United States
Environmental Protection
Agency

Office of
Drinking Water (WH-550D)
Washington DC 20460

EPA 570/9-90-008A
October 1991

EPA

Manual for the Certification of Laboratories Analyzing Drinking Water

Criteria and Procedures Quality Assurance

Third Edition

Prepared by
The Laboratory Certification Program Revision Committee

Change 1 - October 1991

Supersedes EPA/570/9-82/002, October 1982, entitled *Manual for the Certification of Laboratorie: Analyzing Drinking Water*

Printed on Recycled Paper

List of Effective Pages

REMOVE SUPERSEDED PAGES AND INSERT REVISED PAGES

Dates of issue for original and changed pages are as follows:

Original	0	April 1990
Change	1	October 1991

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Notice

This manual has been reviewed by the Office of Drinking Water and the Office of Research and Development and approved for publication. The mention of commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.

Foreword

Each of the approximately 200,000 public water systems in the United States must routinely monitor its drinking water to determine if it is adequately protected from regulated microbiological, chemical, and radiochemical contaminants. Because of the need to safeguard public health, it is imperative that every laboratory analyzing drinking water generate accurate data on a continuing basis. This laboratory certification manual will help the laboratory accomplish this task and also provide a means for the U.S. Environmental Protection Agency (EPA) to evaluate laboratory quality.

Specifically, this manual describes the operational and technical criteria and procedures that EPA will use to evaluate a laboratory for its ability to properly analyze regulated drinking water contaminants. The certification program described in this manual extends to the EPA Regional laboratories, principal State laboratories in States which have primary enforcement responsibility (primacy), and to all laboratories that perform analyses under the Safe Drinking Water Act in the few States without primacy. The vast majority of primacy States have their own laboratory certification programs. Although many of them use the EPA's program as presented in this manual, individual State programs may vary. Therefore, any laboratory outside the EPA's program that wants to become certified should contact the State program.

This manual is the result of sustained work by many individuals, representing EPA, States, and water systems. I hope the manual will be used as a practical tool for upgrading and maintaining laboratory quality. Your comments or suggestions will be considered in developing subsequent revisions of this manual.

James R. Elder, Director
Office of Ground Water
and Drinking Water

Preface

Since 1978, the U.S. Environmental Protection Agency (EPA) has had a program for certifying Regional laboratories, principal State laboratories in primacy States, and local laboratories in non-primacy States performing drinking water analyses required by regulations issued pursuant to the Safe Drinking Water Act. This document is the third edition of the manual describing the program's implementation procedures and technical criteria. It supersedes the *Manual for the Certification of Laboratories Analyzing Drinking Water*, EPA-570/9-82-002 (October 1982).

This revision was necessary to address the increased complexity of the revised drinking water regulations, clarify Regional responsibilities concerning State laboratory certification programs, reduce the time a laboratory can be "provisionally certified," and improve feedback to EPA on how laboratories perform on a routine basis. This edition is based on an ongoing review of the laboratory certification program to improve implementation and technical criteria in light of newly approved methodology and six additional years of experience with the program.

The document was prepared by a committee chaired by the EPA's Office of Drinking Water (ODW). Comments from the Regions and States were solicited and considered at several points in the preparation of this revision. These included recommendations from a workshop held in April 1987, at which all Regions and States were invited to share their views about both the implementation strategy and the technical criteria. Regions and States were represented on the revision steering committee and its various subcommittees and subgroups.

The EPA quality assurance program covers all activities relating to data collection, processing, and reporting. This is managed by the Office of Research and Development, Quality Assurance Management Staff (QAMS). This manual represents ODW's implementation of the QAMS program applicable to laboratories conducting drinking water analyses.

Like the previous edition, this program is not regulatory in nature (except for analytical methodology and requirements in the primary drinking water regulations), but rather offers guidance describing the recommended procedures and criteria for assuring data validity. Laboratories may use equivalent criteria, if these criteria are approved by the certifying authority.

EPA is currently developing new regulations for laboratory certification and certain pre-laboratory and post-laboratory activities. The Agency is undertaking this effort to ensure that all primacy States include in their certification programs those few basic elements that the Agency regards as critical to assuring data validity (e.g., certification downgrading procedures, training of on-site evaluators). EPA does not expect that the recommended procedures and criteria in this manual will conflict with these forthcoming regulations.

Unlike previous editions, this edition is in a loose-leaf format which will allow EPA to more easily update it from time to time. EPA will furnish revised pages to each State drinking water administrator and State laboratory director. Holders of this manual should check with the EPA Region or the State occasionally to make sure their manual is current.

In conclusion, EPA will use the certification criteria in this manual for evaluating all laboratories that it certifies (Regional laboratories, principal State laboratories, and local laboratories in non-primacy States). The Agency will also use this manual as guidance in determining the adequacy of State certification programs for local laboratories.

TO USERS OF THE MANUAL FOR THE CERTIFICATION OF LABORATORIES ANALYZING DRINKING WATER (1990 EDITION).

Enclosed is Change 1 to the manual, which revises Chapter V. This revision includes the certification criteria for the revised Total Coliform Rule and Surface Water Treatment Requirements. The change is to be inserted into the manual, and the previous edition of Chapter V completely removed, along with the other pages indicated on page ii of the update.

Enclosure

Acknowledgments

This manual was prepared through the efforts of many individuals, including representatives from U.S. Environmental Protection Agency program offices and laboratories, Regional offices, States, and utility laboratories. The principal contributors are listed below.

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Chapter V
Microbiology

Note: quality control items are designated as "QC" and necessitate written records which are to be retained for five years.

1. Personnel

1.1 Supervisor/Consultant

The supervisor or consultant is a professional scientist experienced in water microbiology. If a supervisor is not available, a consultant having the same qualifications may be substituted. State laboratory personnel would be a primary source for consultants.

1.1.1 Academic Training: Minimum of a bachelor's degree in science.

1.1.2 Job Training: Minimum of two weeks training from a Federal agency, State agency, or academic institution in microbiological analysis of drinking water.

1.2 Analyst (or equivalent job title)

The analyst performs microbiological tests with minimal supervision.

1.2.1 Academic training: Minimum of high school education.

1.2.2 Job training: Training in microbiological analysis of drinking water, acceptable to the State (or EPA for nonprimacy States), plus a minimum of 30 days on-the-job training. Personnel should take advantage of workshops and training programs available from Federal and State regulatory agencies and professional societies.

1.2.3 Experience: At least six months of bench experience in sanitary, water, milk, or food microbiology.

2. Laboratory Facilities

Laboratory facilities are clean and temperature and humidity controlled, and have adequate lighting at bench tops. The laboratory has provisions for disposal of microbiological waste. It is recommended that the laboratory contain 150-200 square feet and 5 to 6 linear feet of usable bench space per analyst. Laboratory facilities should include sufficient bench-top area for processing samples; storage space for media, glassware, and portable equipment; floor space for stationary equipment (incubators, waterbaths, refrigerators, etc.); and associated area(s) for cleaning glassware and sterilizing materials.

While safety criteria are not an aspect of laboratory certification, laboratory personnel should be aware of general and customary safety practices for laboratories. Each laboratory is encouraged to have a safety plan available.

3. Laboratory Equipment and Supplies

3.1 pH Meter

3.1.1 Accuracy and scale graduations within ±0.1 units.

3.1.2 Use pH buffer aliquot only once.

3.1.3 Maintain electrodes according to manufacturer's recommendations.

QC **3.1.4** Standardize pH meter each use period with pH 7.0 and pH 4.0 standard buffers.

QC **3.1.5** Date commercial buffer solution container upon receipt, and when opened. Discard before expiration date.

3.2 Balance (top loader or pan)

3.2.1 Balance detects 100 mg at a 150 gram load.

QC **3.2.2** Calibrate balance monthly using Class S or S-1 reference weights (minimum of three traceable weights which bracket laboratory weighing needs) or weights traceable to Class S or S-1 weights. Calibrate non-reference weights annually with Class S or S-1 reference weights.

Correction data necessary with S or S-1 reference weights.

QC 3.2.3 Maintain service contract or internal maintenance protocol and maintenance records. Maintenance conducted annually at a minimum.

3.3 Temperature Monitoring Device

3.3.1 Use glass/mercury or dial thermometers graduated in 0.5°C increments or less in incubator units. Mercury column in glass thermometers is not separated.

QC 3.3.2 Check calibration of in-use glass/mercury thermometers annually and in-use dial thermometer quarterly, at the temperature used, against a reference National Institute of Standards and Technology (formerly National Bureau of Standards) (NBS) thermometer or one that meets the requirements of NBS Monograph SP 250-23.

QC 3.3.3 Recalibrate continuous recording devices annually which are used to monitor incubator temperature. Use same reference thermometer described in QC 3.3.2.

3.4 Incubator Unit

3.4.1 Incubator unit has an internal temperature monitoring device and maintains a temperature of 35° ± 0.5°C and, if used, 44.5° ± 0.2°C. For nonportable incubators, place thermometers on the top and bottom shelves of the use area with the thermometer bulb immersed in liquid. If an aluminum block is used, culture dishes and tubes fit snugly.

QC 3.4.2 Record temperature for days in use at least twice per day with readings separated by at least 4 hours.

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

3.5 Autoclave

3.5.1 Autoclave has a temperature gauge with a sensor on the exhaust, a pressure gauge, and an operational safety valve. Autoclave maintains sterilization temperature during the sterilizing cycle and completes an entire cycle within 45 minutes when a 12-15 minute sterilization period is used. Autoclave depressurizes slowly to ensure media do not boil over and bubbles do not form in inverted tubes.

3.5.2 Because of safety concerns and difficulties with operational control, pressure cookers and vertical autoclaves are not acceptable.

QC 3.5.3 Record date, contents, sterilization time, and temperature for each cycle. Establish service contract or internal maintenance protocol, and maintain records.

QC 3.5.4 Use maximum-temperature-registering thermometer, heat-sensitive tape, or spore strips or ampoules during each autoclave cycle and record temperature. Avoid overcrowding.

QC 3.5.5 Check automatic timing mechanism with stopwatch quarterly.

3.6 Hot Air Oven

3.6.1 The oven maintains a stable sterilization temperature of 170°-180°C for at least two hours. Sterilize only dry items and avoid overcrowding. The oven thermometer is graduated in 10°C increments or less, with the bulb placed in sand during use.

QC 3.6.2 Record date, contents, and sterilization time and temperature of each cycle.

3.7 Colony Counter

Use colony counter, dark field model, to count Heterotrophic Plate Count colonies.

3.8 Conductivity Meter

Suitable for checking laboratory pure water. Readable in ohms or mhos, with a range from at least 2 ohms to 2 megohms or equivalent micromhos ± 2%. Unit may be in-line/bench or portable/battery operated.

QC 3.8.1 Conductivity meter is calibrated monthly with a 0.01 M KCl solution, or lower concentration if desired (see Method 120.1 in *Methods for Chemical Analyses of Water and Wastes*, 1979, EPA/600/4-79/020 (revised 1983); or Section 205, "Conductivity," pp. 76-80, in *Standard Methods for the Examination of Water and Wastewater* (16th ed.), 1985). An inline conductivity meter need not be calibrated.

3.9 Refrigerator

3.9.1 Refrigerator maintains a temperature of 1° to 5°C. Thermometer graduated in at least 1°C increments with the thermometer bulb immersed in liquid.

QC 3.9.2 Record temperatures for days in use at least once per day.

3.10 *Inoculating Equipment*

Metal or plastic loops, or wood applicator sticks sterilized by dry heat. The metal inoculating loops and/or needles are made of nickel alloy or platinum.

3.11 *Membrane Filtration Equipment (if MF procedure is used)*

3.11.1 MF units are stainless steel, glass, or autoclavable plastic, not scratched or corroded, and do not leak.

3.11.2 10X to 15X magnification device with fluorescent light source used to count sheen colonies.

3.11.3 Membrane filters approved by the manufacturer for total coliform water analysis. Approval based on data from tests for toxicity, recovery, retention, and absence of growth-promoting substances. Filters are cellulose ester, white, gridmarked, 47 mm diameter, and 0.45 µm pore size, or alternate pore sizes if manufacturer provides performance data equal to or better than the 0.45 µm pore size. Membrane filters are purchased presterilized or autoclaved before use.

QC **3.11.4** Record the lot number and date received for membrane filters. If the quality and performance of membrane filters are questionable, new lot(s) of membrane filters can be checked by comparing recovery of coliform organisms against membrane filters from a previously acceptable lot. (Suggested procedure: Obtain a natural coliform-positive water sample or prepare a laboratory water sample using a pure coliform culture. New lots of membrane filters are evaluated by passing a sufficient volume of water sample through a membrane filter from a new lot and a membrane filter known to be acceptable so that 30 to 60 coliform colonies are observed on the acceptable membrane filter after 24 hours incubation at 35°C. The colony counts on the membranes are evaluated using the formula:

$$\text{Critical value}^* = \frac{A - B - 1}{\sqrt{A + B}}$$, where

A is the count on the acceptable membrane filter, and B is the count on the membrane filter from a new lot.

If the critical value is not less than 1.96, the new membranes should be considered unacceptable.) Unacceptable membrane filters are returned to the vendor with a request to replace them with membrane filters from a different lot number. Replacement membranes are submitted to the same comparative procedure. (This comparative procedure will demonstrate gross differences between the membranes; other, more stringent comparative procedures are acceptable).

QC **3.11.5** Record the lot number and date received for membrane filters.

QC **3.11.6** Check sterility of each lot number of membranes by placing one membrane in 50 mL volume of non-selective broth medium (e.g., tryptic soy broth) and check for growth after 24 hours incubation at 35° ± 0.5°C.

3.12 *Culture Dishes (loose or tight lid)*

3.12.1 Use presterilized plastic or sterilizable glass culture dishes. To maintain sterility of glass culture dishes, use stainless steel or aluminum canisters, or wrap dishes in a heavy aluminum foil or char-resistant paper.

3.12.2 Incubate loose-lid dishes in a tight-fitting container, e.g., plastic vegetable crisper, to prevent dehydration of membrane filter and medium.

3.12.3 Reseal opened packs of disposable culture dishes between major use periods.

3.13 *Pipets*

3.13.1 To sterilize and maintain sterility of glass pipets, use stainless steel or aluminum canisters, or wrap individual pipets in char-resistant paper.

3.13.2 Pipets have legible markings and are not chipped or etched.

3.13.3 Opened packs of disposable sterile pipets are resealed between major use periods.

3.14 *Culture Tubes, Containers, and Closures*

3.14.1 Tubes and containers are made of borosilicate glass or other corrosion-resistant glass, or plastic.

*Hald, *Statistical Theory with Engineering Applications*. John Wiley and Sons, Inc., New York, NY, 1960, p. 725.

3.14.2 Culture tubes and containers are of a sufficient size to contain medium plus sample without being more than three quarters full.

3.14.3 Tube and container closures are stainless steel, plastic, aluminum, or screw caps with non-toxic liners. Cotton plugs are not acceptable.

3.15 Sample Containers

3.15.1 Sample bottles are wide mouth plastic or non-corrosive glass with a non-leaking ground glass stopper or a cap with a non-toxic liner which will withstand repeated sterilization, or other appropriate sample containers. Capacity of sample containers is at least 120 mL (4 oz.).

3.15.2 Glass stoppered bottle closures are covered with aluminum foil or char-resistant paper for sterilization.

3.15.3 Glass and plastic bottles are sterilized by autoclaving; glass bottles may also be sterilized by dry heat. Moisten empty containers with several drops of distilled water before autoclaving to prevent an "air lock" sterilization failure.

3.16 Glassware and Plasticware

3.16.1 Glassware is borosilicate glass or other corrosion-resistant glass and free of chips and cracks. Markings on graduated cylinders and pipets are legible. Plastic items are clear and non-toxic.

3.16.2 Graduated cylinders for measurement of sample volumes have a tolerance of 2.5% or less. In lieu of graduated cylinders, use pre-calibrated containers that have clearly marked volumes of 2.5% tolerance. Spot check the calibration of each lot of pre-calibrated containers.

3.16.3 Pipets delivering volumes of 10 mL or less are accurate within a 2.5% tolerance or less.

3.17 Ultraviolet Lamp (if used)

3.17.1 Disconnect unit monthly and clean lamps by wiping with soft cloth moistened with ethanol.

QC 3.17.2 If UV lamp is used for sanitization, test lamp quarterly with UV light meter and replace if it emits less than 70% of initial output or if agar spread plates containing 200 to 1000 microorganisms, exposed to the UV light for 2 minutes, do not show a count reduction of 99%. Other methods may be used to test a lamp if they are as effective as the two suggested methods.

4. General Laboratory Practices

4.1 Sterilization Procedures

4.1.1 The times for autoclaving materials at 121°C are listed below. Except for membrane filters and pads and carbohydrate-containing media, indicated times are minimal times which may necessitate adjustment depending upon volumes, containers, and loads.

Item	Time (minutes)
Membrane filters & pads	10
Carbohydrate containing media	12-15
Contaminated test materials	30
Membrane filter assemblies	15
Sample collection bottles	15
Individual glassware	15
Dilution water blank	15
Rinse water	15

4.1.2 Remove autoclaved membrane filters and pads and all media immediately after completion of sterilization cycle.

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

4.1.4 Ultraviolet light (germicidal lamp, 2537 angstroms) may be used alternatively to sanitize equipment, if all supplies are presterilized and QC checks are conducted as indicated in paragraph 3.17. Ultraviolet light or boiling water may also be used to control bacterial carry-over between samples during a filtration series. If boiling water is used, membrane filter equipment should be submerged for about two minutes and then cooled to room temperature before filtering the next sample.

4.2 Sample Containers

4.2.1 Add sodium thiosulfate ($Na_2S_2O_3$; anhydrous, 100 mg/L) to sample containers before sterilization (0.1 mL of 10% $Na_2S_2O_3$ solution per 120 mL capacity).

QC 4.2.2 Select at least one sample container at random from each batch of sterile sample bottles, or other appropriate containers, and confirm sterility by adding approximately a 25 mL volume of a sterile non-selective broth (e.g., tryptic soy, trypticase soy, or tryptone broth).

Incubate at 35° ± 0.5°C for 24 hours and check for growth.

4.3 Reagent Water

4.3.1 Use only satisfactorily tested reagent water from stills or deionization units to prepare media, reagents, and dilution/rinse water for performing bacteriological analyses.

QC **4.3.2** Test the quality of the reagent water or have it tested by a certified laboratory to assure it meets the following:

Parameter	Limits	Frequency
Conductivity	> 0.5 megohms resistance or < 2 micromhos/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[1]	Nondetectable	Monthly
Heterotrophic Plate Count[2]	< 500/mL	Monthly
Bacteriological Quality of Reagent Water[3]	Ratio of growth rate 0.8-3.0	Annually

[1] DPD Method not required if source water is not chlorinated.
[2] Pour Plate Method.
[3] Test for bacteriological quality of reagent water (*Standard Methods for the Examination of Water and Wastewater*, 16th Edition p. 835; also *Microbiological Methods for Monitoring the Environment*, EPA-600/8-78-017, p.200). Control water for test is defined as double distilled water using a glass still.

4.4 Dilution/Rinse Water

4.4.1 Prepare stock buffer solution or peptone water using reagent water according to *Standard Methods for the Examination of Water and Wastewater*, 16th edition, p 855.

4.4.2 Stock buffer is autoclaved or filter-sterilized. Label and date containers. Ensure stored stock buffer is free of turbidity.

4.4.3 Dilution/rinse water is prepared by adding 1.25 mL volume of stock buffer solution and 5 mL volume of magnesium chloride ($MgCl_2$) solution (81.1 g $MgCl_2 \cdot 6\ H_2O$/L) per liter of reagent water.

QC **4.4.4** Check each batch of dilution/rinse water for sterility by adding 50 mL of water to a 50 mL of a double strength non-selective broth (e.g., tryptic soy, trypticase soy or tryptose broth). Incubate at 35° ± 0.5°C for 24 hours and check for growth.

4.5 Glassware Washing

4.5.1 Use distilled or deionized water for final rinse.

QC **4.5.2** Perform the Inhibitory Residue Test (*Standard Methods for the Examination of Water and Wastewater*, 16th edition, p. 834, and *Microbiological Methods for Monitoring the Environment*, U.S. EPA-600/8-78-017 p. 199) on the initial use of a washing compound and whenever a different formulation of washing compound, or washing procedure, is used to ensure that glassware is free of toxic residue.

4.6 Analytical Media

4.6.1 General

4.6.1.1 Use of dehydrated or prepared media manufactured commercially is strongly recommended due to concern about quality control. Store dehydrated media in a cool, dry location and discard caked or discolored media.

4.6.1.2 Date bottles of dehydrated media upon receipt and also when initially opened. Discard dehydrated media 6 months after opening; if stored in a desiccator, storage is extended to 12 months. Discard any dehydrated media that has passed the manufacturer's expiration date.

QC *4.6.1.3* For media prepared in the laboratory, record the date of preparation, type of medium, lot number, sterilization time and temperature, final pH, technician's initials.

QC *4.6.1.4* For liquid media prepared commercially, record date received, type of medium, lot number, and pH verification. Discard medium by manufacturer's expiration date.

QC *4.6.1.5* Check each lot of commercial and each batch of laboratory-prepared medium before use for performance with positive and negative culture controls. These control organisms can be stock cultures (periodically checked for purity)

or commercially available disks impregnated with the organism.

4.6.1.6 Refrigerate prepared plates in sealed plastic bags or containers to minimize evaporation.

4.6.2 Membrane Filter (MF) Media (for total coliforms)

4.6.2.1 Use m-Endo broth or agar or m-Endo LES agar in the single step or enrichment techniques. Ensure that ethanol used in rehydration procedure is not denatured. Prepare medium in a sterile flask and use a boiling water bath or, if constantly attended, a hot plate with a stir bar to bring medium just to the boiling point. Do not boil medium. Final pH 7.2 ± 0.2.

4.6.2.2 Refrigerate MF broth no longer than 96 hours, poured MF agar plates no longer than 2 weeks, and ampouled m-Endo broth in accordance with manufacturer's expiration date. Discard earlier if growth or surface sheen is observed.

4.6.3 Multiple Tube Fermentation (MTF or MPN) Media (for total coliforms)

4.6.3.1 Use lauryl tryptose broth or lactose broth in the presumptive test and single strength brilliant green lactose bile (BGLB) broth in the confirmed test. The appropriate presumptive test medium concentration should be adjusted to compensate for sample volume so that the resulting medium after sample addition is single strength. Autoclave media at 121°C for 12-15 minutes (see *Standard Methods* for more specific guidance). Final pH: presumptive test media, 6.8 ± 0.2; BGLB broth, 7.2 ± 0.2).

4.6.3.2 Examine tubes following sterilization to ensure that the inverted vials are one-third to one-half covered by the medium and free of air bubbles.

4.6.3.3 If MPN media are refrigerated after sterilization, incubate overnight at 35°C before use. Discard tubes/bottles showing growth and/or bubbles. Use MPN media prepared in tubes/bottles with loose-fitting closures within one week. If prepared broth media are stored, maintain in the dark at 4°C in screw-cap tubes/bottles no longer than 3 months. Discard media if evaporation exceeds 10% of original volume.

4.6.3.4 Use m-Endo agar LES for the completed test. Use refrigerated medium on plates within two weeks, and examine before use. If growth is observed, discard plates. Protect medium from light.

4.6.4 Presence-Absence (P-A) Coliform Test Medium

4.6.4.1 Autoclave for 12 minutes at 121°C. Allow space between bottles.

4.6.4.2 If prepared medium is stored, maintain in a culture bottle at 4°C in the dark for no longer than 3 months. Discard earlier if evaporation exceeds 10% of original volume.

4.6.4.3 Final pH: 6.8 ± 0.2.

4.6.5 EC Medium (for fecal coliforms)

4.6.5.1 Autoclave for 12-15 minutes at 121°C.

4.6.5.2 Examine tubes following sterilization to ensure that the inverted vials are one-third to one-half covered by the medium and free of air bubbles.

4.6.5.3 Use prepared medium in tubes with loose-fitting closures within one week. If prepared medium is stored, maintain in tightly closed screw-cap tubes at 4°C no longer than 3 months. Incubate refrigerated sterilized medium overnight at 35°C; discard tubes that show growth and/or bubbles.

4.6.5.4 Final pH: 6.9 ± 0.2.

4.6.6 MMO-MUG Test Medium (for total coliforms)

4.6.6.1 Do not prepare this medium from basic ingredients.

4.6.6.2 Protect medium from light.

4.6.6.3 Ingredients and tubes supplied by manufacturer(s) are sterile. Do not autoclave.

4.6.7 EC Medium + MUG (for *E. coli*)

QC *4.6.7.1* Incubate control cultures at 35° ± 0.5°C for 24 hours in lauryl tryptose

broth. Transfer a loopful to EC Medium + MUG and incubate at 44.5° ± 0.2°C for 24 hours. Read and record the results.

4.6.7.2 Check test tubes and autoclaved medium before use with a 366-nm ultraviolet light to ensure they do not fluoresce. If the tubes or medium exhibits faint fluorescence, either use non-fluorescing tubes or another lot of medium that does not fluoresce, or include a MUG-positive (*E. coli*) and MUG-negative (e.g., uninoculated) control for each analysis.

4.6.7.3 Do not use an inverted vial; gas production is not relevant to test, and the use of an inverted vial may cause confusion on test interpretation.

4.6.7.4 MUG may be added to EC Medium before autoclaving. EC Medium + MUG is also available commercially. Final MUG concentration is 50 µg/mL.

4.6.7.5 Final pH: 6.9 ± 0.2.

4.6.7.6 Use prepared medium in tubes with loose-fitting closures within one week. If prepared medium is stored, maintain in tightly closed screw-cap tubes at 4°C no longer than 3 months. Incubate refrigerated sterile medium overnight at 44.5°C; discard tubes with growth.

4.6.8 Nutrient Agar Medium + MUG (for *E. coli*)

QC *4.6.8.1* In accordance with paragraph 4.6.1.5, spot-inoculate control cultures onto a membrane filter on m-Endo LES agar and incubate at 35° ± 0.5°C for 18-24 hours. Then transfer the membrane filter to Nutrient Agar + MUG and incubate at 35°C for four hours. Read and record the results.

4.6.8.2 Sterilize medium in 100-mL volumes at 121°C for 15 minutes. MUG may be added to Nutrient Agar before autoclaving. Nutrient Agar + MUG is also available commercially. Final MUG concentration is 100 µg/mL.

4.6.8.3 Final pH: 6.8 ± 0.2.

4.6.8.4 If sterile medium is stored, refrigerate the medium in petri dishes, in a plastic bag or tightly closed container, and use within two weeks. Incubate stored medium overnight at 35°C before use; discard plates with growth.

4.6.9 Heterotrophic Plate Count (HPC) Media (includes tryptone glucose extract agar, plate count agar, and R2A agar)

4.6.9.1 Autoclave agar medium at 121°C for 15 minutes. Final pH: Plate Count Agar 7.0 ± 0.2; TGE Agar 6.8 - 7.0; R2A Agar 7.2.

4.6.9.2 Temper melted agar at 44-46°C before pouring. Hold melted agar no longer than 3 hours. Do not melt sterile agar medium more than once.

4.6.9.3 Store sterile refrigerated medium in bottles and petri dishes for up to 2 weeks. Store prepared petri dishes with R2A medium for up to one week, inverted and refrigerated, in a plastic bag or tight container. Incubate stored medium overnight at 35°C before use; discard plates with growth.

4.6.10 A-1 Medium (for fecal coliform enumeration in source water only)

4.6.10.1 Sterilize by autoclaving at 121°C for 10 minutes.

4.6.10.2 Final pH: 6.9 ± 0.1.

4.6.10.3 Examine tubes following sterilization to ensure that the inverted vials are one-third to one-half covered by the medium and free of air bubbles.

4.6.10.4 Store in dark at room temperature not more than one week.

4.6.11 Fecal Coliform Membrane Filter (M-FC) Broth/Agar (for fecal coliform enumeration in source water only)

4.6.11.1 Sterilize M-FC broth (with or without agar) by bringing it to boiling point. Do not autoclave.

4.6.11.2 Final pH: 7.4.

4.6.11.3 Refrigerate prepared medium. Discard broth medium after 96 hours,

and agar medium after two weeks. Discard earlier if growth is observed.

5. Analytical Methodology

5.1 General

5.1.1 Use only the analytical methodology specified in the Total Coliform Rule (40 CFR 141.21(f)) and the Surface Water Treatment Requirements (40 CFR 141.74(a)) (see Appendix H).

5.1.2 A laboratory should be certified for all analytical methods indicated below 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. In addition, for principal State laboratories and other laboratories that may enumerate heterotrophic bacteria for compliance purposes, the laboratory should be certified for the Pour Plate Method.

5.1.3 When impregnating an absorbent pad with a liquid medium, ensure that pad is saturated.

5.1.4 Shake water sample vigorously before analyzing.

QC **5.1.5** Each month, perform the coliform procedure normally used on a known coliform-positive, and fecal- or *E. coli*-positive sample.

5.2 Membrane Filter Technique (for total coliforms in drinking water)

5.2.1 Sample volumes analyzed by the MF procedure must be 100 mL ± 2.5 mL.

QC **5.2.2** Conduct sterility check at the beginning and end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter. If control indicates contamination, reject all data from affected samples and request immediate resampling.

5.2.3 Incubate at 35° ± 0.5°C for 22-24 hours.

5.2.4 Invalidate all samples resulting in confluent or TNTC (too numerous to count) growth. 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, without evidence of sheen colonies (total coliforms), covering the entire membrane filter. TNTC is defined as greater than 200 colonies on the membrane filter in the absence of detectable coliforms. Do not invalidate sample when the membrane filter contains at least one total coliform colony.

5.2.5 Verify all sheen colonies (up to at least ten colonies) using either single strength lactose or LTB and then single strength BGLB broth (same media used in MPN procedure), or EPA-approved cytochrome oxidase and β-galactosidase rapid test procedure. Colonies can be transferred with a sterile needle or applicator stick.

5.2.6 Test total coliform colonies for either fecal coliforms or *E. coli.* When EC Medium or EC Medium + MUG is used, transfer the colonies by using one of the options specified by §141.21(f) (5) (see Appendix H). If a swab is used to transfer a total coliform-positive culture, a single swab can be used to inoculate up to three different media (e.g., EC Medium, lauryl tryptose broth, and BGLB broth).

5.3 Multiple Tube Fermentation Technique (for total coliforms in drinking water)

5.3.1 Various testing configurations can be used (see 40 CFR 141.21 (f) (3) (i) and (4)), as long as a total sample volume of 100 mL is examined for each test.

5.3.2 Incubate at 35° ± 0.5°C for 24 ± 2 hours. If no gas is detected, incubate for another 24 hours.

5.3.3 Invalidate all samples which produce a turbid culture in the absence of gas production, in lauryl tryptose broth or lactose broth. Collect, or request the system collect, another sample from the same location as the original invalidated sample. (The laboratory may streak the turbid, total coliform-negative culture onto m-Endo agar, incubate, and examine for total coliforms, and perform HPC. Although not required, this information may help the system assess its problem.)

5.3.4 Confirm 24 and 48 hour gas-positive tubes using BGLB broth.

QC **5.3.5** Perform completed test on not less than 10% of all coliform-positive samples per quarter. If no coliform-positive samples have been observed during a quarter, obtain one and perform the method through the completed test.

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

5.4.1 Inoculate 100-mL sample into P-A culture bottle.

5.4.2 Incubate at 35° ± 0.5°C, and observe for yellow color after 24 and 48 hours.

5.4.3 Confirm yellow cultures in BGLB broth.

5.4.4 Invalidate all samples which produce a non-yellow turbid culture in P-A medium. Collect, or request the system collect, another sample from the same location as the original invalidated sample. (The laboratory may streak the non-yellow, turbid, total coliform-negative culture onto m-Endo agar, incubate, and examine for total coliforms, and perform HPC. Although not required, this information may help the system assess its problem.)

5.5 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)

5.5.1 Use EC medium for determining whether a total coliform-positive culture taken from the distribution system contains fecal coliforms, in accordance with the Total Coliform Rule. Transfer a total coliform-positive culture from a presumptive tube/bottle or colony to a tube containing EC Medium and an inverted vial, as specified by §141.21 (f) (5) (see Appendix H).

5.5.2 Use EC Medium to enumerate fecal coliforms in source water, in accordance with the Surface Water Treatment Requirements. Initially, conduct MTF test, presumptive phase. Use three sample volumes of source water (10 mL, 1 mL, 0.1 mL), 5 or 10 tubes/sample volume. Transfer culture from each total coliform-positive tube to a tube containing EC Medium and an inverted vial.

5.5.3 Use A-1 Medium as an alternative to EC Medium to enumerate fecal coliforms in source water, in accordance with the Surface Water Treatment Requirements. Do not use A-1 Medium for drinking water samples. Use three sample volumes of source water (10 mL, 1 mL, 0.1 mL), 5 or 10 tubes/sample volume. Unlike EC Medium, A-1 Medium can be directly inoculated with a water sample.

5.5.4 Ensure water level of water bath is above upper level of medium in the culture tubes.

5.5.5 Incubate EC Medium at 44.5° ± 0.2°C for 24 ± 2 hours. Incubate A-1 Medium at 35° ± 0.5°C for 3 hours, then at 44.5° ± 0.2°C for 21 ± 2 hours.

5.5.6 Any amount of gas detected in the inverted vial of a tube that has turbid growth is considered fecal coliform-positive.

5.6 MMO-MUG Test (for total coliforms in source water or drinking water)

5.6.1 When using bulk medium, prepare and incubate a sterility control for each analysis. Control should consist of a test tube with the MMO-MUG medium to which sterile water has been added.

5.6.2 (for enumerating total coliforms in source water) Use 5 or 10 tubes for each sample volume tested. Dilution water (for the MMO-MUG test only), if used, is sterile dechlorinated tap water, deionized water, or distilled water.

5.6.3 (for determining presence of total coliforms in drinking water) Use 10 tubes, each containing 10-mL water sample, or a single vessel containing 100-mL water sample.

5.6.4 Incubate sample at 35° ± 0.5°C for 24 hours. A yellow color in the medium indicates the presence of total coliforms.

5.6.5 If sample color is indeterminate after incubation for 24 hours, incubate another 4 hours (do not incubate more than 28 hours total). If the color is still indeterminate with a reference comparator after 28 hours, invalidate sample and request another.

5.6.6 Do not use the MMO-MUG test to verify total coliforms on membrane filters. The filtration step not only concentrates coliforms, but also non-coliforms and turbidity, which, at high levels, can suppress coliforms or cause false-positive results in the MMO-MUG test.

5.6.7 Do not use the MMO-MUG test to confirm total coliforms in the MTF or Presence-Absence (P-A) Coliform Test. High densities of non-coliforms in the inoculum may overload the MMO-MUG suppressant reagent system and cause false-positive results.

5.6.8 Avoid prolonged exposure of inoculated test to direct sunlight. Sunlight may hydrolyze indicator compounds, causing a false-positive result.

5.6.9 Laboratories are encouraged to perform parallel testing between the MMO-MUG 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 MMO-MUG test for the wide variety of water types submitted for analysis.

*5.7 EC Medium + MUG Test (for E. coli)**

5.7.1 Transfer a total coliform-positive culture from a presumptive tube/bottle or colony to EC Medium + MUG, as specified by §141.21 (f) (5) (see Appendix H).

5.7.2 Ensure water level of water bath is above upper level of medium.

5.7.3 Incubate at 44.5° ± 0.2°C for 24 ± 2 hours.

5.7.4 Detect fluorescence using an ultraviolet lamp (366-nm), preferably with a 6-watt bulb. Ensure that weak autofluorescence of medium, if present, is not misinterpreted as positive for *E. coli*. A MUG-positive (*E. coli*) and MUG-negative (e.g., uninoculated) control may be necessary for each analysis where the medium autofluoresces.

QC **5.7.5** Verify at least 5% of both MUG-positive results and turbid MUG-negative results for *E. coli*. Verification of a pure culture may be conducted, for example, by the use of a multi-test system (API 20E or equivalent); standard biochemical tests (e.g., citrate, indole, and urease tests); serotyping after biochemical identification, if desired; or the indole test at 44.5°C and growth in citrate.

*5.8 Nutrient Agar + MUG Test (for E. coli)**

5.8.1 Transfer membrane filter containing coliform colony(ies) from total coliform medium to surface of Nutrient Agar + MUG medium. Mark each sheen colony. A portion of the colony may be transferred with a needle to 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 on the Nutrient Agar + MUG medium after the 4-hour incubation time, with a sterile cotton swab, and transfer to the total coliform verification test.

5.8.2 Incubate at 35° ± 0.5°C for 4 hours.

5.8.3 Detect fluorescence using an ultraviolet lamp (366-nm), preferably with a 6-watt bulb. Any amount of fluorescence in a halo around a sheen colony is considered positive for *E. coli*.

QC **5.8.4** Verify at least 5% of both MUG-positive results and MUG-negative, total coliform-positive results for *E. coli*. Also verify any non-sheen colonies that fluoresce. Verification may be conducted with any of the tests in 5.7.5.

5.9 Pour Plate Method (for enumerating heterotrophic bacteria; see §141.74 (a) (3) in Appendix H. Also for use of R2A medium, see variance criteria in preamble of Federal Register *notice 56:1556-1557, January 15, 1991)*

5.9.1 For most potable water samples, countable plates can be obtained by plating 1.0 mL or 0.1 mL volume of the undiluted sample. Use at least two replicate plates.

5.9.2 Aseptically pipet sample into bottom of 100 mm x 15 mm petri dish. Add 12-15 mL of tempered melted (44° - 46°C) agar to each petri dish. Mix the sample and melted agar carefully to avoid spillage. After agar plates have solidified on a level surface, invert plates and incubate at 35° ± 0.5°C for 48 ± 3 hours (except for R2A Medium). Stack plates in incubator to allow proper air circulation to maintain uniform incubation temperature. Do not stack plates more than four high. Remelt sterile agar medium only once.

5.9.3 Count colonies manually using a counting aid such as a Quebec colony counter. Consider only plates having 30 to 300 colonies in determining plate count, except for plates inoculated with 1.0 mL volume 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.)

5.9.4 Check each batch or flask of agar for sterility by pouring final control plate. Reject data if control is contaminated.

5.10 Spread Plate Method (for enumerating heterotrophic bacteria as part of variance criteria)

5.10.1 Pour 15 mL of R2A agar medium into a petri dish (100 x 15 mm or 90 x 15 mm) and let agar solidify. Inoculate agar and incubate as described by 16th edition of *Standard Methods*, Method 907B. Use at least two replicate plates.

5.10.2 Same as paragraph 5.9.3.

**Detailed procedure can be found in "Test Methods for Escherichia coli in Drinking Water," EPA/600/4-91/016, July 1991. To receive a copy, write Resource Center (WH-550A), USEPA, 401 M Street SW, Washington, DC 20460.*

5.10.3 Same as paragraph 5.9.4.

5.11 Membrane Filter Method (for enumerating heterotrophic bacteria as part of variance criteria

5.11.1 Filter a volume that will yield between 20-200 colonies. Transfer filter to a 50 x 9 mm petri dish containing 5 mL of solidified R2A medium. Incubate at 35°C or lower for 5-7 days in a close fitting box containing moistened paper towels. Use at least two replicate plates.

5.11.2 Count colonies using a stereoscopic microscope at 10 to 15 magnification.

5.11.3 Check each batch or flask of agar for sterility by pouring final control plate. Reject data if control is contaminated.

5.12 MF Procedure (for enumeration of total coliforms in source water)

5.12.1 Same as paragraphs 5.2.2 - 5.2.5, except that in paragraph 5.2.4, laboratories should invalidate any sample which results in confluent growth or TNTC, even when total coliform colonies are present, since coliform density is to be determined.

5.12.2 Use appropriate sample dilutions which will yield no more than 80, and preferably at least 20, total coliform colonies per membrane.

5.12.3 Adjust initial counts based upon verification data.

QC **5.12.4** If two or more analysts are available, each analyst should count monthly the total coliform colonies on the same membrane. Colony counts should agree within 10%.

5.13 Multiple Tube Fermentation Technique (for enumeration of total coliforms in source water)

5.13.1 Use 3 sample volumes of source water (10 mL, 1 mL, 0.1 mL), and 5 or 10 tubes/sample volume.

5.13.2 Incubate at 35° ± 0.5°C for 24 ± 2 hours.

5.13.3 Invalidate all samples which produce a turbid culture in the absence of gas production, in lauryl tryptose broth or lactose broth. Collect, or request the system collect, another sample. The laboratory may use another method to test the second sample.

Alternatively, if a sample produces a turbid culture in the absence of gas production, perform a confirmed test. If the confirmed test is total coliform-positive, report the most probable number. If a confirmed test is total coliform-negative, invalidate the sample and request another one.

QC **5.13.4** Perform the completed test quarterly on a coliform-positive tube(s)/bottle.

5.14 Fecal Coliform Membrane Filter Procedure (for enumerating fecal coliforms in source water)

5.14.1 Use appropriate sample volumes which will yield 20 - 60 fecal coliform colonies per membrane.

QC **5.14.2** Conduct sterility check at the beginning and end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter. If control indicates contamination, reject all data from affected samples and request immediate resampling.

5.14.3 Incubate at 44.5° ± 0.2°C for 24 ± 2 hours.

QC **5.14.4** If two or more analysts are available, each analyst should count monthly the fecal coliform colonies on the same membrane. Colony counts should agree within 10%.

6. Sample Collection, Handling, and Preservation

(Applicable to those laboratories that collect samples; all laboratories are responsible for paragraphs 6.4 and 6.5.)

6.1 Sample Collector

Collector is trained in sampling procedures and, if required, approved by the appropriate regulatory authority or its designated representative.

6.2 Sampling

Samples must be representative of the potable water distribution system. Water taps used for sampling are free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. Maintain a steady water flow for at least 2 minutes to clear the service line before sampling. Collect at least a 100 mL sample volume, allow at least 1/2-inch air space to facilitate mixing of sample by shaking.

6.3 Sample Icing

Sample collectors who deliver samples directly to the laboratory should ice samples immediately after sample collection.

6.4 Sample Holding/Travel Time

Holding/travel time between sampling and analysis is not to exceed 30 hours. If laboratory is required by State regulation to analyze samples after 30 hours and up to 48 hours, the laboratory is to indicate that the data may be invalid because of excessive delay before sample processing. No samples received after 48 hours are to be analyzed for compliance. All samples received in the laboratory are to be analyzed on the day of receipt.

6.5 Report Form

Immediately after collection, enter on the sample report form the sample site location, sample type (e.g., routine, repeat), date and time of collection, free chlorine residual, collector's initials, and any remarks. Also include the date and time of sample arrival at the laboratory and the date and time analysis begins. Record additional information as required by the National Primary Drinking Water Regulations.

6.6 Chain-of-Custody

Follow applicable State regulations pertaining to chain-of-custody.

7. Quality Assurance

The laboratory prepares and follows a written QA plan (see Chapter III's discussion of QA plans) which is to be available for inspection by the certification officer.

8. Records and Data Reporting

Records of microbiological analyses are kept by the laboratory or are accessible to the laboratory for at least five years. Actual laboratory reports may be kept, or data may be transferred to tabular summaries, provided that the following information is included:

- Date, place, and time of sampling, name of persons who collected the sample.
- Identification of sample as to whether it is a routine distribution system sample, repeat sample, raw or process water sample, or other special purpose sample.
- Date and time of sample receipt and analysis.
- Laboratory and persons responsible for performing analysis.
- Analytical technique/method used.
- Results of analysis.

  Total coliform MPN data based on confirmed or completed test (for broth media) and verified counts (for MF media)

9. Action Response to Laboratory Results

9.1 Testing Total Coliform-Positive Cultures

(for Total Coliform Rule) Laboratory must test all total coliform-positive cultures for presence of either fecal coliforms or *E. coli*.

9.2 Notification of Positive Results

(for Total Coliform Rule) Laboratory must notify proper authority promptly 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), 40 CFR 141.31, etc.). Total coliform-positive result is based on confirmed phase for the Multiple Tube Fermentation Technique and Presence-Absence (P-A) Coliform Test, or verified test for Membrane Filter Technique. No requirement exists for confirmation of positive MMO-MUG tests, fecal coliform test, or *E. coli* tests.

9.3 Invalidation of Total Coliform-Negative Sample

(for Total Coliform Rule) Laboratory must notify proper authority when results indicate that noncoliforms may have interfered with the total coliform analysis, as described by 40 CFR 141.21(c) (2).

Sample Forms for On-Site Evaluation of Laboratories Analyzing Public Water Supplies—Microbiology

Laboratory _____

Street _____

City _____ State _____

Telephone Number _____

Survey by _____

Affiliation _____

Date _____

Codes for Marking On-Site Evaluation Forms

S - Satisfactory **X - Unsatisfactory** **U - Undetermined** **NA - Not Applicable**

1. Personnel

Position/Title	Name	Time in Present Position	Academic Training and/or Degree	Present Specialty	Experience (years/area)
Laboratory Director					
Supervisor/ Consultant					
Professional (note discipline)					
Technician/ Analyst					

2. Laboratory Facilities

Laboratory facilities clean, temperature and humidity controlled _____

Adequate lighting at bench top _____

Laboratory has provision for disposal of microbiological wastes _____

3. Laboratory Equipment, Supplies, and Materials

3.1 pH Meter

Manufacturer_____ Model_____

Accuracy ± 0.1 units _____

Scale graduation, 0.1 units _____

Maintains electrodes according to manufacturer's recommendations _____

pH buffer solution aliquots used only once _____

QC Commercial buffer solutions dated when received and discarded before expiration date _____

QC Standardize pH meter each use period with pH 7.0 and 4.0 standard buffers _____

3.2 Balances (Top Loader or Pan)

Manufacturer_____ Model_____

Detects 100 mg at a 150 gram load _____

QC Calibrate balance monthly using Class S or S-1 reference weights or weights traceable to Class S or S-1 weights. If non-reference weights are used, calibrate non-reference weights with Class S or S-1 reference weights _____

QC Correction data available with S or S-1 weights _____

QC Annual service contract or internal maintenance protocol and record maintained _____

3.3 Temperature Monitoring Device

Use glass/mercury or dial thermometer in incubator. Units graduated in no more than 0.5°C increments _____

No separation in mercury column _____

QC Check calibration of glass/mercury thermometers annually and dial thermometers quarterly at the temperature used against a reference NBS thermometer or one meeting the requirements of NBS Monograph SP 250-23 _____

QC Recalibrate continuous recording devices used to monitor incubator temperature annually against a NBS thermometer or one meeting the requirements of NBS Monograph SP 250-23 _____

3.4 Incubator Unit

Manufacturer_____ Model_____

Maintains internal temperature of 35° ± 0.5°C, and if used, 44.5° ± 0.2°C _____

Place thermometers on top and bottom shelves in use area of non-portable incubators

Immerse thermometer bulb in liquid _____

Culture dishes and tubes fit snugly in aluminum block incubator _____

QC Record temperature twice daily for days in use, with readings separated by at least four hours _____

3.5 Autoclave

Manufacturer_____ Model_____

Temperature gauge with sensor on exhaust _____

Operational safety valve _____

Maintains sterilization temperature during cycle _____

Completes entire cycle within 45 minutes when a 12-15 minute sterilization period is used _____

Depressurizes slowly to ensure media do not boil over and bubbles do not form in fermentation tubes _____

QC Record date, contents, sterilization time, and temperature for each cycle _____

QC Establish service contract or internal maintenance protocol _____

QC Heat-sensitive tape, spore strips or ampoules, or maximum temperature registering thermometer used during each autoclave cycle _____

QC Check automatic timing mechanism accuracy with stop-watch quarterly _____

3.6 Hot Air Oven

Manufacturer_____ Model_____

Hot air oven maintains a temperature of 170°-180°C _____

Thermometer graduated in no more than 10°C increments _____

Place thermometer bulb in sand _____

QC Records include date, sterilization time, and temperature of each cycle _____

3.7 Colony Counter

Manufacturer_____ Model_____

A dark field colony counter available to count Heterotrophic Plate Count colonies _____

3.8 Conductivity Meter

Manufacturer_____ Model_____

Suitable for checking laboratory pure water. Readable in ohms or mhos, has a range of 2 ohms to 2 megohms or equivalent micromhos ± 2% _____

QC Conductivity meter is calibrated monthly with a 0.01 M KCl solution or lower concentration _____

3.9 Refrigerator(s)

Manufacturer_____ Model_____

Maintains temperatures of 1° to 5°C _____

Thermometer(s) graduated in 1°C increments or less _____

Thermometer bulb(s) immersed in liquid _____

QC Temperature recorded for days in use _____

3.10 Inoculating Equipment

Metal or plastic loops, or applicator sticks sterilized by dry heat _____

Metal loops and/or needles are made of nickel alloy or platinum _____

3.11 Membrane Filtration Equipment, Membrane Filters and Pads

Manufacturer_____ Model_____

MF units of stainless steel, glass, or autoclavable plastic _____

Units do not leak, not scratched or corroded _____

10 to 15X magnification device with fluorescent light source _____

Forcep tips without corrugations _____

Membrane filters from cellulose ester material, white, gridmarked, 47 mm diameter, 0.45 µm pore size _____

Alternate pore size used _____

Membrane filters recommended by manufacturer for total coliform analysis _____

Membrane filters and pads are purchased presterilized or autoclaved before use _____

QC Record lot numbers of membrane filters and date received _____

QC Determine sterility of each lot of membrane filters by placing one membrane filter in non-selective broth medium _____

3.12 Culture dishes

Use presterilized plastic or sterilized glass dishes _____

Incubate loose-lid dishes in a tight fitting container _____

Sterilize glass culture dishes in stainless steel or aluminum canisters or in heavy aluminum foil or char-resistant paper _____

Reseal open packs of disposable culture dishes between uses _____

3.13 Pipets

Sterilize glass pipets in stainless steel or aluminum canisters or individual pipets wrapped in char-resistant paper _____

Reseal packs of disposable sterile pipets between major use periods _____

Pipets not etched, mouthpiece and tip are not chipped, graduation markings legible _____

3.14 Culture Tubes, Containers and Closures

Tubes and containers are borosilicate glass or other corrosion-resistant glass or plastic _____

Tubes and containers are of sufficient size that medium plus sample does not exceed 3/4 full _____

Tube and container closures are stainless steel, plastic, aluminum, or screw caps with non-toxic liner _____

3.15 Sample Containers

Capacity at least 120 mL (4 oz) _____

Sample bottles are wide mouth plastic with a non-toxic cap liner, or borosilicate glass with a ground glass stopper, or other appropriate sample containers such as single-service sterilized plastic sampling bags with sodium thiosulfate _____

Cover glass-stoppered bottle top with aluminum foil or char-resistant paper prior to sterilization _____

Glass bottles sterilized by autoclaving or dry heat. Plastic bottles sterilized by autoclaving. Empty containers moistened before autoclaving _____

3.16 Glassware and Plasticware

Glass made of borosilicate or other corrosion-resistant glass _____

Free of chips and cracks _____

Graduation marks are legible _____

Plastic items are clear and non-toxic _____

Graduated cylinders and other pre-calibrated containers used to measure sample volume have clearly marked volumes with a 2.5% tolerance or less _____

Calibration of pre-calibrated containers spot checked _____

Pipets used to measure sample volumes have a 2.5% tolerance or less _____

3.17 Ultraviolet lamp (if used)

Lamps cleaned monthly with a soft cloth moistened with ethanol _____

QC Lamp used for sanitization tested every quarter _____

4. General Laboratory Practices

4.1. Sterilization and Sanitation Procedures

Item	Autoclave Time 121°C	
Membrane filter and pads	10 min	_____
Carbohydrate media	12-15 min	_____
Contaminated test materials	30 min	_____
Membrane filter assemblies	15 min	_____
Sample collection bottles	15 min	_____
Individual glassware	15 min	_____
Dilution water blanks	15 min	_____
Rinse water	15 min	_____

Remove autoclaved MF filters and pads and all media immediately after sterilization cycle _____

Membrane filter assemblies are autoclaved at start of each filtration series _____

If ultraviolet light is used to sanitize equipment, all supplies are presterilized and QC checks conducted _____

Ultraviolet light or boiling water used to control bacterial carry-over between samples during filtration series (optional) _____

If boiling water is used to control bacterial carry-over, membrane filter equipment is submerged for two minutes and then cooled to room temperature before filtering next sample _____

4.2 Sample Containers

Stock 10% sodium thiosulfate solution free of turbidity _____

Add sodium thiosulfate to sample containers prior to sterilization _____

QC Determine sterility of each lot of sample bottles or presterilized sample bags by adding non-selective broth, incubating at 35°C for 24 hours and checking for growth _____

4.3 Reagent Water

Use reagent water to prepare media, reagents, and dilution/rinse water _____

QC Reagent water is tested to assure the following minimum criteria are met: _____

Parameter	Limits	Frequency	
Conductivity	> 0.5 megohms or < 2 micromhos at 25°C	monthly	_____
Metals—Pb, Cd, Cr, Cu, Ni, Zn	Not greater than 0.05 mg/L per contaminant. Collectively not greater than 0.1 mg/L	annually	_____
Total chlorine residual	None detected	monthly	_____
Heterotrophic Plate Count	< 500/mL	monthly	_____
Bacteriological quality of reagent water	Ratio 0.8-3.0	annually	_____

4.4 Dilution/Rinse Water

Prepare stock buffer solution or peptone water according to *Standard Methods*, 16th Edition, p. 855 _____

Stock buffer autoclaved or filter sterilized, labeled, dated, and free of turbidity _____

10% peptone stock solution autoclaved or filter sterilized, labeled, dated, and free of turbidity _____

Prepare dilution/rinse water by adding 1.25 mL volume of stock buffer solution and 5 mL volume of $MgCl_2$ stock solution per liter of reagent water _____

Prepare 0.1% peptone water by adding 10 mL of 10% stock solution per liter of reagent water _____

QC pH of stock phosphate buffer solution is 7.2 ± 0.2 _____

QC pH of peptone water is 6.8 ± 0.2 _____

QC Check dilution/rinse water for sterility _____

4.5 Glassware Washing

Use distilled or deionized water for final rinse _____

QC Perform inhibitory residue test on clean glassware _____

4.6 Analytical Media

4.6.1 General

Commercially available dehydrated or prepared media used _____

Dehydrated media stored in cool, dry location _____

Caked or discolored media discarded _____

Dehydrated media dated when received and when initially opened _____

Dehydrated or commercially prepared media that have passed manufacturer's expiration date are discarded _____

Open dehydrated media discarded after 6 months (12 months if stored in desiccator). _____

QC Media preparation records include:

(a) Date of preparation _____

(b) Type of medium _____

(c) Lot number _____

(d) Sterilization time and temperature _____

(e) Final pH _____

(f) Technician's initials _____

QC For liquid media prepared commercially, the following are recorded:

(a) Date received _____

(b) Type of medium _____

(c) Lot number _____

(d) pH verification _____

QC Each commercial lot of medium and each batch of laboratory-prepared medium is checked before use with positive and negative controls, and results recorded

Prepared plates are refrigerated in sealed plastic bags or containers _____

4.6.2 Membrane Filter Media (for total coliforms)

m-Endo broth or agar, final pH: 7.2 ± 0.1 or m-Endo LES agar, final pH: 7.2 ± 0.2 _____

Medium dissolved using:

(a) Boiling water bath _____

(b) Hot plate with stir bar, constantly attended _____

Media prepared in sterile flasks _____

Ethanol not denatured _____

MF broth refrigerated no longer than 96 hours _____

MF agar plates refrigerated no longer than 2 weeks _____

Ampouled m-Endo refrigerated in accordance with manufacturer's expiration date _____

Uninoculated media discarded if growth or surface sheen observed _____

4.6.3 Multiple Tube Fermentation (MTF or MPN) Media (for total coliforms)

Lauryl tryptose (lauryl sulfate) or lactose broth used for presumptive phase _____

Concentration adjusted so that medium is single strength after sample addition _____

Autoclaved at 121°C for 12-15 min. Final pH: 6.8 ± 0.2 _____

Inverted vials in sterilized medium are one-third to one-half covered by medium and free of gas bubbles _____

Brilliant green lactose bile broth used for confirmed phase _____

Autoclaved at 121°C for 12-15 min. Final pH 7.2 ± 0.2 _____

Sterilized media stored at 1° to 5°C in the dark _____

Refrigerated media in tubes/containers with loose-fitting closures used within one week _____

If stored, broth media are refrigerated in screw cap tubes/ containers, and used within 3 months _____

Stored broth media is discarded if evaporation exceeds 10% of original volume _____

Refrigerated sterile media are incubated overnight at 35°C before use, and tubes/containers showing growth or bubbles are discarded _____

m-Endo LES agar used for the completed phase _____

If stored, medium is refrigerated. Refrigerated medium used within two weeks, and discarded if growth is observed. Protect medium from light _____

4.6.4 Presence-Absence (P-A) Coliform Test Medium

Medium is autoclaved for 12 minutes at 121°C, with space allowed between bottles. Final pH: 6.8 ±0.2 _____

Space allowed between bottles _____

Stored medium is refrigerated in culture bottles in dark, used within 3 months, and discarded if evaporation exceeds 10% of original volume _____

4.6.5 EC Medium (for fecal coliforms)

Autoclaved for 12-15 minutes at 121°C. Final pH: 6.9 ± 0.2 _____

Inverted tubes following sterilization are one-half to one-third covered by the medium and free of air bubbles _____

If stored, sterile medium is refrigerated in tightly closed screw cap tubes and used within 3 months _____

Stored sterile media incubated overnight at 35°C before use, and tubes with growth and/or bubbles discarded _____

4.6.6 MMO-MUG Test Medium (for total coliforms)

Commercial preparation used _____

Medium protected from light _____

Medium is not autoclaved _____

4.6.7 EC Medium + MUG (for *E. coli*)

QC Each lot of commercially-prepared medium, or batch of laboratory-prepared medium, is checked with culture controls, and results recorded _____

Tubes and autoclaved medium observed for fluorescence before use with 366-nm ultraviolet light. If weak fluorescence is observed, either another lot of medium is used, or MUG-positive and MUG-negative controls are used with analysis _____

Inverted vial in test tube is not used _____

Final MUG concentration: 50 µg/ml _____

Final pH: 6.9 ± 0.2 _____

If sterile medium is stored, it is refrigerated in tightly closed screw cap tubes and used within 3 months _____

Stored media is incubated overnight at 44.5°C before use, and tubes with growth discarded _____

4.6.8 Nutrient Agar Medium + MUG (for *E. coli*)

QC Quality of medium lot/batch evaluated by spot-inoculating control bacteria _____

Medium sterilized in 100-ml volumes at 121°C for 15 minutes. Final pH 6.8 ± 0.2 _____

Final MUG concentration: 100 μg/ml _____

If media are stored in petri dishes, they are refrigerated in plastic bag or tightly closed container, and used within 2 weeks _____

Refrigerated medium incubated overnight at 35°C before use, and plates with growth discarded _____

4.6.9 Heterotrophic Plate Count (HPC) Medium

Autoclaved at 121°C for 15 minutes. Final pH: Plate Count Agar 7.0 ± 0.2; TGE Agar 6.8- 7.0; R2A Agar 7.2 _____

Melted agar used within 3 hours, and agar tempered (44-46°C) before pouring _____

Sterile agar medium melted not more than once _____

If media are stored in petri dishes, they are refrigerated in plastic bag or tightly closed container, and used within 2 weeks (one week for R2A medium) _____

Refrigerated medium incubated overnight at 35°C before use, and plates with growth discarded _____

4.6.10 A-1 Medium (for fecal coliforms)

Medium is sterilized at 121°C for 10 minutes. Final pH: 6.9 ± 0.1 _____

Inverted tubes are one-third to one-half covered by the medium and free of air bubbles _____

Sterilized medium is stored in dark at room temperature and used within one week _____

4.6.11 Fecal Coliform Membrane Filter (M-FC) Broth/Agar

Sterilized by bringing to boiling point; not autoclaved. Final pH 7.4 _____

If medium is stored, it is refrigerated and used within 96 hours (if broth) or two weeks (if agar) _____

Refrigerated medium is incubated overnight at 44.5°C before use, and plates with growth discarded _____

5. Analytical Methodology

5.1 General

Only analytical methodology specified in the National Primary Drinking Water Regulations (see Appendix H) is used _____

Laboratory is to be certified for at least one total coliform method plus one fecal coliform or *E. coli* method _____

Laboratory is to be certified for HPC (Pour Plate Method), if it conducts method for compliance purposes _____

Absorbent pad, if used, is saturated with liquid medium _____

Water sample shaken vigorously before analyzing _____

QC Coliform test conducted monthly on known coliform-positive and fecal- or *E. coli*-positive sample _____

5.2 Membrane Filter Technique (for total coliforms in drinking water)

Sample volume analyzed is 100 mL ± 2.5 mL _____

QC Sterility check conducted at beginning and end of each filtration series. If control indicates contamination, all data rejected and another sample obtained _____

Inoculated medium incubated at 35° ± 0.5°C for 22-24 hours _____

All samples with confluent or TNTC growth invalidated, unless total coliform-positive, and new sample obtained _____

All sheen colonies verified (up to at least 10 colonies) _____

Total coliform colonies tested for either fecal coliforms or *E. coli*, using approved medium and transfer technique _____

5.3 Multiple Tube Fermentation Technique (for total coliforms in drinking water)

Concentration of inoculated medium is correct _____

Sample volume analyzed is 100 mL ±2.5 mL _____

Inoculated medium incubated at 35° ± 0.5°C for 24 ± 2 hours _____

If no gas is detected, incubate for another 24 hr _____

All turbid gas-negative cultures are invalidated, and another sample obtained _____

Cultures from gas-positive tubes incubated in BGLB broth _____

QC Completed test performed on at least 10% of all coliform positive samples/quarter. If no positive samples were observed, then one obtained and method conducted through the completed test _____

5.4 Presence-Absence (P-A) Coliform Test

Sample volume analyzed is 100 mL ± 2.5 mL _____

Inoculated medium incubated at 35° ± 0.5°C and observed for yellow color after 24 and (if necessary) 48 hours _____

Yellow cultures confirmed in BGLB broth _____

All non-yellow turbid cultures are invalidated, and another sample obtained _____

5.5 Fecal Coliform Test

(For distribution system samples) Positive culture from total coliform medium is transferred to EC Medium, using an approved _____

transfer technique

(For source water samples) Positive culture from total coliform medium is transferred to EC medium, using approved transfer technique, or A-1 Medium is directly inoculated with a water sample. Three sample volumes used, 5 or 10 tubes/sample volume _____

Water level of water bath is above upper level of medium in culture tubes _____

EC Medium incubated at 44.5° ± 0.2°C for 24 ± 2 hours. A-1 Medium incubated at 35° ± 0.5°C for 3 hours, then at 44.5° ± 0.2°C for 21 ±2 hours _____

Any gas detected in inverted vial of tube that has turbid growth is considered fecal coliform-positive _____

5.6 MMO-MUG Test (for total coliforms in source water or drinking water)

If bulk medium is employed, a sterility control is used with each analysis _____

(For source water) 5 or 10 tubes for each sample volume. Dilution water, if used, is dechlorinated tap water, deionized water or distilled water _____

(For drinking water) 10 tubes used, each containing 10-mL water sample, or a single vessel containing 100-mL water sample _____

Inoculated medium incubated at 35° ± 0.5°C for 24 hours _____

If color indeterminate after 24 hours, medium incubated an additional 4 hours. If color still questionable after 28 hours, reference comparator used _____

If sample color remains indeterminate, sample declared invalid and another sample requested from same site _____

Inoculated test not exposed to prolonged direct sunlight _____

MMO-MUG test is not used to verify/confirm coliforms on membrane filters or in broth cultures (e.g., lauryl tryptose broth)

Parallel testing performed for several months or over several seasons between the MMO-MUG test and another EPA-approved procedure (optional) _____

5.7 EC Medium + MUG Test (for *E. coli*)

Positive culture from total coliform presumptive medium is transferred to EC Medium, using an approved transfer technique _____

Water level of water bath is maintained above upper level of medium in culture tubes _____

Inoculated medium incubated at 44.5° ± 0.2°C for 24 ± 2 hours _____

Fluorescence examined with ultraviolet lamp (366-nm). MUG-positive and MUG-negative controls used when needed _____

QC At least 5% of both MUG-positive results and turbid MUG-negative results are verified for *E. coli* _____

5.8 Nutrient Agar + MUG Test (for *E. coli*)

Total coliform-positive membrane filters transferred to Nutrient Agar + MUG _____

Each total coliform-positive colony marked before incubation on Nutrient Agar + MUG _____

Inoculated medium incubated at 35° ± 0.5°C for 4 hours _____

Fluorescence examined with ultraviolet lamp (366-nm). Any amount of fluorescence in a halo around a sheen colony is considered *E. coli*-positive _____

QC At least 5% of both MUG-positive results and MUG-negative, total coliform-positive results are verified for *E. coli* _____

QC Non-sheen colonies that fluoresce are verified for *E. coli* _____

5.9 Pour Plate Method (for heterotrophic bacteria)

Appropriate volume of sample added to plate _____

Agar tempered to 44-46°C before adding to plate _____

Sample and melted agar mixed carefully _____

At least two replicate plates prepared for each sample _____

Plates incubated in inverted position at 35° ± 0.5°C for 48 ± 3 hours (except for R2A Medium; see *Standard Methods*) _____

Plates stacked no more than four high _____

Sterile agar medium remelted only once _____

Colonies counted manually using a counting aid such as a Quebec colony counter _____

Counts reported for plates having 30 - 300 colonies. (If 1.0 ml of undiluted sample results in fewer than 30 colonies, that count is acceptable) _____

Sterility check performed by pouring a final agar control plate. Data rejected if control is contaminated _____

5.10 Spread Plate Method (for heterotrophic bacteria)

R2A agar medium used _____

Plates with solidified medium dried before use _____

Medium inoculated in accordance with *Standard Methods* _____

At least two replicate plates used for each sample _____

Plates incubated in inverted position at 28°C for 7 days _____

Plates stacked no more than four high _____

Colonies counted manually using a counting aid such as a Quebec colony counter _____

Counts reported for plates having 30 - 300 colonies. (If 1.0 mL of undiluted sample results in fewer than 30 colonies, that count is acceptable) _____

Sterility check performed on an uninoculated control plate. Data rejected if control is contaminated _____

5.11 Membrane Filter Method (for heterotrophic bacteria)

Sample volume filtered yields filters with 20-200 colonies _____

Filter transferred to R2A agar medium _____

Plates incubated at 35°C or lower for 5-7 days in a close fitting box containing moistened paper towels _____

At least two replicate plates prepared for each sample _____

Stereoscopic microscope used to count colonies _____

Sterility check performed on a filter in a control plate. Data rejected if control is contaminated _____

5.12 MF Procedure (for total coliforms in source water)

Sample volume filtered yields 80 or fewer colonies/membrane _____

QC Sterility check conducted at beginning and end of each filtration series. If control indicates contamination, all data rejected and another sample obtained _____

Inoculated medium incubated at 35° ± 0.5°C for 22-24 hours _____

All samples with confluent or TNTC growth invalidated, and new sample obtained _____

All sheen colonies verified (up to at least 10 colonies) _____

Initial counts adjusted, based upon verification data _____

QC If two or more analysts are available, each counts the total coliform colonies on same membrane at least monthly. Colony counts agree within 10% _____

5.13 Multiple Tube Fermentation Technique (for total coliforms in source water)

Three sample volumes of source water (10 ml, 1 ml, 0.1 ml) used _____

Five or ten tubes/sample volume used _____

Inoculated medium incubated at 35° ± 0.5°C for 24 ± 2 hours _____

Any sample which produces a turbid culture with no gas is invalidated, and another sample collected or requested. Alternatively, if confirmed test is conducted on each turbid, _____

gas-negative tube, and result is total coliform-positive for each tube, then Most Probable Number is reported. If any turbid, gas-negative tube is coliform-negative, sample is invalidated, and another sample requested

QC Completed test is performed quarterly on coliform-positive tube(s)/bottle _____

5.14 Fecal Coliform Membrane Filter Procedure (for fecal coliforms in source water)

Sample volumes used which will yield 60 or fewer fecal coliform colonies/membrane (and preferably at least 20) _____

Inoculated medium incubated at 44.5° ± 0.2°C for 24 ± 2 hrs _____

QC Sterility check conducted at beginning and end of each filtration series. If control indicates contamination, data rejected and another sample obtained _____

QC If two or more analysts are available, each counts the total coliform colonies on same membrane at least monthly. Colony counts agree within 10% _____

6. Sample Collection, Handling, and Preservation

6.1 Follow sample procedures described in *Standard Methods for the Examination of Water and Wastewater* or *Microbiological Methods for Monitoring the Environment*,U.S. EPA-600/8-78-017 _____

6.2 Sample collectors receive training _____

6.3 Samples representative of distribution system _____

6.4 Water taps free of any attachments and mixing type faucets _____

6.5 Water run to waste for at least two minutes _____

6.6 Sample volume is at least 100 mL with sufficient space for mixing sample _____

6.7 Sample report form completed by collector _____

6.8 Samples iced when carrying samples directly to laboratory _____

6.9 Record date and time of sample arrival at laboratory and date and time analysis begins _____

6.10 Transit time does not exceed 30 hours _____

If laboratory is required by State regulation to examine samples after 30 hours and up to 48 hours, data are indicated as possibly invalid _____

All samples arriving in laboratory after 48 hours are not analyzed for compliance use _____

6.11 Compliance with State chain-of-custody regulations, if required _____

7. Quality Assurance Program

7.1 Written QA Plan implemented and available for review _____

7.2 Quality control records maintained for five years _____

QC 7.3 PE sample is satisfactorily analyzed annually (if available) _____

8. Data Reporting

8.1 Data entered on the sample report form is checked and initialed _____

8.2 Sample report forms are retained by laboratory or State program for five years _____

Report forms include identification of sample, date and time of sample receipt and analysis, laboratory and person(s) responsible for performing analyses, analytical method used and results of analysis _____

8.3 Results of analyses

Total coliform MPN data based on confirmed or completed test (for broth media) and verified counts (for MF media) _____

9. Action Response by Laboratory

All total coliform-positive cultures tested for the presence of either fecal coliforms or *E. coli* (for Total Coliform Rule only) _____

Proper authority notified of a positive total coliform, fecal coliform, or *E. coli* result (for Total Coliform Rule) _____

Proper authority notified when results indicate that high levels of noncoliforms may have interfered with the total coliform analysis (for Total Coliform Rule) _____

Appendix E

Required Analytical Capability for Principal State Laboratory Systems[1] (As of October 1, 1991)

Volatile Organic Chemicals (40 CFR 141.24)

Benzene
Carbon tetrachloride
1,2-Dichloroethane
1,1-Dichloroethylene
p-Dichlorobenzene
1,1,1-Trichloroethane
Vinyl chloride
Trichloroethylene
trans-1,2-Dichloro-ethylene
Ethylbenzene
Styrene
Toluene
Total Trihalomethanes
- Chloroform
- Bromodichloromethane
- Dibromochloromethane
- Bromoform

o-Dichlorobenzene
cis-1,2-Dichloroethylene
1,2-Dichloropropane
Monochlorobenzene
Tetrachloroethylene
Xylenes (total)

Organics other than VOCs (40 CFR 141.24)

Alachlor
Aldicarb
Aldicarb sulfone
Aldicarb sulfoxide
Atrazine
Carbofuran
Chlordane
Dibromochloro-propane (DBCP)
Endrin
Ethylene dibromide (EDB)
Heptachlor
Heptachlor epoxide
Lindane
Methoxychlor
PCBs
Pentachlorophenol
Toxaphene
2,4-D
2,4,5-TP (Silvex)

Inorganics (40 CFR 141.23, 141.89)

Asbestos
Arsenic
Barium
Cadmium
Chromium
Copper
Fluoride
Lead
Mercury
Nitrate-N
Nitrite-N
Selenium
Silver (until July 1992)

Radionuclides (40 CFR 141.25)

Gross alpha
Gross beta
Radium 226
Radium 228
Tritium
Strontium 89
Strontium 90
Gamma radiation
- Cesium 134
- Cesium 137
- Chromium 51
- Cobalt 60
- Iodine 131
- Ruthenium 106
- Zinc 65

Microorganisms (40 CFR 141.21)

Total coliforms
Escherichia coli or fecal coliforms
Heterotrophic bacteria

[1] If principal State laboratories or other laboratories analyze compliance samples for sodium or §1445 chemicals, they must be certified for these contaminants.

Appendix F

Additional Contaminants Scheduled for Rules in 1992-1993

Volatile Organic Chemicals

1,2,4-Trichlorobenzene
1,1,2-Trichloroethane
Methylene chloride (Dichloromethane)

Organics (other than VOCs)

Adipate, di(2-diethylhexyl)
Dalapon
Dinoseb
Diquat
Endothall
Glyphosate
Hexachlorobenzene
Hexachlorocyclopentadiene
Oxamyl (vydate)
PAHs (benzo(a)pyrene)
Phthalates (2-diethylhexyl)
Picloram
Simazine
2,3,7,8-TCDD (dioxin)

Inorganics

Antimony
Beryllium
Cyanide
Nickel
Thallium
Sulfate

Radionuclides

Radon
Uranium

Appendix G

§1445 Unregulated Chemicals to be Monitored

40 CFR 141.40 (final rule July 8, 1987)

Bromobenzene
Bromodichloromethane
Bromoform
Bromomethane

Chlorodibromomethane
Chloroethane
Chloroform
Chloromethane
o-Chlorotoluene
p-Chlorotoluene

Dibromomethane
m-Dichlorobenzene
1,1-Dichloroethane
Dichloromethane

1,3-Dichloropropane
2,2-Dichloropropane
1,1-Dichloropropene
1,3-Dichloropropene

1,1,2,2-Tetrachloroethane
1,1,1,2-Tetrachloroethane
1,1,2-Trichloroethane
1,2,3-Trichloropropane

40 CFR 141.40 (final rule January 30, 1991)

Monitoring is required for the following contaminants. If the State determines the system is not vulnerable to contamination, the system can receive a waiver.

Synthetic Organics

Aldrin
Hexachlorobenzene*
Dalapon*
Dinoseb*
Picloram*
Oxamyl (vydate)*
Simazine*
Glyphosate*
Hexachlorocyclopentadiene*
Benzo (a) pyrene*
Metolachlor
di(2-ethylhexyl) Phthalate*
2,3,7,8-TCDD (Dioxin)*

Diquat*
Dieldrin
Dicamba
Carbaryl
3-Hydroxycarbofuran
Methomyl
Butachlor
Endothall*
Metribuzin
Propachlor
di(2-ethylhexyl) Adipate*

Inorganics

Antimony*
Beryllium*
Cyanide*
Nickel*
Sulfate*
Thallium*

* Being considered for regulation in 1992.

Appendix H

Analytical Methods for Microbiology

1. Total Coliform Rule (40 CFR 141.21(f))

(1) The standard sample volume required for total coliform analysis, regardless of analytical method used, is 100 ml.

(2) Public water systems need only determine the presence or absence of total coliforms; a determination of total coliform density is not required.

(3) Public water systems must conduct total coliform analyses in accordance with one of the following analytical methods:

(i) Multiple-Tube Fermentation (MTF) Technique, as set forth in *Standard Methods for the Examination of Water and Wastewater*, 1985, American Public Health Association *et al.*, 16th edition, Method 908, 908A, and 908B--pp. 870-878, except that 10 fermentation tubes must be used; or *Microbiological Methods for Monitoring the Environment, Water and Wastes*, U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268 (EPA-600/8-78-017, December 1978, available from ORD Publications, CERI, U.S. EPA, Cincinnati, Ohio 45268), Part III, Section B.4.1-4.6.4, pp. 114-118 (Most Probable Number Method), except that 10 fermentation tubes must be used; or

(ii) Membrane Filter (MF) Technique, as set forth in *Standard Methods for the Examination of Water and Wastewater*, 1985, American Public Health Association, *et al.*, 16th edition, Method 909, 909A and 909B--pp. 886-896; or *Microbiological Methods for Monitoring the Environment, Water and Wastes*, U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268 (EPA-600/8-78-017, December 1978, available from ORD Publications, CERI, U.S. EPA, Cincinnati, Ohio 45268), Part III, Section B.2.1-2.6, pp. 108-112; or

(iii) Presence-Absence (P-A) Coliform Test, as set forth in *Standard Methods for the Examination of Water and Wastewater*, 1985, American Public Health Association *et al.*, 16th edition, Method 908E--pp. 882-886; or

(iv) Minimal Medium ONPG-MUG (MMO-MUG) Test as set forth in the article "National Field Evaluation of a Defined Substrate Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with Presence-Absence Techniques" (Edberg et al.), Applied and Environmental Microbiology, Volume 55, pp. 1003-1008, April 1989. (Note: The MMO-MUG Test is sometimes referred to as the Autoanalysis Colilert System.)

(4) In lieu of the 10-tube MTF Technique specified in paragraph (f)(3)(i) of this section, a public water system may use the MTF Technique using either five tubes (20-ml sample portions) or a single culture bottle containing the culture medium for the MTF Technique, i.e., lauryl tryptose broth (formulated as described in *Standard Methods for the Examination of Water and Wastewater*, 1985, American Public Health Association *et al.*, 16th edition, Method 908A--pp. 872), as long as a 100-ml water sample is used in the analysis.

(5) Public water systems must conduct fecal coliform analysis in accordance with the following procedure. When the MTF Technique or Presence-Absence (P-A) Coliform Test is used to test for total coliforms, shake the lactose-positive presumptive tube or P-A vigorously and transfer the growth with a sterile 3-mm loop or sterile applicator stick into brilliant green lactose bile broth and EC medium to determine the presence of total and fecal coliforms, respectively. For EPA-approved analytical methods which use a membrane filter, transfer the total coliform-positive culture by one of the following methods: remove the membrane containing the total coliform colonies from the substrate with sterile forceps and carefully curl and insert the membrane into a tube of EC medium (the laboratory may first remove a small portion of

selected colonies for verification), swab the entire membrane filter surface with a sterile cotton swab and transfer the inoculum to EC medium (do not leave the cotton swab in the EC medium), or inoculate individual total coliform-positive colonies into EC medium. Gently shake the inoculated tubes of EC medium to insure adequate mixing and incubate in a water bath at 44.5 ± 0. 2°C for 24 ± 2 hours. Gas production of any amount in the inner fermentation tube of the EC medium indicates a positive fecal coliform test. The preparation of EC medium is described in *Standard Methods for the Examination of Water and Wastewater*, 1985, American Public Health Association, 16th edition, Method 908C--p. 879, paragraph 1a. Public water systems need only determine the presence or absence of fecal coliforms; a determination of fecal coliform density is not required.

(6) Public water systems must conduct analysis of *Escherichia coli* in accordance with one of the following analytical methods:

(i) EC medium supplemented with 50 µg/ml of 4-methylumbelliferyl-beta-D-glucuronide (MUG) (final concentration). EC medium is described in *Standard Methods for the Examination of Water and Wastewater*, 1985, American Public Health Association *et al.*, 16th edition, p. 879. MUG may be added to EC medium before autoclaving. EC medium supplemented with 50 µg/ml of MUG is commercially available. At least 10 ml of EC medium supplemented with MUG must be used. The inner inverted fermentation tube may be omitted. The procedure for transferring a total coliform-positive culture to EC medium supplemented with MUG shall be as specified in paragraph (f)(5) of this section for transferring a total coliform-positive culture to EC medium. Observe fluorescence with an ultraviolet light (366 nm) in the dark after incubating tube at 44.5 ± 0.2°C for 24 ± 2 hours; or

(ii) Nutrient agar supplemented with 100 µg/ml 4-methylumbelliferyl-beta-D-glucuronide (MUG) (final concentration). Nutrient Agar is described in *Standard Methods for the Examination of Water and Wastewater*, 1985, American Public Health Association *et al.*, 16th edition, p. 874. This test is used to determine if a total coliform-positive sample, as determined by the Membrane Filter Technique or any other method in which a membrane filter is used, contains *E coli*. Transfer the membrane filter containing a total coliform colony(ies) to nutrient agar supplemented with 100 µg/ml (final concentration) of MUG. After incubating the agar plate at 35°C for 4 hours, observe the colony(ies) under ultraviolet light (366 nm) in the dark for fluorescence. If fluorescence is visible, *E. coli* are present.

2. Surface Water Treatment Requirements (40 CFR 141.74(a))

Only the analytical method(s) specified in this paragraph, or otherwise approved by EPA, may be used to demonstrate compliance with the requirements of §§141.71, 141.72, and 141.73. Measurements for pH, temperature, turbidity, and residual disinfectant concentrations must be conducted by a party approved by the State. Measurements for total coliforms, fecal coliforms, and HPC must be conducted by a laboratory certified by the State or EPA to do such analysis. Until laboratory certification criteria are developed for the analysis of HPC and fecal coliforms, any laboratory certified for total coliform analysis by EPA is deemed certified for HPC and fecal coliform analysis. The following procedures shall be performed in accordance with the publications listed in the following section. This incorporation by reference 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 methods published in *Standard Methods for the Examination of Water and Wastewater* may be obtained from the American Public Health Association et al., 1015 Fifteenth Street, NW, Washington, DC 20005; copies of the Minimal Medium ONPG-MUG Method as set forth in the article "National Field Evaluation of a Defined Substrate Method for the Simultaneous Enumeration of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with the Standard Multiple Tube Fermentation Method" (Edberg et al.), Applied and Environmental Microbiology, Volume 54, pp. 1595-1601, June 1988 (as amended under Erratum, Applied and Environmental Microbiology, Volume 54, p. 3197, December, 1988), may be obtained from the American Water Works Association Research Foundation, 6666 West Quincy Avenue, Denver, Colorado 80235; and copies of the Indigo Method as set forth in the article "Determination of Ozone in Water by the Indigo Method" (Bader and Hoigne), may be obtained from Ozone Science & Engineering, Pergamon Press Ltd., Fairview Park, Elmsford, New York 10523. Copies may be inspected at the U.S. Environmental Protection Agency, Room EB15, 401 M Street, SW., Washington, DC 20460 or at the Office of the Federal Register, 1100 L Street NW, Room 8401, Washington, DC.

(1) Fecal coliform concentration--Method 908C (Fecal Coliform MPN Procedures), pp. 878-880, Method 908D (Estimation of Bacterial Density), pp. 880-882, or Method 909C (Fecal Coliform Membrane Filter Procedure), pp. 896-898, as set forth in *Standard Methods for the Examination of Water*

and Wastewater, 1985, American Public Health Association et al., 16th edition.

(2) Total coliform concentration--Method 908A (Standard Total Coliform Multiple--Tube (MPN) Tests), pp. 872-876, Method 908B (Application of Tests to Routine Examinations), pp. 876-878, Method 908D (Estimation of Bacterial Density), pp. 880-882, Method 909A (Standard Total Coliform Membrane Filter Procedure), pp. 887-894, or Method 909B (Delayed--Incubation Total Coliform Procedure), pp. 894-896, as set forth in *Standard Methods for the Examination of Water and Wastewater*, 1985, American Public Health Association et al., 16th edition; Minimal Medium ONPG-MUG Test, as set forth in the article "National Field Evaluation of a Defined Substrate Method for the Simultaneous Enumeration of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with the Standard Multiple Tube Fermentation Method" (Edberg et al.), Applied and Environmental Microbiology, Volume 54, pp. 1595-1601, June 1988 (as amended under Erratum, Volume 54, p. 3197, December, 1988).

(**Note:** The Minimal Medium ONPG-MUG Test is sometimes referred to as the Autoanalysis Colilert System.) Systems may use a five-tube test or a ten-tube test.

(3) Heterotrophic Plate Count--Method 907A (Pour Plate Method), pp. 864-866, as set forth in *Standard Methods for the Examination of Water and Wastewater*, 1985, American Public Health Association et al, 16th edition.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
October 1991

3. REPORT TYPE AND DATES COVERED

4. TITLE AND SUBTITLE
Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures; Quality Assurance - Change 1

5. FUNDING NUMBERS

6. AUTHOR(S)

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Environmental Protection Agency
Office of Ground Water and Drinking Water
401 M St., SW
Washington, DC 20460

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
570/9-90-008A

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Since 1978, the U.S. Environmental Protection Agency (EPA) has had a program for certifying regional laboratories, principal state laboratories in primacy states, and local laboratories in non-primacy states performing drinking water analyses required by regulations issued pursuant to the Safe Drinking Water Act. The manual describing the program's implementation procedures and technical criteria, THE MANUAL FOR THE CERTIFICATION OF LABORATORIES ANALYZING DRINKING WATER, is now in its third edition (April 1990, EPA 570/9-90-008).

This document is change 1 to the 1990 MANUAL FOR THE CERTIFICATION OF LABORATORIES ANALYZING DRINKING WATER. Change 1 revises Chapter V. This revision includes the certification criteria for the revised Total Coliform Rule and Surface Water Treatment Requirements. The updated Chapter V is to be inserted into the 1990 manual and the previous edition of Chapter V completely removed, along with the other pages indicated on page ii of this document.

14. SUBJECT TERMS
Drinking Water, Laboratory Certification, Quality Control

15. NUMBER OF PAGES
52

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT
unclassified

18. SECURITY CLASSIFICATION OF THIS PAGE
unclassified

19. SECURITY CLASSIFICATION OF ABSTRACT
unclassified

20. LIMITATION OF ABSTRACT

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA 570/9-90/008	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE Manual for the Certification of Laboratories Analyzing Drinking Water		5. REPORT DATE April 1990
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Office of Drinking Water (WH-550D) US EPA 401 M Street, SW Washington, DC 20460		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same as #9		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

This manual describes the operational and technical criteria and procedures EPA will use to evaluate a laboratory for its ability to properly analyze a regulated microbiological, chemical, or radiochemical drinking water contaminant. The certification program described in this manual extends to the EPA Regional laboratories, principal State laboratories in States which have primary enforcement responsibility (primacy), and to all laboratories that perform analyses under the SDWA in the few States without primacy. The vast majority of primacy States have thir own laboratory certification programs. Although many of them use the EPA's program as presented in this manual, individual State programs should be contacted to insure equivalency with State requirements.

This document is the third edition of the manual, and supersedes EPA 570/9-82-002, of the same title, which was issued in 1982.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Laboratory Certification Safe Drinking Water Act (SDWA)	Water Supply Drinking Water Quality Assurance Sanitary Microbiology Chemistry Radiochemistry	

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 88
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

☆U.S. GOVERNMENT PRINTING OFFICE:1990-717-003/28006