot of medium used?	5.3.1.2.4				
Are glass and plastic bottles and test tubes checked before use with a 365-366-nm ultraviolet light source with a 6-watt bulb to ensure that they do not fluoresce?	5.3.1.3				
If they fluoresce, does the laboratory use another lot of containers that does not fluoresce?					
If a Whirl-Pak® bag is used to incubate the Colilert or Colitag medium or any other medium which changes to a yellow color to indicate a positive result, is a type used that has a barrier (e.g., B01417) to prevent gaseous emissions to other Whirl-Pak® bags during incubation?	5.3.1.4				
QC If a small air-type incubator is used, are samples brought to room temperature before incubation?	5.3.1.5				
If a water bath is used, is the water level above the upper level of the medium?	5.3.1.6				
For E. coli testing, are all total coliform-positive samples placed under a UV lamp (365-366 nm) in a darkened area?	5.3.1.7				
Does the laboratory refrain from using the enzyme substrate test to confirm a presumptive total coliform-positive culture in a fermentation broth or on a membrane filter?	5.3.1.8				
Does the laboratory invalidate any sample that produces an atypical color change (in the absence of a yellow color) and then collect, or request that the system collect, another sample from the same location as the original invalidated sample?	5.3.1.9				
Does the laboratory use another method to test the second sample?					
Is the reference comparator provided by the manufacturer discarded by the manufacturer's expiration date?	5.3.1.10				
Criteria for specific media	5.3.2				
For the Colilert test, are samples incubated at 35°±0.5°C for 24 hours?	5.3.2.1				
Is a sample with a yellow color in the medium equal to or greater than reference comparator recorded as total coliform-positive?					
Is a sample with a yellow color lighter than comparator incubated for another four hours but no longer than 28 hours total?					

Element	Number	Yes	No	NA	Comments
Is a sample with a yellow color lighter than the comparator after 28 hours of incubation recorded as total coliform-negative?					
Are coliform-positive samples that fluoresce under a UV light marked as *E. coli*-positive?					
For the Colilert-18 test, are samples incubated for 18 hours (up to 22 hours if the sample after 18 hours is yellow, but lighter than the comparator)?					
For enumerating total coliforms in source waters, does the laboratory use the Colilert test, a 5- or 10-tube configuration, Quanti-Tray, or Quanti-Tray 2000 for each sample dilution tested?	**5.3.2.1.1**				
When dilution water is used, is it either sterile deionized or sterile distilled water, not buffered water?					
QC If the Quanti-Tray or Quanti-Tray 2000 test is used, is the sealer checked monthly by adding a dye to the water?	**5.3.2.1.2**				
For the Colisure test, are samples incubated at 35°±0.5°C for 24-48 hours?	**5.3.2.2**				
If the medium changes from a yellow color to a red/magenta color, is the sample noted as total coliform-positive?					
Is a coliform-positive sample that fluoresces under a UV light marked as *E. coli*-positive?					
For the E*Colite test, is the sample incubated at 35°±0.5°C for 28 hours?	**5.3.2.3**				
If the medium changes from a yellow color to a blue or blue-green color, or a blue color in the corners of the bag, is the sample marked as total coliform-positive?					
If the medium fluoresces under a UV light, is the sample considered as *E. coli*-positive?					
If fluorescence is not observed, is the sample reincubated for an additional 20 hours (for a total incubation time of 48 hours) and checked again for fluorescence?					
If the medium becomes red in color, is the sample discarded and another sample requested?					
For the Readycult Coliforms 100 Presence-Absence test, are the contents of a snap pack added to a 100-mL sample and then incubated at 35°±0.5°C for 24±1 hours?	**5.3.2.4**				
If the medium changes color from a slightly yellow color to blue-green, is the sample marked as coliform-positive?					

Element	Number	Yes	No	NA	Comments
If the medium fluoresces a bright light-blue color when subjected to long wave UV (365-366 nm) light, is the sample marked as *E. coli*-positive?					
For the Fluorocult LMX test, is the medium added to purified water, mixed, and the mixture then boiled to dissolve the medium completely in the water?	5.3.2.5				
Are 100-mL aliquots transferred to 250-mL bottles and then autoclaved for 15 minutes?					
Are the autoclaved bottles cooled before adding the 100-mL water sample?					
Is the *E. coli*/Coliform Supplement not added to the medium?					
Is the sample then incubated at 35°±0.5°C for 24±1 hours?					
If the medium changes color from a slightly yellow color to blue-green, is the sample marked as coliform-positive?					
If the medium fluoresces a bright light-blue color when subjected to long wave UV (365-366 nm) light, is the sample marked as *E. coli*-positive?					
For the Colitag test, are samples incubated at 35°±0.5°C for 24±2 hours?	5.3.2.6				
If the medium changes to a yellow color, is the sample marked as coliform-positive?					
If the medium fluoresces under a UV light, is the sample marked as *E. coli*-positive?					
EC Medium + MUG (for detection of *E. coli*)	5.3.3				
If EC medium + MUG is used, is a total coliform-positive culture transferred from a presumptive tube/bottle or colony to this medium?	5.3.3.1				
Is the final pH of EC medium + MUG 6.9±0.2?	5.3.3.2				
Is the medium plus sample incubated at 44.5°±0.2°C for 24±2 hours and then tested for fluorescence?	5.3.3.4				
Enterolert test (for detection of enterococci in ground water)	5.3.4				
Is the medium stored in the dark at 4°-30°C until used?	5.3.4.1				
Is Enterolert reagent added to a 100-mL sample and the sample/medium incubated at 41°±0.5°C for 24-28 hours?	5.3.4.2				

Element	Number	Yes	No	NA	Comments
Is fluorescence under a UV lamp used to indicate the presence of enterococci?					
Membrane Filter (MF) methods	**5.4**				
General	**5.4.1**				
For source water samples (SWTR), do dilutions yield 20 to 80 total coliform colonies or 20 to 60 fecal coliforms for at least one dilution or volume?	**5.4.1.1**				
QC Is at least one membrane filter and filtration unit sterility check conducted at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter and testing for growth?	**5.4.1.2**				
QC If the control indicates contamination, does the laboratory reject all data from affected samples and request an immediate resampling?					
QC Does the laboratory consider a filtration series as ended when 30 minutes or more has elapsed between sample filtrations?					
Are filtration funnels rinsed after each sample filtration with two or three 20-30 mL portions of sterile rinse water to ensure that the entire sample is rinsed off the funnel onto the filter?	**5.4.1.3**				
Are absorbent pads saturated with at least 2 mL of broth and the excess medium removed by "decanting" the plate?	**5.4.1.4**				
MF method for detecting total coliforms and *E. coli* in drinking water, enumerating total coliforms or fecal coliforms in source water, and detecting *E. coli* in ground water	**5.4.2**				
Media for total coliforms, fecal coliforms, and *E. coli*	**5.4.2.1**				
If either M-Endo agar or broth or M-Endo agar LES is used to detect total coliforms in drinking water or enumerating total coliforms in source water, is either the single step or the enrichment technique used?	**5.4.2.1.1**				
Is denatured ethanol used in the rehydration procedure?					
Is the medium prepared in a sterile flask?					
Is a boiling water bath or a constantly attended hot plate with a stir bar used to bring the medium just to the boiling point but not boiled?					
Is the final for M-Endo medium pH 7.2±0.1 and the final pH for M-Endo agar LES 7.2±0.2?					
Is M-Endo medium or M-Endo agar LES incubated at 35°±0.5°C for 22-24 hrs?	**(5.4.2.2)**				

Element	Number	Yes	No	NA	Comments
Are colonies with a metallic (golden) sheen recorded as presumptive total coliforms?	(5.4.2.2)				
If m-ColiBlue24 medium is used to detect total coliforms and *E. coli* in drinking water, are the ampules of broth inverted 2-3 times to mix contents before breaking and the contents then poured evenly over an absorbent pad?	5.4.2.1.2				
Are unopened refrigerated ampules stored in the dark?					
Are unopened ampules discarded before the expiration date, or earlier if contamination is observed?					
Is the medium final pH 7.0±0.2?					
Is m-ColiBlue24 incubated at 35°±0.5°C for 24 hrs?	(5.4.2.2)				
Are red colonies recorded as total coliforms, and blue to purple colonies recorded as *E. coli*?	(5.4.2.2)				
If MI medium (with or without agar) is used to detect total coliforms and *E. coli* in drinking water or enumerate total coliforms in source water, is commercially prepared presterilized bottled MI agar or broth not autoclaved?	5.4.2.1.3				
Is this presterilaized bottled agar medium melted in a boiling water bath (or by other processes recommended by the manufacturer), and care taken not to overheat the agar?					
Is the medium then cooled slightly and poured immediately into sterile plates?					
If dehydrated culture medium is used, is it prepared and autoclaved according to manufacturer's instructions?					
Is this agar medium cooled before adding freshly prepared, filter-sterilized cefsulodin, and then poured immediately into sterile plates?					
Is the final pH of MI agar 6.95±0.20 and the final pH of MI broth 7.05±0.20?					
Is MI medium incubated at 35°±0.5°C for 24±2 hrs?	(5.4.2.2)				
Are fluorescent colonies under UV light recorded as total coliforms, and blue colonies under normal light recorded as *E. coli*?	(5.4.2.2)				
If Chromocult® Coliform Agar is used to detect total coliforms and *E. coli* in drinking water, is the agar medium autoclaved or overheated?	5.4.2.1.4				
Is the final pH of this medium 6.8±0.2?					

Element	Number	Yes	No	NA	Comments
If a heavy background of heterotrophic bacteria is expected, is cefsulodin solution added to 1L of cooled (45°-50°C) medium as a solution of 10 mg cefsulodin dissolved in 2-mL deionized or distilled water?					
Is Chromocult incubated at 36°±1°C for 24±1 hrs?	(5.4.2.2)				
Are salmon to red colonies recorded as total coliforms, and dark-blue to violet colonies recorded as *E. coli*?	(5.4.2.2)				
If Coliscan® is used to detect total coliforms and *E. coli* in drinking water or enumerate total coliforms in source water, is the manufacturer's protocol for reconstitution and antibiotic addition followed?	5.4.2.1.5				
Is the antibiotic, cefsulodin, overheated?					
Is the final pH of Coliscan agar 7.00±0.20?					
Is Coliscan incubated at 32°-37°C for 24-28 hrs?	(5.4.2.2)				
Are pink-magenta colonies recorded as total coliforms, and purple-blue colonies recorded as *E. coli*?	(5.4.2.2)				
If m-FC broth, with or without agar, is used to enumerate fecal coliforms in source water, is the medium autoclaved?	5.4.2.1.6				
Is m-FC broth just brought to the boiling point?					
Is the final pH of m-FC medium 7.4±0.2?					
Is m-FC broth incubated at 44.5°±0.2°C for 24±2 hrs?	(5.4.2.2)				
Are blue colonies recorded as fecal coliforms?	(5.4.2.2)				
Is the prepared medium refrigerated when stored and brought to room temperature before use?	5.4.2.1.7				
Are petri dishes containing medium stored in a plastic bag or tightly closed container, and used within 2 weeks?					
Are plates with laboratory-prepared broth medium discarded after 96 hours, poured agar plates after 2 weeks, and ampuled broth discarded before the manufacturer's expiration date?					
Are the date and time of medium preparation recorded?					
For invalidation of a total coliform-negative drinking water sample, are all samples resulting in confluent growth or TNTC growth invalidated unless at least one total coliform colony is detected?	5.4.2.3				

Element	Number	Yes	No	NA	Comments
If no coliforms are detected, is the sample recorded as "confluent growth" or "TNTC" and an additional sample requested from the same sampling site?					
Does the laboratory perform a verification test on the total coliform-negative culture before invalidation?					
If the verification test is total coliform-positive, does the laboratory report the sample as total coliform-positive?					
If the verification test is total coliform-negative, is the sample invalidated?					
For invalidation of source water samples (SWTR), where coliform density must be determined, does the laboratory invalidate any sample that results in confluent growth or TNTC, even when total coliform or fecal coliform colonies are present?	5.4.2.4				
For drinking water samples on M-Endo type media, are all sheen colonies, up to a maximum of five, verified by using either LB or LTB and then 2% BGLBB or, alternatively, by using a cytochrome oxidase and β-galactosidase procedure?	5.4.2.5				
If no sheen colonies are observed, are up to five red questionable sheen colonies and/or red non-sheen colonies representing different morphological types verified?					
For drinking water samples, are total coliform-positive colonies tested for *E. coli* or fecal coliforms?	5.4.2.6				
When EC Medium or EC + MUG is used, are colonies transferred by employing one of the options specified by the Total Coliform Rule?					
When the swab technique is used, is a single swab used to inoculate a presumptive total coliform-positive sample into EC or EC+MUG first, LTB second, and BGLBB third?					
For source water samples, are the initial total coliform counts adjusted based upon verified data?	5.4.2.7				
QC For source water samples when two or more analysts are available, does each analyst count the total coliform or fecal coliform colonies on the same membrane monthly and do the counts agree within 10%?	5.4.2.8				
Nutrient Agar + MUG Test (for detection of *E. coli* in drinking water or ground water)	5.4.3				
Is the medium autoclaved at 121°C for 15 minutes?	5.4.3.1				
Is the final MUG concentration 100 µg/L?					
Is the final pH of NA + MUG 6.8±0.2?					

Element	Number	Yes	No	NA	Comments
QC Are positive and negative culture controls tested as stated in 5.1.6.4?	5.4.3.2				
QC Are culture controls filtered or spot-inoculated onto a membrane filter on M-Endo broth or agar, or M-Endo agar LES, and incubated at 35°±0.5°C for 24 hours?					
QC Is the filter then transferred to NA + MUG and incubated at 35°±0.5°C for another four hours?					
QC Are these results read and recorded?					
Is the membrane filter containing total coliform colonies transferred to the surface of the Nutrient Agar + MUG medium?	5.4.3.3				
Is the presence of each sheen colony marked on the petri dish lid with permanent marker, and the lid and base marked to realign the lid when removed?					
For the total coliform verification test, is a portion of each colony transferred with needle before the MF transfer or after the four-hour NA + MUG incubation time?					
Alternatively, is the membrane filter surface swabbed with a sterile cotton swab after the four-hour incubation time on NA + MUG and then transferred to a total coliform verification test?					
Is the inoculated NA + MUG medium incubated at 35°±0.5°C for four hours?	5.4.3.4				
Is fluorescence checked by using a UV lamp (365-366 nm) with a six-watt bulb in a darkened room and any fluorescence in the halo around a sheen colony considered positive for *E. coli*?	5.4.3.5				
MF method for detecting enterococci/fecal streptococci in ground water	5.4.4				
Media	5.4.4.1				
When mE agar is used for the detection of enterococci, is basal mE agar prepared, autoclaved, and cooled before the addition of nalidixic acid (or its sodium salt) and triphenyl tetrazolium chloride, both of which are added separately to the medium and mixed?	5.4.4.1.1				
Is the final pH of mE agar 7.1±0.2?					
When m-Enterococcus agar is used for the detection of fecal streptococci (not enterococci), is the medium heated, not autoclaved, to dissolve the ingredients?	5.4.4.1.2				
Is the final pH of m-Enterococcus agar 7.2±0.2?					

Element	Number	Yes	No	NA	Comments
When mEI agar is used for the detection of enterococci, is 0.75g indoxyl-β-D-glucoside added to 1L basal mE agar and then prepared according to 5.4.4.1.1 except that only 0.02 g/L triphenyl tetrazolium chloride is added?	5.4.4.1.3				
Is the final pH of mEI agar 7.1±0.2?					
Is a 100-mL sample filtered and the membrane placed on one of the agar media previously listed?	5.4.4.2				
If m-Enterococcus agar is used, are the plates incubated in an inverted position at 35°±0.5°C for 48 hours?	5.4.4.3				
Using magnification and a fluorescent lamp, are all light and dark red colonies counted as fecal streptococci?					
If mE agar is used, are the plates incubated in an inverted position for 48 hours at 41°±0.5°C?	5.4.4.4				
Is the membrane filter then transferred to EIA medium and incubated at 41°±0.5°C for 20-30 minutes?					
Using magnification and a fluorescent lamp, are all pink to red colonies on mE agar with a black or reddish brown precipitate on the underside of the filter on EIA agar counted as enterococci?					
If mEI agar is used, are plates incubated in an inverted position for 24 hours at 41°±0.5°C?	5.4.4.5				
Using magnification and a fluorescent lamp, is the plate examined, top and bottom, for colonies with a blue halo, and any colony with a blue halo (regardless of colony color) considered as positive for enterococci?					
Heterotrophic Plate Count (for enumerating heterotrophic bacteria in drinking water)	5.5				
Does the laboratory use the Pour Plate Method or the SimPlate Method for enumerating heterotrophic bacteria in drinking water and for testing reagent grade water?	5.5.1				
For systems granted a variance from the TCR's maximum contaminant level, does the laboratory use R2A medium with a method in *Standard Methods*, Section 9215 for enumerating heterotrophic bacteria in drinking water?					
Media	5.5.2				
Is the final pH recorded for plate count agar pH 7.0±0.2, R2A agar 7.2±0.2, and SimPlate 7.2±0.2?					
For the Pour Plate Method, is melted agar tempered at 44°-46°C in a water bath and maintained no more than 3 hours before pouring?	5.5.3				

Element	Number	Yes	No	NA	Comments
Is this sterile medium melted only once?					
For the Spread Plate Method, is 15 mL of R2A medium (or other medium) poured into a sterile petri dish and allowed to solidify?	5.5.4				
Is refrigerated medium in bottles or screw-capped tubes stored for no longer than six months, or in petri dishes for no longer than 2 weeks (one week for prepared petri dishes with R2A medium)?	5.5.5				
For countable plates of most potable water samples, are 1.0 mL and/or 0.1 mL volumes of the undiluted sample plated?	5.5.6				
Are at least duplicate plates prepared per dilution tested?					
For the Pour Plate Method, is the sample pipetted aseptically onto the bottom of a sterile petri dish and then at least 10-12 mL tempered melted agar added?	5.5.7				
Is the sample and melted agar mixed, avoiding spillage?					
After the agar plates have solidified on a level surface, are they inverted and incubated at 35°±0.5°C for 48±3 hours?					
Are plates stacked no more than four high and arranged in the incubator to allow proper air circulation and to maintain a uniform incubation temperature?					
Does the laboratory ensure that incubator does not have excess humidity and that the plates do not lose more than 15% by weight during the 48 hours of incubation?					
For the Spread Plate Method, is 0.1 or 0.5 mL of the sample (or dilution) pipetted onto the surface of the predried agar in the plate and then spread over the entire surface using a sterile bent glass rod?	5.5.8				
Is the inoculum absorbed completely before incubating?					
Are the plates incubated in an inverted position at 20°-28°C for 5-7 days?					
For the Membrane Filter Technique, does the filtered volume yield between 20-200 colonies?	5.5.9				
Is the filter transferred to a petri dish containing 5 mL solidified R2A medium and then incubated at 20°-28°C for 5-7 days?					
Are plates with loose-fitting lids placed in a plastic box with a close-fitting lid and moistened paper towels, and rewetted as necessary?					
Are colonies counted using a stereoscopic microscope at 10-15X magnification?					
SimPlate Method	5.5.10				

Element	Number	Yes	No	NA	Comments
For a single sample Unit Dose, is a 10-mL test sample added to a test tube containing dehydrated SimPlate medium and then poured onto the center of a plate containing 84 small wells?	**5.5.10.1**				
Alternatively, is 9-mL of sterile diluent 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?					
Is this mixture distributed evenly to the 84 wells and is the excess liquid drained into the absorbent pad on the plate?					
Is the plate inverted and incubated at 35°±0.5°C for 45-72 hours?					
Is bacterial density 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?					
If a 10-mL sample is used, is the Unit Dose MPN/mL read directly or, if a 1-mL sample is used, is the MPN/mL value corrected by multiplying it by 10?					
For the Multiple Dose for 10 samples of 1 mL each, is a 100-mL sterile diluent added to the dehydrated SimPlate medium and shaken to dissolve?	**5.5.10.2**				
Is a 1.0-mL test sample then pipetted to the center of a plate, followed by 9 mL of the reconstituted medium?					
Is the plate then gently swirled to mix and distribute the sample and medium mixture evenly to the 84 wells, with the excess liquid then being drained into the absorbent pad on the plate?					
Is the plate inverted and incubated at 35°±0.5°C for 45-72 hours?					
Is bacterial density 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 Multi-Dose MPN table?					
If sample dilutions were made during sample preparation, is the MPN/mL value multiplied by the dilution factor?					
For the Pour Plate and Spread Plate Techniques, are colonies counted manually using a dark field colony counter?	**5.5.11**				
Are only plates having 30 to 300 colonies counted, except for plates inoculated with 1.0 mL of undiluted sample where counts of less than 30 are acceptable?					

Element	Number	Yes	No	NA	Comments
QC Is each batch or flask of agar checked for sterility by pouring a final control plate?	**5.5.12**				
QC Does the laboratory reject data if the control is contaminated?					
Coliphage (Draft Method 1601 and 1602 for the GWR)	**5.6**				
EPA Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment	**5.6.1**				
Is the 100-mL (or 1-L) water sample supplemented with magnesium chloride, log-phase host bacteria, and tryptic soy broth (TSB) as an enrichment step for coliphage?					
After incubation overnight, are samples spotted onto a lawn of host bacteria, incubated, and then examined for circular lysis zones?					
Media	**5.6.1.1**				
For antibiotic stocks, are antibiotics always added to the medium *after* the medium has been autoclaved?	**5.6.1.1.1**				
Are antibiotic stocks stored frozen at -20°C for no longer than one year?					
Are antibiotic stocks thawed at room temperature or rapidly in a water bath up to 37°C and mixed well before using?					
Is 10X tryptic soy broth (TSB) stored at 1°-5°C until used?	**5.6.1.1.2**				
Are 1.5% tryptic soy agar (TSA) plates after antibiotic supplementation and solidification stored inverted at 1°-5°C for no longer than two weeks, if not used immediately?	**5.6.1.1.3**				
Are 0.7% TSA top agar tubes with appropriate antibiotics, dispensed as 5-mL per sterile 10-mL tube, labeled, and kept at 45°-48°C until used?	**5.6.1.1.4**				
Are these tubes used the day of preparation?					
For spot plates that develop condensation during storage, are plates incubated for approximately 10 minutes to reduce condensation prior to inoculation?	**5.6.1.1.5**				
Are spot plates used that day or stored at 1°-5°C for up to four days?					
Coliphage stock	**5.6.1.2**				
Are MS2 (ATCC#15597-B1 male-specific) and phi-X 174 stock coliphage (ATCC#13706-B1, somatic) stored at 2°-8°C for up to five years?	**5.6.1.2.1**				

Element	Number	Yes	No	NA	Comments
Does analysis of raw sewage filtrate begin within 24 hours of collection?	5.6.1.2.2				
Is at least 10 mL of filtered sewage obtained?	5.6.1.2.4				
If the filtrate is stored more than 24 hours, is it re-titered before use?	5.6.1.2.5				
Host bacteria stock cultures	5.6.1.3				
After preparation, are host bacteria stock cultures held at a temperature between -20°C and -70°C?	5.6.1.3.1				
Are bacteria stored no longer than two months at -20°C or no longer than one year at -70°C?					
Are prepared overnight host bacteria stock cultures chilled on wet ice or at 1°-5°C until ready to use?	5.6.1.3.2				
After preparation, are log-phase host bacteria stock cultures chilled on wet ice or at 1°-5°C to slow replication until ready for use?	5.6.1.3.3				
When stored, are these suspensions stored no more than 48 hours?					
Are remaining bacterial host cultures stored at 1°-5°C overnight to inoculate flasks for the preparation of new log-phase bacterial hosts?					
General QC	5.6.1.4				
Initial demonstration of capability (IDC)	5.6.1.4.1				
QC Did the laboratory demonstrated ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples?					
QC Did the IDC test consist of ten reagent water samples spiked with enumerated sewage filtrate or equivalent at 1-2 PFU mL per sample for every sample for each coliphage type used, according to the IDC Table?					
QC Were these IDC tests accompanied by a method blank for each coliphage type used?					
Method Blanks	5.6.1.4.2				
QC Is a method blank (reagent water sample containing no coliphage) analyzed to demonstrate freedom from contamination?					
For each coliphage type used, is a sterile reagent water sample prepared and analyzed using the same procedure used for analysis of field and QC samples?					
QC Is at least one method blank analyzed for every spot plate used for field samples?					

Element	Number	Yes	No	NA	Comments
Positive Controls	5.6.1.4.3				
QC Are positive controls analyzed to ensure that stock coliphage suspensions, host bacterial cultures, and growth media are performing properly?					
QC For each coliphage type used, is a 100 mL reagent water sample spiked with 20 PFU from sewage filtrate or 60 PFU from a pure coliphage stock culture?					
QC Is one positive control analyzed for every spot plate used for field samples?					
QC If multiple spot plates are inoculated with samples on the same day, is a single enriched positive control sample used to inoculate multiple spot plates that day?					
Matrix spikes (MS)	5.6.1.4.4				
QC Is method performance assessed by analyzing one set of MS samples for each coliphage type used when samples are first received from a new ground water source?					
QC For each coliphage type analyzed, are three field samples spiked with 1-2 PFU, with at least one out of the three MS samples being positive for each coliphage type?					
QC Is one set of MS samples analyzed on an ongoing basis after every 20th field sample for each ground water source?					
QC Are these MS samples collected at the same time as routine field samples?					
QC Are these samples spiked in "bulk" at the concentrations indicated in the MS and ODC Sample Spiking Table?					
Ongoing demonstration of capability (ODC)	5.6.1.4.5				
QC Does the laboratory demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples?					
QC Are three reagent water samples spiked with 1-2 PFU for each coliphage type used?					
QC Are ODC test samples analyzed exactly like field samples?					
QC Is a minimum of one out of three ODC samples positive for each coliphage type used?					
QC Is one set of ODC samples analyzed after every 20 field and MS samples or one per week, whichever occurs more frequently?					

Element	Number	Yes	No	NA	Comments
QC Are samples spiked in "bulk" at the concentrations indicated in the MS and ODC Sample Spiking Table?					
Performance studies	**5.6.1.4.6**				
QC Does the laboratory periodically analyze external QC samples when available?					
QC Does the laboratory participate in available interlaboratory performance studies?					
QC Does the laboratory review results, correct unsatisfactory performance, and record corrective actions?					
EPA Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure	**5.6.2**				
Is the 100-mL ground water sample supplemented with magnesium chloride and host bacteria, and then added to 100-mL of double-strength molten tryptic soy agar containing the appropriate antibiotic?					
Is the sample thoroughly mixed and the total volume then poured into five to ten plates?					
After overnight incubation, are circular lysis zones recorded as coliphage?					
Media	**5.6.2.1**				
For antibiotic stocks, are antibiotics always added to the medium *after* the medium has been autoclaved?					
Are antibiotic stocks stored frozen at -20°C for up to one year?					
Are antibiotic stocks thawed at room temperature or rapidly in a water bath up to 37°C and then mixed well before using?					
Is 10X TSB stored at 1°-5°C until used?					
Are 1.5% TSA plates, after antibiotic supplementation and solidification, stored inverted at 1°-5°C for no longer than two weeks, if not used immediately?					
Are 0.7% TSA top agar tubes with appropriate antibiotics, dispensed as 5-mL per sterile 10-mL tube, labeled, and kept at 45°-48°C until used?					
Are these tubes used the day they are prepared?					
Are spot plates that develop condensation during storage, incubated for approximately 10 minutes to reduce condensation prior to inoculation?					

Element	Number	Yes	No	NA	Comments
Are these plates used that day or stored at 1°-5°C for up to four days?					
Is 2X TSA, with appropriate antibiotics, kept molten at 45°-48°C in a water bath?	5.6.2.1.2				
Is this agar used only on the day of preparation?					
Coliphage Stock	5.6.2.2				
Are MS2 (ATCC#15597-B1 male-specific) and phi-X 174 stock coliphage (ATCC#13706-B1, somatic) stored at 2°-8°C for up to five years?					
Does analysis of raw sewage filtrate begin within 24 hours of collection?					
Is at least 10 mL of filtered sewage obtained?					
If the filtrate is stored more than 24 hours, is it re-titered before use?					
Host bacteria stock cultures	5.6.2.3				
After preparation, are host bacteria stock cultures held at a temperature between -20°C and -70°C?					
Are bacteria stored no longer than two months at -20°C, or no longer than one year at -70°C?					
After preparation, are overnight host bacteria stock cultures chilled on wet ice or at 1°-5°C until ready for use?					
After preparation, are log-phase host bacteria stock cultures chilled on wet ice or at 1°-5°C to slow replication until ready to use?					
When stored, are these suspensions stored no longer than 48 hours?					
Are remaining bacterial host cultures stored at 1°-5°C overnight to inoculate flasks for the preparation of new log-phase bacterial hosts?					
General QC	5.6.2.4				
Initial precision and recovery (IPR)	5.6.2.4.1				
QC Has the laboratory demonstrated the ability to perform this method acceptably by performing an IPR test before analyzing any field samples?					
QC Does the IPR Test consist of four reagent water samples tested for each coliphage type used, spiked with enumerated sewage filtrate or equivalent to yield a target spike concentration of 80 PFU per sample?					

Element	Number	Yes	No	NA	Comments
QC Does the relative standard deviation of the recovery (RSD), and the average percent recovery ($\bar{x}$) based on all four sample results for each coliphage type used, meet the acceptance criteria in the QC Acceptance Criteria table?					
Method blanks	5.6.2.4.2				
QC Is one method blank (reagent water sample containing no coliphage) analyzed with each analytical batch to demonstrate freedom from contamination?					
QC For each coliphage type used, are sterile reagent water samples prepared and analyzed using the same procedures used for analysis of the field and QC samples?					
QC Is an analytical batch defined as all samples analyzed during a single day, up to a maximum of 20 samples (field samples and matrix spike samples) per coliphage type used?					
Matrix spikes (MS)	5.6.2.4.3				
QC Is method performance assessed by analyzing one set of MS samples for each coliphage type used when samples are first received from a new ground water source?					
QC Is one set of MS samples routinely analyzed after every 20th field sample for each ground water source?					
QC If the recovery for coliphage falls outside its limit (see the QC Acceptance Criteria table), is method performance considered unacceptable?					
QC If OPR results are not within control limits, is the problem identified and corrected and the data qualified?					
QC Does the laboratory maintain a control chart on recovery and update it on a regular basis?					
Ongoing precision and recovery (OPR)	5.6.2.4.4				
QC Does the laboratory demonstrate acceptable performance through analysis of an OPR sample on an ongoing basis?					
QC For each coliphage type used, is a reagent water sample spiked with approximately 80 PFU?					
QC Is the OPR sample analyzed exactly like a field sample?					
QC Is one OPR sample analyzed for each analytical batch?					
QC Does the laboratory compare the OPR percent recovery (R) with the corresponding limits in the QC Acceptance Criteria table?					

Element	Number	Yes	No	NA	Comments
QC If R falls outside the range for recovery, is analysis stopped until the problem is identified, corrected, and another OPR test is successfully performed?					
Performance studies	5.6.2.4.5				
QC Does the laboratory periodically analyze an external QC sample when available?					
QC Does the laboratory participate in available interlaboratory performance studies?					
QC Does the laboratory review these results, correct unsatisfactory performance, and record corrective actions?					
6. SAMPLE COLLECTION, HANDLING, AND PRESERVATION					
Sample Collector	6.1				
Is the sample collector trained in aseptic sampling procedures and, if required, approved by the appropriate regulatory authority or its designated representative?					
Sampling	6.2				
Are the drinking water samples collected under the Total Coliform Rule representative of the water distribution system?	6.2.1				
Are the water taps used for sampling free of aerators, strainers, hose attachments, mixing type faucets, and purification devices?					
Are only cold water taps used?					
Are service lines cleared before sampling by maintaining a steady water flow for at least 2 minutes or until a steady water temperature is reached?					
Is at least a 100-mL sample volume collected, allowing at least a 1-inch air space in the container to facilitate mixing of the sample by shaking?					
Is a sample information form completed immediately after sample collection?					
If a sample bottle is filled too full to allow for proper mixing, is the entire sample poured into a larger sterile container and mixed before proceeding with the analysis?					
For the SWTR, are the source water samples representative of the source of supply and collected not too far from the intake point, but at a reasonable distance from the bank or shore?	6.2.2				
Is the sample volume sufficient to perform all the tests required?					

Element	Number	Yes	No	NA	Comments
For the analysis of coliphage, *E. coli*, or enterococci under the GWR, is at least a 100-mL sample volume collected?	6.2.3 6.2.4				
Sample Icing	6.3				
For drinking water bacterial samples, is the sampler encouraged to hold samples at <10°C during transit to the laboratory?	6.3.1				
For source water bacterial samples, are samples held at <10°C during transit to the laboratory?					
Does the laboratory reject samples that have been frozen?					
For coliphage analysis under the GWR, are samples shipped at <10°C, stored at 1°-5°C, and not frozen?	6.3.2				
QC For SWTR samples and coliphage samples, does the laboratory record sample temperature upon receipt?	6.3.3				
QC Does the laboratory flag samples that have a temperature upon receipt of >10°C, whether iced or not, unless the time since the sample collection is less than two hours?					
Sample Holding/Travel Time	6.4				
For the analysis of total coliforms in drinking water, does the time between sample collection and placement of the sample in the incubator not exceed 30 hours?	6.4.1				
Are all samples analyzed on the day of receipt?					
Are samples received late in the day refrigerated overnight only if analysis can begin within 30 hours of collection?					
For total coliforms and fecal coliforms in surface water sources, and for heterotrophic bacteria in drinking water, is the time from sample collection to placement in the incubator less than eight hours?	6.4.2				
For coliphage analysis, is the time from sample collection to placement of sample in the incubator less than 48 hours?	6.4.3				
For coliphage analysis, is the time from sewage sample collection to analysis of QC spiking suspension less than 24 hours, unless re-titered and the titer has not decreased by more than 50%?					
If the titer has not decreased by more than 50%, is the sample stored no longer than 72 hours?					
For *E. coli* and enterococci analysis under the GWR, is the time between sample collection and the placement of sample in the incubator less than 30 hours?	6.4.4				
Sample Information Form	6.5				

Element	Number	Yes	No	NA	Comments
After collection, does the sampler enter the following information, in indelible ink, on sample information form? – Name of system (PWSS identification number if available) – Sample identification (if any) – Sample site location – Sample type (e.g., a routine distribution, repeat, raw or process, or other special purpose) – Date and time of collection – Analysis requested – Disinfectant residual – Name of sampler – Any remarks					
Chain-of-Custody	**6.6**				
Are applicable State regulations pertaining to chain-of-custody followed by sample collectors and the laboratory?					
7. QUALITY ASSURANCE					
Does the laboratory have a written QA Plan prepared and available for inspection?	**7.1**				
Does the laboratory follow the written QA Plan?					
Does the laboratory have a Standard Operating Procedure available for review pertaining to its own calibration of equipment or supplies?					
Does the laboratory successfully analyze at least one set of PT samples once every 12 months for each method for which it is certified?	**7.2**				
For methods used to test the presence or absence of an organism in a sample, does the laboratory analyze each PT sample set using a single analytical method only?					
8. RECORDS AND DATA REPORTING					
Legal Defensibility	**8.1**				
Are compliance monitoring data being maintained by the laboratory both thorough and accurate, and thus legally defensible?					
Does the laboratory's QA plan and/or SOPs describe the policies and procedures used by the facility for record retention and storage?					
If samples are expected to become part of legal action, does the laboratory follow chain-of-custody procedures?					
Maintenance of Records	**8.2**				
Does the public water system maintain records of microbiological analyses for five years?					

Element	Number	Yes	No	NA	Comments
Does the laboratory maintain easily accessible records for five years or until the next certification data audit is completed, whichever is longer?					
Does the laboratory notify the client water system before disposing of records so they may request copies if needed?					
Does the laboratory backup all electronic data by protected tape, disk, or hard copy?					
When the laboratory changes its computer hardware or software, are provisions in place for transferring old data to the new system so that data remain retrievable within the specified time frames?					
Sampling Records	**8.3**				
Are all data recorded in ink, with any changes lined through such that the original entry is visible?					
Are changes initialed and dated?					
Does the laboratory have the following sample information readily available? – Date and time of sample receipt by the laboratory – Name of the laboratory person receiving the sample – Information on any deficiency in the condition of the sample	**8.3.1-4**				
Are samples invalidated for the following reasons? – Time between sample collection and receipt by laboratory exceeded – Presence of disinfectant in sample noticed, e.g., odor – Evidence of freezing – Use of a container not approved by the laboratory for the purpose intended – Insufficient sample volume, e.g., <100 mL – Presence of interfering contaminants noticed, e.g., hydrocarbons, cleansers, heavy metals, etc. – Sample temperature exceeding the maximum allowable	**8.3.4**				
Analytical Records	**8.4**				
Are all recorded data in ink with any changes lined through such that original entry is visible?					
Are these changes initialed and dated?					

Element	Number	Yes	No	NA	Comments
Are the following readily available? – Laboratory sample identification information – Information concerning date and time analysis begins – Name of the laboratory and a signature or initials of the person(s) performing analysis – Information concerning the analytical technique or method used – Information concerning all items marked "QC" – Results of the analyses	8.4.1-6				
Preventive Maintenance	8.5				
Does the laboratory maintain preventive maintenance and repair records for all instruments and equipment?					
Are these records kept for five years in a manner that allows for easy inspection?					
9. ACTION RESPONSE TO LABORATORY RESULTS					
Testing Total Coliform-Positive Cultures	9.1				
For the Total Coliform Rule, does the laboratory test all total coliform-positive cultures for the presence of either fecal coliforms or *E. coli*?					
Notification of Positive Results	9.2				
For Total Coliform Rule, does the laboratory promptly notify the proper authority of a positive total coliform, fecal coliform, or *E. coli* result, so that appropriate follow-up actions can be conducted?	9.2.1				
For the Total Coliform Rule, if a sample is fecal coliform- or *E. coli*-positive, does the system notify the State as soon as it is notified of the test result, i.e., at the end of that day or, if the State office is closed, by the end of the next business day?	9.2.2				
Does the laboratory base a total coliform-positive result on the confirmed phase if the Multiple Tube Fermentation Technique or Presence-Absence Coliform Test is used, or the verified test for the Membrane Filtration Technique if M-Endo medium or M-Endo LES agar is used?	9.2.3				
If a presumptive total coliform-positive culture does not confirm/verify as such, but is found to be fecal coliform or *E. coli*-positive, is the sample considered total coliform-positive and fecal coliform/*E. coli*-positive?					
Notification of Total Coliform Interference	9.3				

Element	Number	Yes	No	NA	Comments
For the Total Coliform Rule, does the laboratory promptly notify the proper authority when results indicate non-coliforms may have interfered with total coliform analysis?					

Chapter VI
Critical Elements for Radiochemistry

1. Personnel

1.1 Laboratory Supervisor

At a minimum, the laboratory supervisor should have a bachelor's degree in chemistry or an equivalent degree, and one year of experience in the measurement of radioactive analytes in drinking water. The laboratory supervisor is required to have a working knowledge of Quality Assurance (QA) and Quality Control (QC) principles and apply it to all radiochemical practices and procedures conducted in his or her laboratory. The laboratory supervisor is responsible for ensuring that all laboratory personnel have demonstrated their ability to satisfactorily perform the analyses to which they are assigned and that all data reported by the laboratory meet the required quality assurance criteria.

1.2 Laboratory Analyst

At a minimum, the laboratory analyst should have a bachelor's degree in chemistry or an equivalent degree, and one year of experience in the measurement of drinking water for radiochemical parameters. If the analyst is responsible for the operation of analytical instrumentation, he or she is required to have completed specialized training offered by the manufacturer, another qualified training facility, or served a period of apprenticeship under an experienced analyst. The duration of this apprenticeship is proportional to the sophistication of the instrument. Completion of this apprenticeship period for instrumentation should be documented and maintained in a training file.

1.3 Technician

At a minimum, the laboratory technician should have a high school diploma or its equivalent. Prior to working independently on drinking water samples, technicians should have at least 6 months bench experience in drinking water analyses, and have completed method training programs in the methods they will use on a daily basis. Their completed method training should be recorded in a training file.

1.4 Sampling Personnel

If the laboratory also conducts field sampling activities, the laboratory personnel who collect samples should have training in the proper collection technique for all the types of samples they collect. Their technique will be reviewed by experienced sampling personnel prior to independently collecting drinking water compliance monitoring samples. Both training and technique reviews should be documented in sampling personnel's training files.

1.5 Initial and Ongoing Demonstrations of Proficiency for Analysts and Technicians

Before beginning the analysis of compliance samples, all analysts, and technicians must demonstrate the ability to conduct their measurements with acceptable accuracy, precision, and freedom from interferences and demonstrate this proficiency annually. These demonstrations of proficiency should be recorded in each analyst's and technician's training file. The bench sheets and instrument printouts made during these demonstrations of proficiency should be retained and be available for inspection.

Demonstrations of Proficiency must be done, by conducting an MDL study as described in 40 CFR part 136, Appendix B, or by the following alternate procedure. The analyst should prepare and measure a sample set of at least four reagent blanks and four laboratory fortified blanks that have the radioanalyte of interest added to them at a known concentration for the relevant method. To demonstrate proficiency at quantitation levels appropriate for drinking water samples, the activity level added to the laboratory fortified blanks should be between the radioanalyte's MCL and its required detection limit. To be deemed an acceptable demonstration of proficiency, the mean recoveries and the standard deviation of the recoveries of the replicate measurements should be consistent with the requirements for accuracy and precision described in Section 7.7, and reagent blank measurements must have a mean result below the detection limit for each analyte measured with the method.

Continuing Demonstrations of Proficiencies may be done by repeating the studies described above annually, or can consist of documenting batch QC samples the analyst has processed during the year since the last Demonstration of Proficiency. The data for at least four reagent blanks in different batches and measured on non-consecutive days is used to assess sensitivity, and data from four laboratory fortified blanks not in the same batch and in sample batches measured on non-consecutive days, is used to assess accuracy and precision. The amount of the analyte added to the sample preparation batch laboratory fortified blanks should follow the guidance for the studies described in the preceding paragraph, and use the acceptance criteria found for them in Section 7.7.

All Initial and Ongoing Demonstrations of Proficiency should be recorded in a training file specific for each analyst and technician. Records related to methods training and external training relevant to laboratory operations should also be documented in the training file.

1.6 Data Produced by Analysts and Technicians in Training

Data produced by analysts and technicians who have not completed their training, or do not have a current demonstration of proficiency on record, as well as instrument operators still in the process of obtaining the required training or experience, are acceptable only when reviewed and validated by a fully qualified analyst, or the laboratory supervisor. They should provide a permanent record of their review and the data's acceptability by placing their signature and date in ink on any bench sheets, calculation sheets, or reports generated by staff who lack sufficient training or a current demonstration of proficiency.

1.7 Waiver of Academic Training

The certification officer may waive the need for specified academic training, on a case-by-case basis, for highly experienced analysts.

2. Laboratory Facilities

2.1 General

The analysis of compliance samples must be conducted in a laboratory where the security and integrity of the samples and the data can be maintained. The laboratory facilities should be clean, have adequate temperature and humidity control and adequate lighting at the bench top. The laboratory must have provisions for the proper disposal of chemical and radiological wastes, including liquid scintillation cocktail mixtures. Since many radiochemistry procedures can involve routine use of strong mineral acids, sulfides and/or organic liquids, the appropriate type of exhaust hoods are required. These hoods must be able to provide the analyst or technician with a sufficient hood velocity to remove hazardous fumes from the working area, and adequate bench space inside them to perform sample preparation activities safely and without concern of cross-contaminating samples. Hood velocities should be checked annually.

Analytical and sample storage areas must be isolated from all potential sources of contamination. Any sample having an emission rate in excess of 0.5 mrem/hr must be stored in a secured location away from drinking water samples. There should be sufficient storage space for chemicals, glassware, and portable equipment; sufficient floor and bench space for stationary equipment; and areas for cleaning materials.

2.2 Instrumentation

Instruments should be properly grounded. Counting instruments must be located in a room other than one in which samples and standards are being prepared, or where other types of chemical analyses are being performed. An uninterrupted power supply (UPS) should be available for all radiation measurement instruments and their associated computers used for data acquisition and reduction.

2.3 Preparation of Standards

In areas where radioactive standards are being prepared, care should be taken to minimize contamination of surfaces, other samples, and personnel. Bench surfaces of an impervious material covered with adsorbent paper, or plastic or fiberglass trays lined with adsorbent paper are acceptable.

3. Laboratory Equipment and Instrumentation

The laboratory is required to have the equipment, supplies and instrumentation necessary to perform the approved methods for which it is certified.

3.1 Radiation Counting Instruments

Specific types of radiation counting systems are necessary to conduct the measurements as described in the regulations. All measurement instruments used to measure radioactivity in compliance monitoring samples must have a documented history of stability with regard to their ability to make measurements accurately, with acceptable precision, and sufficiently free from interfering background radiation so the detection limits can be met. The following subsections specify instrumentation that many of the approved methods use for their measurements, when calibration or recalibration is required, and stability checks that are documented and recorded to monitor the laboratories ability to make measurements reliably.

3.1.1 Liquid Scintillation Counting (LSC) system: An LSC system is essential if the laboratory is to be certified for the measurement of tritium in drinking water samples. It is recommended that the liquid scintillation system have spectral analysis capabilities to establish proper regions of energy discrimination. The system should have adequate sensitivity to meet the detection limit of the methods.

Prior to their use to measure compliance monitoring samples, LSCs are initially calibrated for efficiency using NIST-traceable sources, and the background measured. Afterwards, an efficiency check source and an instrument blank to check backgrounds are measured on each day of use prior to any sample measurements to verify the instrument is ready to measure samples. These prior-to-use efficiency check source and the instrument blank measurements are recorded and used to establish control limits for the instrument's performance. During periods when LSCs are idle, the efficiency calibration and background should be checked weekly to confirm the LSC's ready status for sample measurements.

Warning limits and control limits should be calculated from the efficiency and background checks. If any efficiency check source measurement or instrument blank measurement exceeds its control limits, the LSC is placed out of service until the reason for the out of control measurement can be identified and corrected.

3.1.2 Gas-flow proportional counting system: A gas-flow proportional counting system may be used for the measurement of gross alpha and gross beta activities, radium-226, radium-228, strontium-89, strontium-90, radioactive cesium, iodine-131, and uranium as described in the references in 40 CFR part 141.25(a). The detector may be either a "windowless" (internal proportional counter) or a "thin window" type. A combination of shielding and a cosmic (guard) detector operated in anticoincidence with the main detector should be used to achieve low background beta-counting capability. The alpha and beta background count of the system should be low enough so that the sensitivity of the radioanalysis of water samples will meet the requirement of 40 CFR part 141.25(c) with reasonable counting time (not more than 1,000 minutes).

Solids absorption curves that relate the attenuation of alpha or beta particle emission with increasing sample mass in the planchet are essentially initial calibrations for specific analytes measured with gas proportional counters. They are either remeasured and a new curve generated, or the current accuracy of the original curve reverified on a regular basis. EPA requires this be done annually.

EPA recommends the following as an acceptable approach to solids absorption curve reverification. Solids absorption standards are stored in a desiccator, isolated from other samples after their initial use if the laboratory plans to use them later to reverify their solids absorption curves. When solids absorption curves need to be reverified, at least three of the original solids absorption standards made to produce the solids absorption curve currently in use for data reduction are selected for remeasurement. They should span the range of weights used in the original solids absorption curve. For the reverification measurement to be acceptable, the original measurement of the solids standard should occur within the range defined by the uncertainty of the reverification measurement calculated at the 95 percent confidence level. If any of the verification checks are not within this acceptability range when compared to their original measurements, all solids absorption standards must be remeasured for both sample weight and activity, then used to generate a new solids absorption curve to use for data reduction. If a solids absorption standard weight is not within five percent of its previously recorded weight, then it is possible the sample has been contaminated, or suffered loss of sample, and therefore is discarded and a new solids absorption standard manufactured to replace it. If more than one solids absorption standard weight is not within this acceptance range, then the currently used solids absorption curve may be verified as described below, or an entirely new set of solids absorption standards can be manufactured to measure and generate a new solids absorption curve to use for data reduction.

For short lived radioanalytes, or if the original solids absorption standards are suspected of being compromised, at least three new solids absorption standards (one each representative of the low, medium and high weight ranges of the original solids absorption curve) can be manufactured and used as solids absorption verification standards. For the verification measurement to be acceptable, the efficiency for the verification standard calculated from the solids absorption curve presently in use should occur within the range defined by the uncertainty of the efficiency verification measurement calculated at the 95 percent confidence level. If any verification standard's measured efficiency is not within this acceptance range, then additional solids absorption standards are manufactured that span the range of sample weights used at the laboratory. The complete set of solids standards is then measured to produce a new solids absorption curve to use for data reduction.

For stability checks, an efficiency calibration check source and the instrument background is measured and recorded prior to measuring samples on each day of use to verify the instrument is ready to make sample measurements. During periods when gas proportional counters are idle, the efficiency calibration should be checked daily and the background checked weekly to confirm the ready status of the instrument for sample measurements.

If a gas proportional counter measures samples sequentially with an automatic sample changer, it is possible both the size of the batch and long count times creates a sample counting set that requires several days to complete. In these cases, measurements of sample preparation batch Quality Control (QC) samples may substitute for the instrument checks. After 24 hours of continuously measuring samples, a measurement of any preparation batch QC sample with a known amount of the analyte added to it (See section 7.7) may be substituted for the calibration check source measurement, and any QC sample measurement expected to have no activity in it (reagent blanks) may substitute for an instrument blank measurement.

Warning limits and control limits for instrument stability measurements should be calculated. If instrument control measurements exceed their control limits, the proportional counter is placed out of service until the reason for the change in efficiency or background can be determined and corrected. If the out of control measurement is a sample batch QC sample substituting for an instrument control measurement, the entire batch is remeasured after the reason for the out of control event is identified and addressed. If a gas proportional counter is moved, serviced, or had an interruption in either gas flow or electrical power, the plateau voltage for both alpha and beta is verified, its crosstalk factors remeasured, and the solids absorption curves for each analyte reverified or regenerated prior to measuring any compliance monitoring samples.

3.1.3 Alpha scintillation counting system: For measurement of gross alpha activities and radium-226, a scintillation system designed for alpha counting may be substituted for the gas-flow proportional counter described above. In such a system, a Mylar disc coated with a phosphor (silver-activated zinc sulfide) is either placed directly on the sample or on the face of a photo-multiplier tube, enclosed within a light-tight container, along with the appropriate electronics (high voltage supply, pre-amplifier, amplifier, timer, and scalar). Solids absorption curves for each analyte measured by alpha scintillation counting systems are generated or reverified at least annually as described above for gas proportional counters.

Photo Multiplier Tube (PMT) efficiency and background checks are measured and recorded prior to the measurement of a set of compliance monitoring samples, or at least weekly to verify the alpha scintillation system's ready status to measure samples. Warning limits and control limits for both types of instrument stability measurements should be calculated. If any PMT efficiency or background check exceeds its control limits, the instrument is placed out of service until such time the reason for the out of control measurement can be identified and corrected.

3.1.4 Scintillation cell system: An alpha scintillation system designed to accept alpha scintillation flasks ("Lucas cells") should be used for the specific measurement of radium-226 by the radon emanation method. The detection system consists of a light-tight enclosure capable of accepting Lucas cells, a photomultiplier tube detector (phototube), and the appropriate electronics (high voltage supply, amplifier, timers, and scalars). The Lucas cells needed for this measurement may either be purchased from commercial suppliers or constructed by the laboratory.

Lucas cell efficiency calibration constants are checked and recorded at least quarterly or every 10 uses (whichever occurs more frequently) using a standardized solution of Ra-226 and a bubbler exclusively dedicated to this activity. All cell constants are measured relative to a specific scintillation cell counting system. Also, each Lucas cell should have acceptability limits for results that are calculated from the previous cell constants determined for each specific cell. If a laboratory has more than one scintillation cell counting system, the efficiency of each counting system should be determined using a calibrated alpha scintillation source. Their efficiencies are checked at least weekly to verify the ready status of the system to measure samples, or prior to use (whichever is more frequent). Cell backgrounds also are measured and recorded prior to the measurement of compliance monitoring samples. Control limits for both efficiency and background checks are calculated.

If a cell constant or efficiency check exceeds the determined control limits, the affected Lucas cell or counting system must not be used until the out of control parameter is investigated. If, after remeasuring the check source, the change in cell constant or counting system efficiency is confirmed, the laboratory supervisor may allow them

to be used, using the new cell constant or counting system efficiency when reducing counting data from compliance monitoring sample measurements. If the out of control check is the background of the Lucas cell, then the cell must be placed out of service until the excess background decays to levels that allow the required detection limits be achieved within reasonable counting times.

3.1.5 Gamma spectrometer systems: Either a solid-state, lithium-drifted germanium detector or a high-purity intrinsic germanium detector connected to a multichannel analyzer is needed if the laboratory is to be certified for analyses of photon emitting radionuclides.

A system with a lithium-drifted germanium, or a high-purity intrinsic germanium detector may be used for measurement of photon emitting radionuclides if the efficiency of the detector is adequate to meet the detection limits listed at 40 CFR part 141.25(c). These detectors should be shielded with a minimum of 10 cm of iron or equivalent. In addition to appropriate electronics, the multichannel analyzer should contain a memory of not less than 4096 channels and at least one readout device.

Upon receipt, after servicing or if the instrument's settings are changed for any reason, gamma spectrometry systems must be efficiency and energy calibrated, and the background of the detector cave measured for a sufficient time so that any potentially interfering photo-peaks from naturally occurring gamma emitters in the count room environment can be identified, as well as the gross gamma activity. Calibration sources must be in the same geometry the laboratory chooses to measure drinking water samples, and contain NIST-traceable sources for several gamma emitters whose photo-peaks span the width of the gamma spectral window the instrument is set to detect. The cave background must be remeasured at least monthly, and the background gross gamma activity recorded.

A calibration check source is measured prior to sample measurements on each day of use. If gamma detectors are idle, the calibration check source is measured at least weekly to verify the detector's ready status for measuring compliance monitoring samples.

Calibration check sources for intrinsic germanium gamma spectrometers must contain a minimum of two photo-peaks. One photo-peak is located at energies below the maximum efficiency energy of the detector (known as the "knee" of the detector energy vs efficiency curve) where the curve has a positive slope, and another above the detector efficiency knee, where the efficiency curve has a negative slope. Each peak must be evaluated for the detector efficiency at that energy, the channel number of the peak centroid, and the Full Width at Half Maximum (FWHM).

Warning limits and control limits for the efficiency, the channel number of the peak centroid, and the FWHM of each photo-peak in the check source, and the gross total activity of the detector cave background measurement is calculated. If any monitored parameter in the efficiency check source measurement exceeds their control limits, the gamma detector must be recalibrated. If the gross gamma activity of the cave background check exceeds its control limit, the source of the increased background must be identified, removed if possible, and the cave background remeasured prior to analyzing compliance monitoring samples.

3.1.6 Alpha Spectrometer Systems: Alpha spectrometry systems should be used for uranium measurements if specific uranium isotope activities are of interest. Alpha spectrometry systems consist of a solid state device (a silicon surface barrier detector, a passively implanted planar silicon detector, etc.) mounted in a vacuum chamber and connected to a multichannel analyzer system.

The detectors in alpha spectrometry systems are energy and efficiency calibrated, and the background of its sample chamber measured when it is placed into service. Energy calibrations are checked weekly, and efficiency calibrations checked monthly to verify the alpha spectrometry system's stability. The sample chamber background is checked at least monthly, and should be checked after each day of sample measurements to monitor for alpha recoil contamination from samples.

The alpha peaks in the check source are evaluated for the detector efficiency, the channel number of the peak centroid, and the FWHM. These alpha peak properties are recorded and control limits calculated. The background activity check is for total alpha activity for each detector sample chamber and is also recorded and used to set control limits. If any monitored alpha peak property is out of control, the detector is to be recalibrated.

If a background check is out of control, the detector and chamber is placed out of service until the source of the background increase is identified and removed. If excess alpha background cannot be removed, the detector is placed out of service until the contamination decays to a level that allows the required detection limits to be achieved within reasonable count times for the analyte being interfered with by the contamination.

3.1.7 Other Instrumentation: Other radiation measurement devices as mentioned in legislation or referred to in the approved methods can also be used for measuring radioanalytes in drinking water. Their initial calibration periods and checks should be consistent with the methods they are used for and the manufacturer's recommendations.

4. General Laboratory Practices

4.1 Chemicals/reagents

Chemicals and reagents should meet the specifications in the methods. If not specified, then "Analytical Reagent Grade" (AR) or American Chemical Society (ACS) grade chemicals or better should be used. Radioactive standards are to be certified by the National Institute of Standards and Technology (NIST) or traceable to a NIST-certified source.

4.2 Reagent Water

The laboratory should have a source of reagent water meeting the requirements of being an ASTM Type 1,2, or 3 reagent water, having a minimum resistivity of 10 megohms/cm at 25° C. It should be monitored daily by measuring the reagent water's conductivity or resistivity and documented.

Radioactive components have been known to break through reagent water manufacturing units before an increase in resistivity is noted. To monitor the background radioactivity of the reagent water, it is to be screened for radioactivity after each time the treatment unit is serviced, and periodically thereafter depending on volume of reagent water used at the laboratory between unit servicing.

4.3 Glassware/Plasticware

Specific requirements in the methods for the cleaning of glassware should be followed. The purpose of these requirements are to minimize the possibility that glassware can contaminate samples, and should include acid rinsing. Acid rinsing not only mobilizes any metals remaining adhering to their surfaces, but also hydrates the outer silica layer on glassware which inhibits contamination with radioactive materials. If there are no specifications for cleaning glassware in the method, then glassware should first be washed in detergent solution, then thoroughly rinsed in tap water followed by a second rinse in a dilute acid solution, and finally rinsed with reagent water and dried.

Laboratory glassware should also be checked periodically for cracks, scratches, and abrasions. If they are found to be damaged, they are to be discarded. Scratched glassware increases the likelihood of sample contamination or losses due to the increase of surface area that is exposed to the sample.

4.4 Safety

Guidelines in the Laboratory Safety Manual, the Chemical Hygiene Plan, or the Standard Operating Procedures (SOPs) should include safety training and protection information specific to a radiochemistry laboratory. This should include identifying what operations of the laboratory use hazardous materials, identify same, and determine the health risks that are possible if someone is exposed to them. This should also include what workers should do to protect themselves from exposure and possible injury. For radiochemistry laboratories, this should include when and how radiation shielding is used to protect analysts and technicians from harmful levels of radioactivity. It should also define when circumstances warrant the use of protective equipment, and should include the use of gloves, laboratory coats, eye protection and appropriate pipetting techniques to avoid exposure and possible injury from chemicals and radioactive substances.

5. Analytical Methods

The approved methods cited at 40 CFR part 141.25(a) and (b) must be used for the analysis of drinking water compliance samples. These are listed in Table VI-1.

5.1 *Standard Operating Procedures*

A laboratory-specific Standard Operating Procedure (SOP) should be written for each method used by the laboratory for measuring regulated radio-analytes in compliance monitoring samples. These SOPs should be consistent with a referenced approved method. Any EPA-approved modifications should be noted.

6. Sample Collection, Handling, and Preservation

Sample containers, preservatives, and holding times specified in the methods should be followed. Table VI-2 lists critical elements for sample handling, including preservation. Sample preservatives provided by the laboratory should be screened for radioactive content by lot number prior to their use in the laboratory and documented. Samples preserved with reagents not provided by the laboratory are to be accompanied by a blank, radioactive free sample that is preserved in the same manner as the submitted sample.

6.1 Composited Samples

If deemed acceptable by the state, samples may be composited by the utility or the laboratory as an alternate sampling protocol, provided that all sample aliquots are properly preserved at the time of collection. Since the required compliance monitoring measurement is "total activity" samples are not filtered before preservation, as it must represent the maximum potential exposure from drinking water. Samples must be drawn on a quarterly basis and where compositing is not done by the laboratory, there must be documentation submitted with the composite sample detailing on what days each aliquot was obtained, its volume, and when it was preserved. A sample of the preservative should accompany the composited sample to the laboratory to determine the contribution of radioactivity, if any, from the addition of the preservative to the sample. Analysis of composite samples must be completed within one year after the first sample is collected or within normal holding times if the compositing period is less than 90 days. Where possible, the laboratory should be responsible for managing the compositing of samples.

7. Quality Assurance

Laboratory Quality Assurance systems are all the protocols, policies and procedures implemented at a laboratory to ensure the generation of data of known quality. Comprehensive programs should encompass every aspect in the sample processing path through a laboratory, from sample receipt, storage, measurement, to producing the final report for the sample measurements as well as record keeping for all sampling information, raw data and reports for samples. It should also address laboratory support functions, such as specifying procedures to train and qualify technicians, instrument maintenance and calibration procedures, support equipment maintenance, purchasing supplies, and problem solving protocols. Each area discussed should address providing documented evidence of compliance with the specific areas of the Quality Assurance system. Specific areas for Quality Assurance systems are addressed in Chapter III, and areas specifically relevant in radiochemistry laboratories are discussed in the sections below.

7.1 General Requirements

7.1.1 Availability of Records and Documents: The analytical methods references, Quality Assurance Manual, and SOPs are to be readily available to the analysts. All QC data and records are to be available for inspection by the certification officer.

7.2 Balance and Weights

Radiochemistry laboratories should have balances with the appropriate ranges for their operations. Since weights are used to calculate gravimetric recoveries used in the calculation of analytical results for some methods, balances used to weigh samples should have a documented history of accuracy and precision. At a minimum, the laboratory should have an analytical balance with at least a .01 mg sensitivity to measure sample weights. Also, a top loading balance should be available to weigh out chemicals for reagent preparation. Balances must be re-calibrated at least annually with ASTM Type 1 weights. At least once every 31 calendar days, the analytical balance's range appropriate for sample measurements should be checked using three traceable Type 1, Class 1, or Class 2 weights that span the range of sample weighing needs. Prior to use, the calibration of the balance should be checked with the ASTM Type 1, Class 1, or Class 2 weight closest in weight to most sample measurements. Most commonly this is the 10 gram weight because tared steel planchets often weigh between 8 and 10 grams. These calibration checks should be recorded in a permanent log. Weights used for calibration checks should be recertified every five years.

7.3 Method Sensitivity Studies

The laboratory should determine the standard analytical conditions for each method for measuring compliance monitoring samples that can produce detection limits that are equal to or less than those specified in 40 CFR part 141.25(c)(1) Table B, and 40 CFR part 141.25(c)(2) Table C. These procedures should be consistent with Appendix C, of "Prescribed Procedures for the Measurement of Radioactivity in Drinking Water (EPA-600/4-80-032). Once the method standard analytical conditions are determined, the laboratories should then institute a monitoring program to ensure the sensitivity

of each method used to analyze compliance monitoring samples does not exceed the detection limits defined in the CFR references cited above.

Method background levels, as measured from a reagent blank, monitor for potential contamination from several potential sources of interferences in the sample preparation laboratory in addition to the background of the detector used for its measurement. Consequently, sensitivity monitoring for radiochemical methods used to analyze drinking water should use the results of the reagent blank. The activity and uncertainty of each reagent blank prepared with sample preparation batches should be calculated in the same concentration units as the detection limits listed in the regulations referred to above, recorded, then the activity plotted on a control chart to provide a record that will serve as a continuous contamination and sensitivity monitor for the method as it is performed at the laboratory.

7.4 Proficiency Test (PT) Studies

To be certified for the analysis of a radio-analyte in drinking water, a laboratory must successfully participate in at least one PT study for the analyte for which they are seeking certification. To maintain certification for an analyte, the laboratory must pass one study per year thereafter. Scoring criteria for PT samples can be found in the CFR as cited above. The laboratory may choose to participate in either type of study from a provider acceptable to the state or EPA that is described below.

7.4.1 Mixed Alpha and Mixed Beta/Gamma PT Studies: These PT study samples contain either a mixture of known alpha emitting radionuclides whose concentrations are unknown to the laboratory, or a mixture of known beta/gamma emitting radioanalytes whose concentrations are also unknown to the laboratory.

7.4.2 Other PT Studies: These are PT studies whose samples contain a single analyte at an unknown concentration, or varied number of analytes at unknown concentrations that are often associated with each other in drinking water. These studies are distributed several times per year. If the analyte the laboratory wishes to be certified for is not contained in a Mixed PT sample, they must successfully analyze it at least once a year by participating in one of these other PT studies that contain the radioanalyte for which the laboratory wishes certification.

7.4.3 Additional Proficiency Testing Studies: The laboratories may also participate in a second PT study each year for each analyte for which they seek certification. Voluntary interlaboratory round robin studies could meet this recommendation. These additional PT studies should be performed in the half of the year that the blind or analyte-specific PT study the laboratory participates in for annual certification is not performed. This will provide a biannual check of the laboratory's performance.

7.5 Operating Manuals

Operating manuals and calibration protocols for counting instruments should be available to analysts and technicians. These documents should be stored near the instruments for easy reference.

7.6 Maintenance of Records

Calibration data and maintenance records on all radiation instruments and analytical balances should be maintained in a permanent record. If this is a hard copy record, it should be maintained in a permanently bound notebook and all entries made in ink. If it is an electronic record, the information should be stored on a computer system that is password protected, and backed up weekly with a copy of the back up data stored offsite to be considered a permanent record.

7.7 Sample Measurement Quality Control Requirements

Just as the stability of the measurement instruments should be monitored to verify the measurements they produce will have reasonable accuracy, precision and freedom from interferences, similar controls should be used to monitor sample preparation activities. In order to assess the effect of sample preparation activities on the accuracy, precision, and freedom from interferences of drinking water compliance monitoring measurements, the laboratory includes the QC samples listed below at the specified frequencies for each preparation batch of samples that are measured for the radioanalytes for which the laboratory is certified, unless otherwise noted for some methods. A preparation batch of samples are those samples prepared sequentially together within a 24 hour period. The number of compliance monitoring samples in a preparation batch should not exceed 20 (excluding the QC samples described below).

7.7.1 Assessment of preparation batch precision: The laboratory should assess the precision of each sample preparation batch. This can be done in one of two alternative ways. First, the laboratory may measure a second aliquot of a sample for a duplicate measurement (DUP). Alternately, if nondetects for a method are frequent, the laboratory may elect to measure a matrix spike (MS)/matrix spike duplicate (MSD) pair instead. An MS is a second aliquot of a sample that has a known amount of the radioanalyte being measured added to it. An MSD is a third aliquot of the same sample that has the same amount of radioanalyte added to it as the MS. Regardless of sample choice, the DUP or MS/MSD pair must be prepared with the other samples with a frequency no less than 1 every 10 samples or less.

With the exception of gross alpha particle activity, gross beta particle activity, and tritium measurements, if there are insufficient sample volumes for a duplicated sample or a duplicate matrix spike sample in a set of samples, two aliquots from the laboratory's tap water may substitute for the sample aliquots and be used for the duplicated sample.

Precision shall be assessed by calculating the relative percent difference (RPD) for either the sample and its duplicate, or for the MS/MSD pair. An RPD result for either precision assessment measurement that exceeds its calculated control limit, which, ideally should be 20 percent or less, indicates the precision of the sample preparation batch is questionable, and data reported from these results should be flagged as having questionable precision.

If a duplicated sample measurement has an activity that is less than 5 times the radioanalyte's detection limit, and exceeds 20 percent RPD when compared to the first measurement for the sample, it may be reevaluated using the two measurement's replicate error ratio (RER). The RER of two measurements made from the same sample is an assessment of whether they are within two standard deviations of their aggregate measurement uncertainty of each other. The RER is calculated using the following formula;

$$RER = |A - B| / SQRT(s_a^2 + s_b^2) \leq 2$$

where;
A = Net Activity of the first measurement
B = Net Activity of the second measurement made from a different aliquot from the same sample.
s_A = The uncertainty of the first measurement
s_B = The uncertainty of the second measurement

If the RER is less than or equal to two, then the two measurements are within 2 standard deviations of each other, and so are acceptable. If the RER exceeds 2, it is unacceptable since it means there is greater than 2 standard deviations of difference between the two measurements.

If precision assessments exceed their limits, calculations and procedures should be examined and samples recounted. If the precision assessments are still unsatisfactory after the samples are recounted, then all sample results in the preparation batch are to be reported with a qualifier to indicate the measurement has questionable precision. If the client requires unqualified results, then all the samples in the discarded sample preparation batch are then re-measured using new aliquots of the sample if hold times and sufficient volume is available or resampled and reanalyzed.

7.7.2 Assessment of Preparation Batch Accuracy: Matrix Spike (MS) samples are prepared to monitor how sample preparation procedures impact the accuracy of measurements of the samples in a sample preparation batch. A MS sample is prepared by adding a known amount of the radioanalyte being measured to a second aliquot of a sample. The MS sample has radioanalyte activities added to them that are approximately 10 times the anticipated level of the sample activity, or the detection limit, as appropriate for the measured radioanalyte. MS samples should be prepared and processed with the samples at a frequency of one MS per sample preparation batch.

MS performance is assessed using the percent recovery of the known activity of radioanalyte added to the sample.

The percent recovery should be within the control limits calculated from previous MS measurements, which should ideally be ± 20 percent of the amount of activity added to the MS sample. For gross alpha particle activity, gross beta particle activity measurements, and Ra-228 methods of analyses, where experience has shown lower accuracies can be expected, control limits calculated from previous MS results should be within ± 30 percent of the amount of activity added to the MS for the accuracy relative to the sample matrices to be considered acceptable.

MS samples need not be included in preparation batches for certain methods. Since there is no way currently to quantitatively add an inert gas to a liquid, MS samples will not be required for radon in water measurements. Also, gamma screens of drinking water need not include MS samples in their preparation batches of samples. Density, as expressed by the average atomic weight, or Z, of the material surrounding a gamma detector is the principle factor in scattering or absorbing gamma rays. With respect to this property, there is little difference between deionized water and drinking water sample matrices. Consequently, the same information with respect to accuracy can be assessed with the laboratory fortified blank.(LFB).

With the exception of gross alpha particle activity, gross beta particle activity, and tritium measurements, if there are insufficient sample volumes for a matrix spike in a set of samples, two aliquots from the laboratory's tap water may substitute for the sample aliquots and used for the unspiked/spiked MS sample pair.

If MS assessments of accuracy exceed their calculated control limits, calculations and procedures should be examined and samples recounted. If the assessment is still unsatisfactory after the samples are recounted, then all sample results in the batch should be flagged as possibly biased low or high (as the result indicates) due to matrix effects. If the LFB assessment of accuracy independent of matrix effects is also unsatisfactory in the same preparation batch (see below), then all the samples in the discarded sample preparation batch are remeasured using new aliquots of the sample if hold times and sufficient volume is available.

7.7.3 Assessing the preparation batch accuracy independent of matrix effects: In order to assess the method's preparation batch accuracy independent of matrix effects, an (LFB) shall be prepared with each preparation batch of samples. The LFB is prepared by adding a known amount of the radioanalyte being measured to an aliquot of deionized water that is prepared using the same procedures used to measure the samples in the preparation batch. LFBs are prepared at a frequency of one per preparation batch.

LFB accuracy is assessed using the percent recovery of the known activity of radioanalyte added to the sample. The percent recovery should be within the control limits calculated from previous results, which should ideally be within ± 10 percent of the amount of activity added to the LFB sample. For gross alpha particle activity, gross beta particle activity, and Ra-228 measurements, where lower accuracies can be expected, the calculated control limits should not exceed ± 20 percent recovery of the amount of activity added to it.

If LFB assessments exceed their limits, calculations and procedures should be examined and samples recounted. If the LFB assessment is still unsatisfactory after the samples are recounted, the batch must be considered contaminated. All samples in the sample preparation batch must then be discarded. After the source of contamination is identified and addressed, all the samples in the discarded sample preparation batch must then be remeasured using new aliquots of the sample if hold times and sufficient volume is available.

7.7.4 Assessing instrument drift during sample measurements: While most radiation measurements systems are noteworthy for their stability, sudden changes can occur due to instrument component failure, loss of gas pressure, vacuum, or contamination of a detector or sample chamber from a high activity sample can possibly occur while samples are being counted. Instrument drift in detector efficiency and background must be checked both before and after measuring compliance monitoring samples to verify both the calibration and background did not change significantly while the samples were being measured.

A sample measurement, or "counting" batch is a second type of sample batch that can be defined in radiochemical methods of analysis. A sample measurement batch includes those samples that are measured within 24 hours of each other, and their measurements occur in between the detector efficiency and instrument background checks made prior to and at the end of measuring a set of compliance monitoring samples. Often a sample measurement

batch and a sample preparation batch consist of the same number of samples. In some cases, however, it may take several days to measure all the samples in a sample preparation batch. In these instances, efficiency and instrument background checks should not wait until the last sample in a preparation batch has been measured. To do so could possibly result in several days of lost counting time if they are out of control. Instead, a sample count order must be arranged so an efficiency check and an instrument background check is to be made at least every 24 hours while samples are being counted.

To facilitate these daily checks, the sample order may be arranged so a sample spiked with a known activity of the radioanalyte being measured (i.e an LFB, MS, or MSD sample) and a sample not expected to have measurable activity in it (RB) is counted at the end of a 24 hour period, or if the total sample measurement batch counting time does not exceed 24 hours, after all the other samples in the sample preparation batch have been measured. If these QC samples meet their acceptance criteria, then it may be assumed little or no change in the efficiency or background occurred while samples were being measured. If however, sample counting requirements prevent these samples from being counted at the end of each 24 hours sample measurement period or as the last samples measurements of the sample preparation batch, the efficiency calibration check source used for the efficiency calibration checks, and an instrument blank must be measured in their place. Both of these measurements must be within the control ranges produced from their previous measurements.

If either the QC samples or the instrument efficiency or background check exceed their calculated control limits, the instrument is placed out of service until the source of the out of control condition is identified and corrected. If the failed instrument QC check occurs with a measurement made at the end of measuring a sample measurement batch, then all the samples in the measurement batch are recounted after the source of the out of control condition is identified and corrected..

7.8 Instrument and Method Performance Charts/Records

Quality control performance records or control charts should be maintained for each instrument and method used by the laboratory for compliance monitoring sample measurements. For instruments, the initial calibrations and all efficiency calibration and instrument background checks should be maintained in a permanent record. The efficiency calibration and instrument background checks should have control limits calculated, and be control charted separately to monitor the instruments ready status to measure samples. For each method used for compliance monitoring measurements, the assessments of sample preparation batch precision (DUP or MSD), accuracy (MS and MSD), and interferences (RB) should be recorded separately from sample results in a permanent record. Laboratory-specific performance warning and control limits for each parameter monitored for both instruments and methods should be recalculated every 20 measurements for each QC parameter. If a QC result exceeds its calculated control limit, all measurements using the associated method or instrument must cease until the source of the exceedance is identified and corrected.

7.9 Quality Assurance (QA) Plan

The laboratory should prepare and follow a written QA plan (see Chapter III).

8. Records and Data Reporting

8.1 Legal Defensibility

Laboratories are to retain sufficient data and documentation for compliance monitoring samples so that their receipt and any measurement made for this purpose can be reproduced if validation of the data is required. The QA plan and/or SOPs should describe the policies and procedures used by the facility for record retention and storage. Since all compliance monitoring measurements are used to interpret and enforce legal requirements, chain of custody procedures should be used (See Appendix A).

8.2 Maintenance of Records

Public water systems are required to maintain records of radionuclide analyses of compliance samples for 10 years (40 CFR part 141.33). The laboratory should maintain easily accessible records for 10 years. The client water system should be notified before disposing of records so they may request copies if needed. This includes all raw data, calculations, and QC data. These data files may be either hard copy, microfiche, or electronic. Electronic data should always be backed up by protected tape, disk, or hard copy. If the laboratory changes its computer hardware or software, it should make provisions for transferring old data to the new system so that it remains retrievable within the time frames specified above. Data which is expected to become part of a legal action should be maintained for a longer period of time.

8.3 Sampling Records

Data should be recorded in ink with any changes lined through such that original entry is visible. Changes should be initialed and dated. The following information should be readily available in a summary or other record(s):

8.3.1 Date, location (including name of utility and PWSID), site within the system, time of sampling, name, organization and phone number of the sampler, and analyses required.

8.3.2 Identification of the sample as to whether it is a routine distribution system sample, check sample, raw or finished water sample, repeat or confirmation sample or other special purpose sample.

8.3.3 Date of receipt of the sample.

8.3.4 Sample volume/weight, container type, preservation and holding time and condition on receipt.

8.3.5 pH and disinfectant residual at time of sampling (from plant records).

8.3.6 Transportation and delivery of the sample (person/carrier, conditions).

8.4 Analytical Records

Data must be recorded in ink with any changes lined through such that original entry is visible. Changes must be initialed and dated. The following information must be readily available:

8.4.1 Laboratory and persons responsible for performing analysis.

8.4.2 Analytical techniques/methods used.

8.4.3 Date and time of analysis.

8.4.4 Results of sample and quality control analyses.

8.4.5 Calibration and standards information.

8.4.6 Counting data;

8.4.7 Results of analyses, including the activity, uncertainty of the measurement, and the method detection limit.

8.5 Data Reporting

Reports to drinking water suppliers and programs should contain sufficient information to establish the validity of the reported results for the required analyses. It should be designed as a summary form that contains the sampling and analytical information described above. At a minimum, these reports should contain the following information:

8.5.1 Name and location of the water supply, including its PWSID.

8.5.2 Location in the water supply where the sample was taken.

8.5.3 Date and time of sampling.

8.5.4 The name of the person responsible for taking the sample.

8.5.5 Sample receipt date at the laboratory.

8.5.6 Date and time of analysis.

8.5.7 The method used for the analysis.

8.5.8 The laboratory and the initials of the person responsible for performing the analysis.

8.5.9 The analytical result, including the calculated uncertainty of the measurement.

8.5.10 If the result is below the calculated detection limit for that sample, the result should be reported as less than the calculated detection limit.

8.6 Computer programs

Computer programs should be verified initially and periodically by manual calculations and the calculations should be available for inspection. Access to computer programs and electronic data should be limited to appropriate personnel.

9. Action Response to Laboratory Results

When a laboratory is responsible, either by contract or state policy, to report sample results which would cause a system to be out of compliance, the proper authority must be promptly notified and a request made for resampling from the same sampling point.

Table VI-1 Methods for Radionuclide Analysis CFR 141.25

Contaminant	Methodology	Reference (method or page number)									
		EPA[1]	EPA[2]	EPA[3]	EPA[4]	EPA[5]	SM[5]	ASTM[6]	USGS[7]	DOE[8]	Other
Naturally occurring											
Gross alpha[11] and beta	Evaporation	900.0	p 1	00-01	p 1		302, 7110 B	.....	R-1120-76	.....	
Gross alpha[11]	Co-precipitation	.....	.....	00-02	.....		7110 C	.....	.....	.....	
Radium 226	Radon emanation, Radiochemical	903.1 903.0	p 16 p 13	Ra-04 Ra-03	p 19		7500-Ra C 304, 305, 7500-Ra B	D 3454-97 D 2460-97	R-1141-76 R-1140-76	Ra-04	N.Y.[9]
Radium 228	Radiochemical	904.0	p 24	Ra-05	p 19		304, 7500-Ra D	.....	R-1142-76	.....	N.Y.[9] N. J.[10]
Uranium[12]	Radiochemical Fluorometric Alpha spectrometry Laser Phosphorimetry ICP-MS	908.0 908.1 .... 200.8[13]	.... 	.... 00-07 	.... p 33 		7500-U B 7500-U C (17th Ed.) 7500-U C (18th, 19th, or 20th Ed.) 3125	..... D 2907-97 D 3972-97 D 5174-97 D5673-03	..... R-1180-76 R-1181-76 R-1182-76	..... U-04 U-02	
Man-made											
Radioactive cesium	Radiochemical Gamma ray spectrometry	901.0 901.1	p 4	....	..... p 92		7500-Cs B 7120	D-2459-72 D 3649-91	R-1111-76 R-1110-76	..... 4.5.2.3	
Radioactive iodine	Radiochemical Gamma ray spectrometry	902.0 901.1	p 6 p 9	....	..... p 92		7500-I B 7500-I C, 7500-I D 7120 (19th Ed.)	 D 3649-91 D 4785-93	....	..... 4.5.2.3	
Radioactive Strontium 89, 90	Radiochemical	905.0	p 29	Sr-04	p. 65		303, 7500-Sr B	.....	R-1160-76	Sr-01 Sr-02	
Tritium	Liquid scintillation	906.0	p 34	H-02	p. 87		306, 7500-3H B	D 4107-91	R-1171-76	.....	
Gamma emitters	Gamma ray Spectrometry	901.1 902.0 901.0	.....	.....	p 92		7120 7500-Cs B 7500-I B	D 3649-91 D 4785-93	R-1110-76	GA-01-R	

The procedures shall be performed in accordance with the documents listed below. The incorporation by reference of the following documents was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies of the documents may be obtained from the sources listed below. Information regarding obtaining these documents can be obtained from the Safe Drinking Water Hotline at 800-426-4791. Documents may be inspected at EPA's Drinking Water Docket, 401 M Street, SW., Washington, DC 20460 (Telephone: 202-260-3027); or at the Office of Federal Register, 800 North Capitol Street, NW., Suite 700, Washington, DC.

1. "Prescribed Procedures for Measurement of Radioactivity in Drinking Water", EPA 600/4-80-032 , August 1980. Available at U.S. Department of Commerce, National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161 (Telephone 800-553-6847) , PB 80-224744.
2. "Interim Radiochemical Methodology for Drinking Water", EPA 600/4-75-008(revised), March 1976. Available at NTIS, ibid. PB 253258.
3. "Radiochemistry Procedures Manual", EPA 520/5-84-006, December 1987. Available at NTIS, ibid. PB 84-215581.
4. "Radiochemical Analytical Procedures for Analysis of Environmental Samples", March 1979. Available at NTIS, ibid. EMSL LV 053917.
5. "Standard Methods for the Examination of Water and Wastewater", 13th, 17th, 18th, 19th Editions, or 20th edition. 1971, 1989, 1992, 1995, 1998. Available at American Public Health Association, 1015 Fifteenth Street N.W., Washington, D.C. 20005. Methods 302, 303, 304, 305, and 306 are only in the 13th edition. Methods 7110B, 7110C, 7500-Ra B, 7500Ra C, 7500-Ra D, 7500-U B, 7500-Cs B, 7500-I B, 7500-I C, 7500-I D, 7500-Sr B, 7500-3H B are in the 17th, 18th, 19th and 20th editions. Method 7500-U C Fluorometric Uranium is only in the 17th edition, and 7500-U C Alpha spectrometry is only in the 18th 19th and 20th editions. Method 7120 is only in the 19th and 20th editions.
6. Annual Book of ASTM Standards, Vol. 11.02, 1999. Available at American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.
7. "Methods for Determination of Radioactive Substances in Water and Fluvial Sediments", Chapter A5 in Book 5 of Techniques of Water-Resources Investigations of the United States Geological Survey, 1977. Available at U.S. Geological Survey (USGS) Information Services, Box 25286, Federal Center, Denver, CO 80225-0425.
8. "EML Procedures Manual", 28th (1997) or 27th (1990) Edition, Volume I and II. Available at the Environmental Measurements Laboratory, U.S. Department of Energy (DOE), 376 Hudson Street, New York, NY 10014-3621.
9. "Determination of Ra-226 and Ra-228 (Ra-02)", January 1980, Revised June 1982. Available at Radiological Sciences Institute Center for Laboratories and Research, New York State Department of Health, Empire State Plaza, Albany, NY 12201.
10. "Determination of Radium 228 in Drinking Water", August 1980. Available at State of New Jersey, Department of Environmental Protection, Division of Environmental Quality, Bureau of Radiation and Inorganic Analytical Services, 9 Ewing Street, Trenton, NJ 08625.
11. Natural uranium and thorium-230 are approved as gross alpha calibration standards for gross alpha with co-precipitation and evaporation methods; americium-241 is approved with co-precipitation methods.
12. If uranium (U) is determined by mass, a 0.67 pCi/μg of uranium conversion factor must be used. This conservative factor is based on the 1:1 activity ratio of U-234 to U-238 that is characteristic of naturally occurring uranium.
13. "Methods for the Determination of Metals in Environmental Samples - Supplement I," EPA-600/R-94-111, May 1994. Available at NTIS, PB 94-184942.

Table VI-2: Sample Handling, Preservation, and Instrumentation

Parameter	Preservative[1]	Container[2]	Maximum Holding Time[3]	Instrumentation[4]
Gross Alpha	Conc. HCl or HNO_3 to pH <2[5]	P or G	6 mo	A, B, or G
Gross beta	Conc. HCl or HNO_3 to pH <2[5]	P or G	6 mo	A or G
Strontium-89	Conc. HCl or HNO_3 to pH <2[5]	P or G	6 mo	A or G
Strontium-90	Conc. HCl or HNO_3 to pH <2[5]	P or G	6 mo	A or G
Radium-226	Conc. HCl or[5] HNO_3 to pH <2	P or G	6 mo	A, B, D or G
Radium-228	Conc. HCl or HNO_3 to pH <2[5]	P or G	6 mo	A or G
Cesium-134	Conc. HCl to pH <2[5]	P or G	6 mo	A, C or G
Iodine-131	None	P or G	8 da	A, C or G
Tritium	None	G	6 mo	E
Uranium	Conc. HCl or HNO_3 to pH <2[5]	P or G	6 mo	A, B, F, H
Photon emitters	Conc. HCl or HNO_3 to pH <2[5]	P or G	6 mo	C

[1]It is recommended that the preservative be added to the sample at the time of collection unless suspended solids activity is to be measured. If the sample has to be shipped to a laboratory or storage area unpreserved, acidification of the sample (in its original container) may be delayed for a period not to exceed 5 days. A minimum of 16 hours must elapse between acidification and analysis.

[2]P = Plastic, hard or soft; G = Glass, hard or soft.

[3]Holding time is defined as the period from time of sampling to time of analysis. In all cases, samples should be analyzed as soon after collection as possible. If a composite sample is prepared, a holding time cannot exceed 12 months.

[4]A = Low background proportional system; B = Alpha and beta scintillation system; C = Gamma spectrometer [Ge(Hp) or Ge(Li)]; D = Scintillation cell system; E = Liquid scintillation system (section C.2.a); F = Fluorometer (section C.1.1); G = Low background alpha and beta counting system other than gas-flow proportional; H=Alpha spectrometry system.

[5]If HCl is used to acidify samples which are to be analyzed for gross alpha or gross beta activities, the acid salts must be converted to nitrate salts before transfer of the samples to planchets.

Example Checklists for On-Site Evaluation of Laboratories Analyzing Drinking Water for Radiochemistry

General Audit Information

Laboratory_____

Street_____

City_____State_____

Survey By_____

Affiliation_____

Date_____Telephone No._____

Laboratory_____Date_____

Location_____Evaluator_____

PERSONNEL

Position/Title	Name	Academic Training	Present Specialty	Years Experience (chemistry)	Years Experience (radiochemistry)
Laboratory Director					
Quality Assurance Officer					
Section/Division Chief/Director (if applicable)					
Supervisory Analyst					
Chemical Analyst(s)					
Chemical Technician(s)					
Computer Support Technician					
Electronics Support Technician					

Laboratory_____ Date_____

Location_____ Evaluator_____

LABORATORY FACILITIES

Item	Available		Comments
	Yes	No	
Laboratory			
Electrical outlets 120V ac. grounded			
Distilled or deionized water or ASTM type 1, 2, or 3			
Exhaust Hood			
Vacuum source			
Counting Room - separate from wet chemistry, sample and standards preparation area			
Regulated power supply			
Reagents			

Laboratory_____Date_____

Location_____Evaluator_____

GENERAL LABORATORY EQUIPMENT AND INSTRUMENTS

Item	No.of Units	Manufacturer	Model	Satisfactory Yes	Satisfactory No
Analytical Balance 0.1 mg sensitivity stable base ASTM type 1 or 2 weights or better					
pH meter ±0.5 units readability ±0.1 units line or battery					
Conductivity meter Readable in ohms or mhos Range of 2 ohms or mhos Line or battery					
Drying oven gravity or convection controlled from room temp to 180°C or higher (±2°C)					
Infrared lamp may be substituted for drying oven					
Desiccator Glass or plastic					
Hot plate temperature control					
Refrigerator					
Magnetic Stirrer variable speed Teflon coated stir bar					
Balance, top loading					
Glassware					
Thermometers					
Muffle furnace to 450°C					
Centrifuge to 3000 rpm to hold 4 x 50 mL					

Laboratory_____Date_____

Location_____Evaluator_____

ALL INSTRUMENTATION

Item	Yes	No	Comments
Are operating manuals readily available to the operator			
Are calibration protocols available to the operator			
Are calibrations kept in a permanent control chart			
Are permanent service maintenance records kept			

Laboratory_____Date_____

Location_____Evaluator_____

THIN WINDOW GAS-FLOW PROPORTIONAL COUNTER

Instrument number	Manufacturer	Model	Year	Sample Changing		
				Manual	Automatic	Capacity

	Counting Gas	Window Density (g/cm^2)	Instrument Background			
			Operating Voltage	Alpha cpm	Operating Voltage	Beta cpm

Calibration Standard Type: Alpha Beta Supplier: Alpha Beta	Calibration Frequency[1]				Service Maintenance Frequency[2]				Condition[3]		
	D	W	M	Other	Q	S	A	Other	G	R	N

WINDOWLESS GAS-FLOW PROPORTIONAL COUNTER

Instrument number	Manufacturer	Model	Year	Sample Changing		
				Manual	Automatic	Capacity

	Counting Gas	Window Density (g/cm^2)	Instrument Background			
			Operating Voltage	Alpha cpm	Operating Voltage	Beta cpm

Calibration Standard Type: Alpha Beta Supplier: Alpha Beta	Calibration Frequency[1]				Service Maintenance Frequency[2]				Condition[3]		
	D	W	M	Other	Q	S	A	Other	G	R	N

1. Daily, Weekly, Monthly.
2. Quarterly, Semiannually, Annually.
3. Good, operating but needs Repair, Not operating

Laboratory_____Date_____

Location_____Evaluator_____

LIQUID SCINTILLATION COUNTER

Instrument number	Manufacturer	Model	Year	Sample Changing		
				Manual	Auto	Capacity

Calibration Standard Type:	Calibration Frequency[1]				Service Maintenance Frequency[2]				Condition[3]		
	D	W	M	Other	Q	S	A	Other	G	R	N
Supplier:											

ALPHA SCINTILLATION COUNTER

Instrument number	Manufacturer	Model	Year	Sample Changing		
				Manual	Auto	Capacity

Calibration Standard Type:	Calibration Frequency[1]				Service Maintenance Frequency[2]				Condition[3]		
	D	W	M	Other	Q	S	A	Other	G	R	N
Supplier:											

RADON-GAS COUNTING SYSTEM

System number	Gas counting cells/system	Manufacturer of gas counting cell	Counting Instrument		
			Make	Model	Year

	Calibration Frequency[1]				Service Maintenance Frequency[2]				Condition[3]		
	D	W	M	Other	Q	S	A	Other	G	R	N

1. Daily, Weekly, Monthly.
2. Quarterly, Semiannually, Annually.
3. Good, operating but needs Repair, Not operating

Laboratory_____Date_____

Location_____Evaluator_____

GAMMA SPECTROMETER SYSTEM

Detector System	System Number										
	Type		Make		Model			Year		Size	
	Analyzer System										
	Make		Model		Year			Channels			
Calibration Standard Type											
	Calibration Frequency				Service Maintenance Frequency				Condition		
	D	W	M	Other	Q	S	A	Other	G	R	N
Supplier											

OTHER APPROVED DETECTOR

Detector System	System Number										
	Type		Make		Model			Year		Size	
	Analyzer System										
	Make		Model		Year			Channels			
Calibration Standard Type											
	Calibration Frequency[1]				Service Maintenance Frequency[2]				Condition[3]		
	D	W	M	Other	Q	S	A	Other	G	R	N
Supplier											

1. Daily, Weekly, Monthly.
2. Quarterly, Semiannually, Annually.
3. Good, operating but needs Repair, Not operating

Laboratory_____Date_____

Location_____Evaluator_____

SAMPLE HANDLING AND PRESERVATION

Parameter	Container Used	Preservative Used	Comments	Satisfactory Yes	Satisfactory No
Gross Alpha Activity					
Gross Beta Activity					
Strontium-89					
Strontium-90					
Radium-226					
Radium-228					
Cesium-134					
Iodine-131		NONE			
Tritium		NONE			
Uranium					
Photon Emitters					
a.					
b.					
c.					
d.					
e.					

Laboratory_____Date_____

Location_____Evaluator_____

METHODOLOGY

Parameter	Sample Load/Mo	Method[1] Used - Cite Edition, Year, and Page						Satisfactory	
		EPA	SMI	ASTM	USGS	DOE	Other	Yes	No
Gross Alpha Activity									
Gross Beta Activity									
Strontium-89									
Strontium-90									
Radium-226									
Radium-228									
Cesium-134									
Iodine-131									
Tritium									
Uranium									
Photon Emitters Identify:									
a.									
b.									
c.									
d.									
e.									

1 - Methods used must be referenced in the National Primary DrinkingWaterRegulations (40 CFR 141.25)

Laboratory_____Date_____

Location_____Evaluator_____

QUALITY CONTROL

Item	Performance Testing Studies	A[1]	B[2]	Other PE Studies	A[1]	B[2]
Participation in performance evaluation and Blind PE studies Reporting Period: _____ to _____	Gross Alpha			Gross Alpha		
	Gross Beta			Gross Beta		
	Sr-89			Sr-89		
	Sr-90			Sr-90		
	Ra-226			Ra-226		
	Ra-228			Ra-228		
	Uranium			Uranium		
	Cs-134			Cs-134		
	Cs-137			Cs-137		
	Co-60			Co-60		
	Ba-133			Written QA Plan implemented and available for review		
	Zn-65					
	Tritium					
	I-131					

	Frequency	Yes	No	Comment	Satisfactory Yes	Satisfactory No
Duplicate analyses						
Spikes						
Reagent Blanks						
Laboratory Fortified Blanks						
Failed PE studies						
Control charts						
Calibration and Maintenance records						

1 - Scheduled frequency of participation by the laboratory, times per year.

2 - Number of acceptable performance results in the past year, where an acceptable result is a normalized deviation from the known value of ≤3.0 sigma

Laboratory_____Date_____

Location_____Evaluator_____

DATA REPORTING

Item	Comments: systems used, frequency, etc.
Records kept for 10 years Actual laboratory reports	
Tabular Summary	
Information included Date	
Place of sampling	
Time of sampling	
Sampler	
Date of sample receipt	
Date of analysis	
Type of analysis	
Laboratory & person responsible	
Other reported data	
Other reported data	
Method(s) used	
Results	