Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Scientific Laboratory Division

Title:	Micro Handbook
Version:	3.1
Ou Name:	Quality, Safety, Security, and Emergency Preparedness
Authors:	Paul Gray

Uncontrolled Copy

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Scientific Laboratory Division

Microbiological Drinking Water Handbook

v. 3.1 July 2011

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Contents

SECTION 1

Title	Page
Microbiological Drinking Water Laboratory Certification Program	
Individuals Responsible for Certification Program	7
On-Site Laboratory Audit Team	7
Certification Process	7
Types of Certification	8
Drinking Water Laboratories	8
Other Considerations for Laboratory Certification	8
Laboratory Quality Assurance Plan	9
Performance on Routine Water Samples	
Chain of Custody	9
Requirements for Maintaining Certification Status	9
Methodology	9
Sample Numbers	11
Sample Reporting	11
Results that Require Immediate Notification	11
Contact Log	11
On-Site Evaluation	11
Response to On-Site Evaluation	11
Training	12
Alternate Test Procedures (ATP's)	12
Fee Associated With SLD Evaluation	12

SECTION 2

USEPA Manual for the Certification or Laboratories Analyzing Drinking Water Criteria and Procedures Quality Assurance Edition 5 (EPA 815-R-05-004) Chapter 5 (January 2005)

Critical Elements for Microbiology

Section	Title	Page
1.	Personnel	19
2.	Laboratory Facilities	20
3.	Laboratory Equipment and Supplies	20
4.	General Laboratory Practices	25
5.	Analytical Methodology	27
5.1	*General*	27
5.2	*Fermentation broth methods*	31
5.3	*Chromogenic/fluorogenic substrate tests*	34

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

5.4	*Membrane Filter (MF) methods*	**37**
5.5	*Heterotrophic Plate Count (for enumerating heterotrophic bacteria in drinking water)*	**41**
5.6	*Coliphage*	**42**
6.	Sample Collection, Handling, and Preservation	**47**
7.	Quality Assurance	**49**
8.	Records and Data Reporting	**50**
9.	Action Response to Laboratory Results	**51**

Microbiology Laboratory Analysis Review Checklist

Section	Title	Page
Form	General Audit Information	**53**
Form	Laboratory Personnel	**54**
1.	Personnel	**55**
2.	Laboratory Facilities	**56**
3.	Laboratory Equipment and Supplies	**56**
4.	General Laboratory Practices	**62**
5.	Analytical Methodology	**63**
5.1	*General*	**63**
5.2	*Fermentation broth methods*	**65**
5.3	*Chromogenic/fluorogenic substrate tests*	**68**
5.4	*Membrane Filter (MF) methods*	**71**
5.5	*Heterotrophic Plate Count (for enumerating heterotrophic bacteria in drinking water)*	**75**
5.6	*Coliphage*	**77**
6.	Sample Collection, Handling, and Preservation	**82**
7.	Quality Assurance	**83**
8.	Records and Data Reporting	**84**
9.	Action Response to Laboratory Results	**85**

SECTION 3

Appendix A

Section	Title	Page
App A	Chain-of-Custody Evaluations	**87**

Appendix G

Section	Title	Page
App G	Analytical Methods for Microbiology	**93**

Supplement 1 to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water

Page
101

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

This DoH - SLD Microbiological Drinking Water Laboratory Program Manual replaces the previous Microbiological Drinking Water Laboratory Certification Program manual published July 2005 for all laboratories in New Mexico seeking certification through SLD for microbiological drinking water compliance samples. Laboratories seeking certification through Environment Department – Drinking Water Bureau are responsible for following the Drinking Water Bureau Manual.

Section 1 of this Manual is an edited version of Chapter 3 of EPA's ***Manual for the Certification of Laboratories Analyzing Drinking Water:*** *Criteria and Procedures Quality Assurance* (EPA 815-R-05-004 January 2005). Items have been added, expanded or deleted to reflect the current program in New Mexico.

Section 2 is as direct a copy of Chapter 5 of EPA's ***Manual for the Certification of Laboratories Analyzing Drinking Water:*** *Criteria and Procedures Quality Assurance* (EPA 815-R-05-004 January 2005) as can be made converting from Word Perfect (EPA's standard) to MS Word (SLD's standard). The only changes should be line spacing and pagination. The checklist (pages 1-C through 33-C) will be used during on-site evaluations (the "working checklist" may have different spacing and page numbers, but nothing will be added or removed).

Section 3 is direct copies of **Appendix A** ***Chain-of-Custody Evaluation*** and **Appendix G** ***Analytical Methods for Microbiology***. Both of these are referred to in Chapter V. The 2008 Supplement has also been added.

Published
July 2011
by
Department of Health
Scientific Laboratory Division
Office of Quality, Safety, Security and Emergency Preparedness

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Department of Health
Scientific Laboratory Division
Microbiological Drinking Water

Individuals Responsible for the Certification Program

The New Mexico Environment Department – Drinking Water Bureau (ED-DWB) is the agency granted Primacy by EPA for the Safe Drinking Water Act in New Mexico. The ED-DWB has, in turn, authorized the Department of Health Scientific Laboratory Division (DoH-SLD) to perform audits for those microbiology laboratories seeking certification by the certifying authority of the ED-DWB.

- The **Certification Authority (CA)** is the person who has signature authority for all certification decisions. For the DoH-SLD Microbiology Certification Program, the Certification Authority (CA) is a member of the Environmental Department – Drinking Water Bureau.

- The **Certification Officers (COs)** are DoH-SLD personnel who have the responsibility of certifying laboratories under their purview. 40 CFR 142.10(b) (3) (i) requires Primacy States to designate a person certified by the Administrator as the official responsible for the State's certification program.

On-Site Laboratory Audit Team

The DoH-SLD will establish one or more teams of certification officers and auditors to audit laboratories. It is the responsibility of these teams to perform the on-site laboratory audits, review the laboratory **Performance Evaluation (PE)** data and make recommendations to the ED-DWB CA concerning the certification status of the laboratories.

Team members will be experienced professionals, hold at least a bachelor's degree or equivalent education/experience in the discipline (microbiology) for which they certify, and have recent laboratory experience.

Team members will also have experience in laboratory evaluation and quality assurance, be familiar with the drinking water regulations and data reduction and reporting techniques, be technically conversant with the analytical techniques being evaluated and be able to communicate effectively, both orally and in writing.

The on-site team will include at least one Laboratory Certification Officer (CO) knowledgeable in microbiology. EPA policy requires COs to successfully complete the appropriate EPA laboratory certification course.

Certification Process

The certification process begins when the laboratory director makes a formal request to the CA (NMED-DWB) to be certified. This application may be one of the following:

- A request for first-time certification for microbiology;
- A request for certification to analyze additional or newly regulated Contaminants;
- A request to reapply for certification after correction of deficiencies which resulted in the downgrading/revocation of certification status.

The response to a formal application for any of the above requests should be given by the ED-DWB CA within 30 days. At this time a mutually agreeable date and time should be set for the on-site laboratory audit between the laboratory and SLD. If this is not a first-time certification, an on-site visit may not be necessary.

Subsequent audits may be initiated by the CA (or CO) or the Laboratory. If initiated by the CA (or CO), the audit may be announced or unannounced. Reasonable access by the certification team must be granted during regular work days and hours.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Types of Certification

After review of PE sample results and an on-site visit, the CO will provide a written report within 60 days and recommend classification of the laboratory for each contaminant or group of contaminants according to the following rating scheme:

- ***Certified*** - a laboratory that meets the minimum requirements of this manual and all applicable regulatory requirements.

- ***Provisionally Certified*** - a laboratory that has deficiencies but demonstrates its ability to consistently produce valid data within the acceptance limits specified in the National Primary Drinking Water Regulations, and within the policy required by their certification authority. A provisionally certified laboratory may analyze drinking water samples for compliance purposes, if the said clients are notified of its downgrade status in writing, on any report. Provisional certification may not be given if the evaluation team believes that the laboratory cannot perform an analysis within the acceptance limits specified in the regulations.

- ***Not Certified*** - a laboratory that possesses major deficiencies and, in the opinion of the CO, cannot consistently produce valid data.

  Interim Certification - interim certification may be granted in certain circumstances when it is impossible or unnecessary to perform an on-site audit. Interim certification status may be granted only when the CA judges that the laboratory has the appropriate instrumentation, is using the approved methods, has adequately trained personnel to perform the analyses, and has satisfactorily analyzed PE samples, if available, for the contaminants in question. The CO should perform an on-site audit as soon as possible but in no case later than three years. An example of a situation where this type of certification is warranted might be a laboratory that has requested certification for the analysis of additional analytes that involves use of a method for which it already has certification or a very similar method. The CO should review the laboratory's quality control data before granting this type of certification.

Drinking Water Laboratories

For the purpose of certification, any laboratory which analyzes drinking water compliance samples is considered a drinking water laboratory. This includes Federal laboratories that analyze compliance samples and any laboratories that analyze compliance samples for Federal facilities. **All** such laboratories must be certified by the State or EPA (40 CFR 141.21, .23, .24, .25). If requested by the State, a Region may certify Federal laboratories in its Region.

Other Considerations for Laboratory Certification

Laboratory Personnel

The laboratory should have sufficient supervisory and other personnel, with the necessary education, training, technical knowledge and experience for their assigned functions.

- ***Laboratory Director/Manager or Technical Director***
  The laboratory director/manager should be a qualified professional with the technical education and experience and managerial capability commensurate with the size/type of the laboratory. The laboratory director/manager is ultimately responsible for ensuring that all laboratory personnel have demonstrated proficiency for their assigned functions and that all data reported by the laboratory meet the required quality assurance (QA) criteria and regulatory requirements.
- ***Quality Assurance Officer/Manager***
  The QA officer/manager should be independent from the laboratory management if possible and have direct access to the highest level of management. The QA officer/manager should have a bachelor's degree in science, training in quality assurance principles commensurate with the size and sophistication of the laboratory and at least one year of experience in quality assurance/control. The QA officer/manager should have at least a working knowledge of the statistics involved in quality control of laboratory analysis and a basic understanding of the methods which the laboratory employs.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Laboratory Quality Assurance Plan

All laboratories analyzing drinking water compliance samples must adhere to the QC procedures specified in the methods being used. This is to ensure that routinely generated analytical data are scientifically valid and defensible and are of known and acceptable precision and accuracy. To accomplish these goals, each laboratory must prepare a written description of its QA activities (a QA plan). It is the responsibility of the QA manager to keep the QA plan up to date. All laboratory personnel must be familiar with the contents of the QA plan. This plan should be submitted to the auditors for review prior to the on-site visit or should be reviewed as part of the on-site visit.

The laboratory QA plan should be a separately prepared text. However, documentation for many of the listed QA plan items may be made by reference to appropriate sections of this manual, the laboratory's standard operating procedures, (SOPs) or other literature (e.g., promulgated methods, *Standard Methods for the Examination of Water and Wastewater*, etc.). The QA Plan should be updated at least annually.
At a minimum, the following items should be addressed in each QA plan:

1. **Laboratory organization and responsibility**
   - Include a chart or table showing the laboratory organization and lines of responsibility, including QA managers
   - List the key individuals who are responsible for ensuring the production of valid measurements and the routine assessment of measurement systems for precision and accuracy (e.g., who is responsible for internal audits and reviews of the implementation of the plan and its requirements)
   - Reference the job descriptions of the personnel and describe training to keep personnel updated on regulations and methodology, and document that laboratory personnel have demonstrated proficiency for the methods they perform.

2. **Process used to identify clients' Data Quality Objectives.**

3. **SOPs with dates of last revision.**
   - The laboratory should maintain SOPs that accurately reflect all phases of current laboratory activities.
   - Keep a list of SOPs
   - Ensure that current copies of SOPs are in the laboratory and in the QA Managers files
   - Ensure that SOPs are reviewed annually and revised as changes are made
   - Ensure that SOPs have signature pages and revisions dated

4. **Field sampling procedures**
   - Describe the process used to identify sample collectors, sampling procedures and locations, required preservation, proper containers, correct sample container cleaning procedures, sample holding times from collection to analysis, and sample shipping and storage conditions
   - Ensure that appropriate forms are legibly filled out in indelible ink or hard copies of electronic data are available
   - Describe how samples are checked when they arrive for proper containers and temperature
   - Ensure that sampling protocol is written and available to samplers

5. **Laboratory sample handling procedures**
   - Use laboratory note books, filled out in ink; entries dated and signed (A secure, password protected, electronic data base is acceptable)
   - Store unprocessed and processed samples at the proper temperature, isolated from laboratory contaminants, standards and highly contaminated samples and, sometimes, each other; holding times may not be exceeded
   - Maintain integrity of all samples, (e.g., by tracking samples from receipt by laboratory through analysis to disposal)
   - Specify criteria for rejection of samples which do not meet shipping, holding time and/or preservation requirements and procedures for notification of sample originators
   - Require Chain-of Custody procedures for samples likely to be the basis for enforcement action (i.e. repeat samples)

6. **Analytical procedures (may reference SOP)**
   - Cite complete method manual
   - Describe quality control procedures required by the methods that must be followed

7. **Data reduction, validation, reporting and verification (may reference SOP)**
   - Describe data reduction process: method of conversion of raw data to coliforms/100 mL, etc.
   - Describe data validation process
   - Describe reporting procedures, include procedures and format
   - Describe data verification process
   - Describe procedure for data corrections

8. **Type of quality control (QC) checks and the frequency of their use.**
   Parameters for microbiology should include or reference:
   - Positive and negative culture controls

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

- Confirmation/verification of presumptive total coliform positive samples
- Sterility controls
- Performance evaluation and quality control samples

9. **List schedules of internal and external system and data quality audits and inter laboratory comparisons** (may reference SOP).

10. **Preventive maintenance procedures and schedules**
    - Describe location of instrument manuals and schedules and documentation of routine equipment maintenance
    - Describe availability of instrument spare parts in the laboratory
    - List any maintenance contracts in place

11. **Corrective action contingencies**
    - Describe response to obtaining unacceptable results from analysis of PE samples and from internal QC checks
    - Name persons responsible for the various corrective actions
    - Describe how corrective actions taken are documented

12. **Record keeping procedures**
    - Describe procedures and documentation of those procedures
    - List length of storage, media type (electronic or hard copy)
    - Describe security policy of electronic databases
    - All Electronic data should have software support so it may be regenerated

If a particular item is not relevant, the QA plan should state this and provide a brief explanation. A laboratory QA plan should be responsive to the above items while remaining brief and easy to follow. Minimizing paperwork, while improving dependability and quality of data, are the intended goals.

PERFORMANCE ON ROUTINE WATER SAMPLES

Chain-of-Custody Procedures

Certified laboratories will use adequate chain-of-custody procedure as defined by NMED-DWB. The procedure used must be documented.

Requirements for Maintaining Certification Status

- **Performance Evaluation (PE) Samples**

  All certified drinking water laboratories **must** satisfactorily analyze PE samples to maintain certification. If the laboratory does not analyze the PE sample within the acceptance limits of:

  - time – within twelve months of the previous PE sample and
  - results – report at least nine of the ten samples correctly for both total coliforms and fecal coliforms/*E. coli,*

  the CA must follow the procedure discussed in the section entitled, "Criteria and Procedures to Downgrade/Revoke Certification Status" (Section 2.8 of the NMED-DWB Drinking Water Laboratory Certification Program Guidance Manual).

  If a laboratory wishes to be certified for a contaminant by more than one method, it must analyze the PE samples by each method for which it wishes to be certified. The methods listed on the laboratory's certification certificate must be the methods by which the PE samples were analyzed.

  The laboratory should be able to provide documentation to the CA that the person(s) analyzing any PE sample is a laboratory employee who routinely analyzes drinking water compliance samples.

- **Methodology**

  Laboratories must use the methods specified in the drinking water regulations at 40 CFR part 141.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

➢ **Sample Numbers**

To maintain certification, a laboratory must process a minimum of twenty (20) samples each month, by each method of certification. The laboratory must also have documentation of the confirmation of fifteen (15) positive (total AND fecal/*E. coli*) samples per calendar quarter (starting in January). If the laboratory does not process enough compliance samples to fulfill the quota, non-compliance samples (lab/spike and PE samples) may be added in. If enough positive samples are not processed in the quarter, both **POSITIVE** PE samples and spike samples may be used to reach the quota. A spike sample must confirm positive for both total and fecal/*E. coli*.

➢ **Sample Reporting**

Laboratories must provide a copy of all compliance sample results to the submitter of the sample and the appropriate NMED-DWB or EPA (or EPA designate) office within five (5) working days from the completion of the analyses. The form supplied by the laboratory and submitted to ED must conform to Section 6. and Section 8. of EPA's Chapter V (following this portion of the DoH Handbook). In addition, the Laboratory form must have the ED laboratory number on the form.

➢ **Results that require immediate notification:**

- Any sample with a result of "Present" for total coliform or Total Coliform and Fecal Coliform/*E. coli* must be reported to the water system and NMED-DWB or EPA (or EPA designate) by the end of the business day the analyses is completed.
- Any sample processed by membrane filter with a result of Too Numerous to Count (TNTC) or Confluent Growth of **Non-Coliforms** must be reported to the water system and NMED-DWB or EPA (or EPA designate) by the end of the business day the confirmation is completed.
  - ♦ If total coliform or fecal coliform/*E. coli* are confirmed, the sample is reported as **"Present"** for the group confirmed **AND** TNTC or Confluent Growth of Non-Coliform. Coliforms are present and the system may be out of compliance.
  - ♦ If no confirmation is performed, or confirmation is negative, report TNTC or Confluent Growth of Non-Coliform **with no reference to the presents or absence of coliforms**.
- Any sample that is rejected by the Laboratory for not meeting submission criteria (i.e. Leaking, frozen, >10°C, or >30 hours from collection) must be reported to the water system and NMED-DWB or EPA (or EPA designate) within twenty-four hours.
- Any sample that will be reported by the Laboratory as "Laboratory Accident" must be reported to the water system and NMED-DWB or EPA (or EPA designate) within twenty-four hours.

➢ **Contact Log**

A laboratory must keep a written record of contacts made to report positive results, invalid results or samples rejected by the laboratory. The record must contain information identifying the sample contact was made about, who was contacted (name and affiliation), when the contact was made (date and time) and how the contact was made (in person, by phone or email).

➢ **On-Site Evaluation**

The CA should be satisfied that a laboratory is maintaining the required standard of quality for certification. Normally, this should be based on a recommendation from an actual on-site evaluation. If the laboratory undergoes a major change, or if it fails a PE sample or other unknown test sample, the CA should consider conducting an evaluation at any time during the three-year certification period.

➢ **Response to On-Site Evaluation**

The Laboratory will have thirty (30) days to respond to the SLD CO, in writing, specifying what immediate corrective actions are being taken AND what proposed corrective actions will occur. The SLD CO and NMED-DWB CA will consider the adequacy of the response. If the response, and accompanying documentation, seems to correct the deviations, the laboratory will be issued notification of its certification status by NMED-DWB. If the response does not address the deviations, or lacks appropriate documentation, the laboratory will be notified of its downgraded status by ED-DWB. If no response is received within the thirty (30) day time limit, the NMED-DWB CA will be notified to initiate the process to revoke the laboratory's certification.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Training

State and Federal Drinking Water Programs constantly change, new methods are approved and new regulations are imposed. SLD will provide training programs, on request, to the Laboratories it Certifies. SLD Certified laboratories should attend at least one SLD provided training program (or a drinking water microbiology training program approved by the SLD CA) each calendar year.

Alternate Test Procedures (ATPs)

EPA promulgates analytical methods for all regulated drinking water contaminants. A regulation for a particular contaminant will include one or more methods that must be used to determine that contaminant. Subsequently, the Agency may approve additional methods or modifications of EPA approved methods in another rule. EPA may also authorize the use of alternate analytical methods as provided in 40 CFR 141.27, "With the written permission of the State, concurred by the Administrator of the EPA, an alternate analytical technique may be employed. An alternate technique may be accepted only if it is substantially equivalent to the prescribed test in both precision and accuracy as it relates to the determination of compliance with any MCL."

Anyone can request that EPA approve a new method or modification of a method already approved by EPA, by submitting EPA-specified data and other information to the Director, Analytical Methods Staff,(MS 4303T) Office of Science and Technology, Office of Water, EPA, 1200 Pennsylvania Ave., NW, Washington DC 20460. EPA will evaluate the material to determine whether the method or method modification meets EPA criteria. In the case of “acceptable versions” of methods, (minor modifications to approved methods), a letter of approval will be issued by the EPA Office of Water (OW). A list of these approved minor modifications can be found on the OW website at http://www.epa.gov/OGWDW/methods.

Fee Associated With SLD Evaluation

As of July 1, 2011 the NM Environment Department Drinking Water Bureau will no longer have an agreement with the Scientific Laboratory Division to defer the cost of services to certify drinking water laboratories. Now drinking water certification is split into two separate parts, the on-site evaluation, performed by SLD, and certification, issued by NMED-DWB. As a result, SLD will pass the cost of laboratory evaluation, reporting and recommendation of certification to NMED-DWB to facilities requesting certification. Payment of fees does not guarantee that SLD will recommend certification or that NMED-DWB will accept the recommendation of SLD. The current price list may be found at: http://www.sld.state.nm.us/safewater.asp or http://www.sld.state.nm.us/ > Lab fee schedule > Safe Drinking Water Act. The facility will receive an invoice after the on-site with prompt payment expected. Nonpayment will result in SLD requesting NMED-DWB to either revoke certification from, or not issue certification to, the facility. As stated in the price list, the fee will cover the first evaluation and, if necessary, a follow-up to that evaluation to determine compliance. If a third, or further, on-site is required to attain certification, the fee will be calculated using the schedule at the bottom of the page. If there are any questions, please contact SLD for clarification.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

United States
Environmental Protection
Agency

Manual for the Certification of Laboratories Analyzing Drinking Water

Criteria and Procedures Quality Assurance

Fifth Edition

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

EPA 815-R-05-004
January 2005

Manual for the Certification of Laboratories Analyzing Drinking Water

Criteria and Procedures Quality Assurance

Fifth Edition

Supersedes EPA/570/9-90/008, April 1990,
EPA 814-B-92-002, September 1992, and
EPA 570/9-90-008A, October 1991
EPA 815-B-97-001, March 1997

US Environmental Protection Agency
Office of Water
Office of Ground Water and Drinking Water
Technical Support Center
Cincinnati, Ohio 45268

tape on recycle
logo

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24
Authorised on: 22-Aug-2011. Authorised by: Paul Gray. SOP Unique Reference: 582-17895560. No review required.
Author(s): Paul Gray

Uncontrolled Copy

DISCLAIMER

The U.S. Environmental Protection Agency's (EPA's) Office of Ground Water and Drinking Water, in the Office of Water, and the Office of Research and Development of the U.S. Environmental Protection Agency prepared this manual. Those Offices as well as EPA's ten Regional Offices have reviewed this manual. EPA intends to use this manual for its own use in certifying laboratories for analysis of drinking water contaminants. In order to assume primary enforcement responsibility for the drinking water regulations, a State must either have available laboratory facilities, certified by the Administrator, capable of conducting analytical measurements of drinking water contaminants, or establish and maintain its own program for certification of laboratories. States wishing to adapt the procedures and criteria of this manual for their own certification program should revise it to accurately reflect accurately their State certification program.

This is a guidance manual and not a regulation. It does not change or substitute for any legal requirement. While EPA has made every effort to ensure the accuracy of the manual's discussion, the obligations of the regulated community are determined by the relevant statutes, regulations or other legally binding requirements. The manual obviously can only reflect the regulations in place at this time of its preparation. Consequently, for any definitive description of current legal obligations, the public should not rely only on the discussion in the manual. This manual is not a rule, is not legally enforceable, and does not confer legal rights or impose legal requirements upon any member of the public, States or any other Federal agency. In the event of a conflict between the discussions in this manual and any statute or regulation, this document is not controlling. The word "should" in this manual does not connote a requirement but does indicate EPA's strongly preferred approach to ensure the quality of laboratory results. EPA may decide to revise this manual without public notice to reflect changes to its approach or to clarify and update the text.

The mention of commercial products in this manual does not constitute an endorsement of the use of that product by EPA.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Acknowledgments

This edition of the manual was prepared through the efforts of many individuals, including representatives from the U.S. Environmental Protection Agency's Office of Ground Water and Drinking Water (OGWDW), Office of Research and Development (ORD), Regional Offices and the States. It has as its foundation previous editions of the manual. Contributors to the previous editions of the manual are listed in EPA documents EPA 815-B-97-001 March 1997, EPA/570/9-90/008 April 1990, EPA-570/9-82-002 October 1982 and EPA 600/8-78-008 May 1978. Contributors to this edition are listed below.

Manual Revision Committee:
Paul Berger, OGWDW
Edward Glick, OGWDW
Patricia Hurr, OGWDW
Caroline Madding, OGWDW

Microbiology Subcommittee:
Paul S. Berger, Chair
Kristen Brenner, ORD
Terry C. Covert, ORD
Mary Ann Feige, OGWDW
Nancy H Hall, UI HL
Stephanie Harris, Region 10
Margo Hunt, OEI
Kimberly K Laurie, IN DOH
Ted Pass II, KY DEP
Gene Rice, ORD
Mark Rodgers, ORD
Peggy Ryker, Consultant
Lois Shadix, OGWDW
Jim Sinclair, OGWDW
John Standridge, WI SLH
Phillip R Zillinger, IN DOH

Radiochemistry Subcommittee:
John Dehart, Dyncorp
John Griggs, OAR
Richard Sheibley, PA DEP

Other Manual Reviewers:
Ann Marie Allen, MA DEP
Dale Alley, RI DOH
Cheryl Baker, ME DHS
Rusty Baldwin, AR DOH
Brenda Barnett, WV DOH
Aimee Bennett, WA
Eve Berry AR DOH
Dave Bingham, WA
George Bowman, WI LOH
Ellen Braun-Howland, NYSDOH
David Brierley, MA DEP
Douglas Brune, Region 7
John Bourbon, Region 2
Donald Buckley, MA DPH
Joan Burkart, LA DOHH
Dwayne Burkholder, PA DEP
Isa Chamberlain, Region 10
Fred Choske, CA ELAP
Art Clark, Region 1
Terry Covert, ORD
Chris Dean, NM DOH
Veronica DeBoer, AK
Mary Dillon, NJDHSS
Ron Donaly, ID
Charles Dyer, NH DES
Stan Falk, AR DOH
Charles Feldmann, OGWDW
Ray Flores, Region 6
Paul Gray, NM DOH
Stephanie Harris, Region 10
Garry Haworth, NH DES
Paul Hickey, NYSDOH
Myron Honda, HI DOH
Jane Jensen, CA ELAP
Charles Jones, Jr., Region 3
Cecylia Karch, VT DOH
Ewa King, RI DOH
Michael Kitto, NYSDOH

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24
Authorised on: 22-Aug-2011. Authorised by: Paul Gray. SOP Unique Reference: 582-17895560. No review required.
Author(s): Paul Gray

Uncontrolled Copy

RJ Kiyokane, HI DOH
Sharon Kluender, WDNR
Margaret Knight, Region 10
George Kulasingam, CA ELAP
Don LaFara, NV BLC
Mary Beth Lawhorn, IL EPA
Andrew Lincoff, Region 9
Alan Love, AK
Nicholas Macelletii, CT DHS
Jim O'Dell, ORD
Alicia P. Ordona, VA DCLS
Pavin Parekh, NYSDOH
Virgina Palomo, OR
Oscar Pancorbo, MA DEP
Bahman Parsa, NJDHSS
Gary Perryman, Region 8
Barry Pharoah, ID
Max Phillips, TCEQ
Sandra Radwin, ID
Sara Rairick, AK
Robert Rieck, Region 10
J.Jane Roll, AR DOH
Irene Ronning, OR PHL
Jack Ruckman, NV BLC
David Russell, Region 3
Eileen Sanders, VA DCLS
Tim Sanders, Region 6
Philip Schlossberg, CT DPH
Richard Sheibley, PA DEP
Thomas Semkow, NYSDOH
Jody Shoemaker,ORD
Sandra Spence, Region 8
Mary E. T. Stancavage, MD HMH
David Stockton, Region 6
Miguel R. Suarez, Region 5
Marilyn Thornton, Region 4
Lisa Touet, MA DEP
Laura Traas, WDOTCP
Timothy Troup, AR DOH
Wayne Turnbull, Region 4
Debra Waller, NJDEP
Ted Witten, OK DEQ
Bruce Woods, Region 10
Kenneth Wunschel, RI DOH
DeLois Manor, AR DOH
Ted Martin, ORD
Gerald McKenna, Region 2
Joseph Mierzwicki, NJDHSS
William George Mills, VT DOH
Cathy Moore, AR DOH
Jean Munch, ORD
David Munch, OGWDW
Thomas O'Connell, MA DPH
Yue,Zhang,TDH

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Section 2 EPA's Chapter V

Critical Elements for Microbiology

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

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

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

1. Personnel

1.1 Supervisor/Consultant

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

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

1.2 Analyst (or equivalent job title)

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

1.3 Waiver of Academic Training

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

1.4 Personnel Records

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

2. Laboratory Facilities

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

3. Laboratory Equipment and Supplies

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

3.1 pH Meter

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

3.1.2 pH buffer aliquots should be used only once.

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

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

QC 3.1.5 Record pH meter slope monthly, after calibration.

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

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

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

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

3.2 *Balance (top loader or pan)*

3.2.1 Balances should have readability of 0.1 g.

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

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

3.3 Temperature Monitoring Device

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

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

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

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

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

3.4 Incubator Unit

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

3.5 Autoclave

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

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

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

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

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

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

3.6 Hot Air Oven

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

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

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

3.7 Colony Counter

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

3.8 Conductivity Meter

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

3.9 Refrigerator

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

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

3.10 Inoculating Equipment

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

3.11 Membrane Filtration Equipment (if MF procedure is used)

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

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

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

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

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

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

3.12 Culture Dishes (loose or tight lids)

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

3.13 Pipets

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

3.13.2 Pipets should have legible markings and should not be chipped or etched.
3.13.3 Opened packs of disposable sterile pipets should be resealed between use periods.

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

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

3.14 Glassware and Plasticware

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

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

3.14.3 Culture tubes and containers containing fermentation medium should be of sufficient size to contain medium plus sample without being more than three quarters full.
3.14.4 Tube closures should be stainless steel, plastic, aluminum, or screw caps with non-toxic liners. Cotton plugs and foam plugs should not be used.

3.15 Sample Containers

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

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

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

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

3.16 Ultraviolet lamp **(if used)**

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

3.17 Spectrophotometer or colorimeter (if used)

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

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

4. General Laboratory Practices

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

4.1 Sterilization Procedures

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

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

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

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

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

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

4.2 Sample Containers

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

4.3 Reagent-Grade Water

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24
Authorised on: 22-Aug-2011. Authorised by: Paul Gray. SOP Unique Reference: 582-17895560. No review required.
Author(s): Paul Gray

Uncontrolled Copy

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

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

Parameter	Limits	Frequency
Conductivity	>0.5 megohms resistance or <2 micromhos/cm (microsiemens/cm) at 25°C	Monthly[4]
Pb, Cd, Cr, Cu, Ni, Zn	Not greater than 0.05 mg/L per contaminant. Collectively, no greater than 0.1 mg/L	Annually
Total Chlorine Residual[1]	<0.1 mg/L	Monthly
Heterotrophic Plate Count[2]	< 500 CFU/mL[5]	Monthly
Bacteriological Quality of Reagent Water[3]	Ratio of growth rate 0.8 to 3.0	Annually

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

4.4 Dilution/Rinse Water

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

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

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

4.5 Glassware Washing

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

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

5. Analytical Methodology

5.1 General

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

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

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

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

5.1.5 Sample volume analyzed for total coliforms in drinking water must be 100 mL.

5.1.6 Media (or defined substrate)

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

[1] *S. aureus, P. aeruginosa* - not lactose fermenter
[2] *P. vulgaris* - not lactose fermenter; uses hydrolyzed lactose, indicating "overcooked" medium
[3] *E. aerogenes* - ferments lactose, but is not typically thermotolerant
[4] *K. pneumoniae* - ferments lactose, but does not hydrolyze MUG
[5] Do not use closely related strains from genus *Streptococcus* as a positive control
[6] *S. aureus* - sensitive to nalidixic acid in medium
[7] *E. coli* - sensitive to sodium azide in medium
[8] *S. marcescens* - will not hydrolyze fluorogenic compound in medium
[9] Examples of appropriate ATCC strains include the following:

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

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

Maximum Holding Times and Temperatures for Prepared Media

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

QC5.1.7 Laboratories are encouraged to perform parallel testing between a newly approved test and another EPA-approved procedure for enumerating total coliforms for at least several months and/or over several seasons to assess the effectiveness of the new test for the wide variety of water types submitted for analysis. During this testing, spiking the samples occasionally with sewage or a pure culture may be necessary to ensure that some of the tests are positive.

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Escherichia coli						
Enzyme substrate method	5.3.2	Colilert® or Colilert-18®	SM 9223	X		X
	5.3.2	Colisure®	SM 9223	X		X
	5.3.2	E*Colite®		X		X
	5.3.2	Readycult® or Fluorocult LMX®		X		
	5.3.3	LTB, P/A broth, M-Endo ⇒ EC-MUG	(SM 9221B,D; SM 9222B) SM 9221F	X		X
	5.3.2	Colitag®		X		
Membrane filter method	5.4.2	MI Medium	SM 9222	X		X
	5.4.2	m-ColiBlue24®		X		X
	5.4.2	Chromocult®		X		
	5.4.2	Coliscan®		X		
	5.4.3	M-Endo or LES Endo ⇒ NA-MUG	(SM 9222B) & SM 9222G	X		X

Enterococci[3]						
Enzyme substrate method	5.3.4	Enterolert	ASTM D6503-99			X
Fermentation broth method	5.2.5	Azide Dextrose ⇒ BEA/BHI	SM 9230B			X
Membrane filter method	5.4.4	mE EIA m-Enterococcus	SM 9230C			X
	5.4.4	mEI	EPA 1600			X
Heterotrophic Bacteria						
Pour plate method	5.5	Plate count agar	SM 9215B		X	
Multiple enzyme substrate	5.5	SimPlate®			X	
Pour plate, spread plate, or membrane filter methods	5.5	R2A		X[4]		

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Male-Specific and Somatic Coliphage[3]						
Agar plate method	5.6.2	Two-Step Enrichment	EPA 1601			X
	5.6.3	Single Agar Layer	EPA 1602			X

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

5.2 Fermentation broth methods

5.2.1 General

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

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

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

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

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

5.2.2.3 Media

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

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

5.2.2.7 Invalidation of total coliform-negative samples

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

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

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

5.2.3.1 Medium

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

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

5.2.3.1.3 If prepared medium is stored, it should be maintained in a culture bottle at 1°-3°C in the dark for no longer than three months. If evaporation exceeds 10% of original volume, the medium should be discarded.

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

5.2.4.1 EC Medium

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

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

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

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

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

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

5.2.4.2 A-1 Medium

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

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

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

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

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

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

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

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

5.3 Enzyme (Chromogenic/fluorogenic) substrate tests

5.3.1 General

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

5.3.1.2 Media

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

5.3.1.2.2. The media must be protected from light.

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

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

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

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

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

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

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

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

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

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

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

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

5.3.2 Criteria for specific media

5.3.2.1 For the Colilert test, samples must be incubated at 35°±0.5°C for 24 hours. A yellow color in the medium equal to or greater than the reference comparator indicates that the sample is total coliform-

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

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

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

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

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

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

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

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

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

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

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

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

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

5.4 Membrane Filter (MF) methods

5.4.1 General

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

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

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

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

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

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

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

5.4.2.1.2 m-ColiBlue24 medium for detecting total coliforms and *E. coli* in drinking water. Ampules of broth should be inverted 2-3 times to mix contents before breaking. Then contents should be poured evenly over absorbent pad. Unopened refrigerated ampules may be stored in the dark until the expiration date, but should be discarded earlier if growth is observed. The final pH of medium should be 7.0±0.2.

5.4.2.1.3 MI Medium (with or without agar) for detecting total coliforms and E. coli in drinking water or enumerating total coliforms in source water. Do not autoclave commercially made, presterilized bottled MI agar or broth. Melt bottled agar in a boiling water bath (or by other processes recommended by the manufacturer). As soon as complete melting has occurred, cool slightly and pour immediately into sterile plates. Care should be taken to prevent

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

overheating the agar, as excessive heat destroys the effectiveness of the antibiotic, cefsulodin. If dehydrated culture medium is used, it should be prepared and autoclaved according to the manufacturer's instructions. Cool the agar, add freshly prepared, filter-sterilized cefsulodin, and pour immediately into sterile plates. The final pH of MI agar should be 6.95±0.20; the final pH of MI broth should be 7.05±0.20. The preparation and use of MI agar and MI broth is described in the article, "New medium for the simultaneous detection of total coliform and *Escherichia coli* in water" by Brenner, K.P., *et al.*, 1993, *Applied and Environmental Microbiology* 59:3534-3544. EPA Method 1604, which can be found online at www.epa.gov/microbes, is identical.

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

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

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

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

5.4.2.2 Incubation conditions and colony color of inoculated medium:

Medium	Incubation	Total coliforms[1]	*E. coli*
M-Endo medium or M-Endo agar LES	35°±0.5°C for 22-24 hrs	Metallic (golden) sheen colonies (presumptive)	N/A
m-ColiBlue24	35°±0.5°C for 24 hrs	Red colonies	Blue to purple colonies
MI	35°±0.5°C for 24±2 hrs	Fluorescent colonies under UV light	Blue colonies under normal light
Chromocult	36°±1°C for 24±1 hrs	Salmon to red colonies	Dark-blue to violet colonies[2]
Coliscan	32°-37°C for 24-28 hrs	Pink-magenta colonies	Purple-blue colonies
m-FC	44.5°±0.2°C for 24±2 hrs	N/A	Blue colonies (fecal coliforms)

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

[1] Without the presence of *E. coli*. If an *E. coli* colony is present, as indicated by the last column, it should be counted as a total coliform-positive colony.
[2] If confirmation of *E. coli* is desired, add one drop of Kovac's reagent to each dark-blue to violet colony; the formation of a cherry-red color within seconds confirms the presence of *E. coli*.

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

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

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

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

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

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

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

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

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

5.4.4.1 Media

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

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

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

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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

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

5.5.2 Media

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

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

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

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

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

5.5.10 (For SimPlate Method)

5.5.10.1 Unit Dose (for a single sample). A10-mL volume of test sample is added to a test tube containing dehydrated SimPlate medium. Then the dissolved medium should be poured onto the center of a plate containing 84 small wells (provided by the manufacturer, IDEXX Laboratories, Inc.). Alternatively, 9 mL of sterile diluent (D.I. water, distilled water, or buffered water [*Standard Methods*, 9050 C, 1a]) can be added to the tube, followed by 1-mL sample. Then follow the procedure as indicated above for the 10-mL sample. The mixture should be distributed evenly to the 84 wells on the plate, and the excess liquid drained into an absorbent pad on the plate. The plate should then be inverted (the fluid in each well is held in place by surface tension), and incubated for 45-72 hours at 35°±0.5°C. Bacterial density is determined by counting the number of wells that fluoresce under a 365-366 nm UV light, and converting this value to a Most Probable Number using the Unit Dose MPN table provided by the manufacturer. If 10-mL sample is used, read the Unit Dose MPN/mL directly. If a 1-mL sample is used, then correct the MPN/mL value by multiplying it by 10.

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

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

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

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

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

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

Method Summary: A 100-mL (or 1-L water sample) is supplemented with magnesium chloride, log-phase host bacteria (*E. coli* F_{amp} for male-specific coliphage and *E. coli* CN-13 for somatic coliphage), and Tryptic Soy Broth (TSB) as an enrichment step for coliphage. After an overnight incubation, samples are "spotted" onto a lawn of host bacteria specific for each type of coliphage, incubated, and examined for circular lysis zones, which indicate the presence of coliphage.

5.6.1.1 Media

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

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

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

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

5.6.1.2 Coliphage stock

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

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

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

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

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

5.6.1.3 Host bacteria stock cultures

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

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

5.6.1.3.3 Log-phase host bacteria stock cultures—After preparation, chill on wet ice or at1°-5°C to slow replication until ready for use. The suspension may be stored up to 48 hours. However, the best results occur when cultures are used immediately (within 6 hours). Store remaining bacterial host culture at 1°-5°C overnight to inoculate flasks for the preparation of new log-phase bacterial hosts.

5.6.1.4 General QC

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

5.6.1.4.2 Method blanks. *The laboratory must analyze method blanks (reagent water sample containing no coliphage) to demonstrate freedom from contamination.* For each coliphage type used, prepare and analyze a sterile reagent water sample containing no coliphage using the same procedure used

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

for analysis of the field or QC samples. At a minimum, the laboratory must analyze one method blank for each spot plate used for field samples. In an effort to determine if cross-contamination is an issue, the sterile method blank should be spotted onto the lawn of host bacteria immediately following the positive control spot.

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Initial demonstration of laboratory capability (IDC) for Method 1601

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

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

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

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

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

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

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

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

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

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

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

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

5.6.2.4 General QC

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

5.6.2.4.1 Initial precision and recovery (IPR). *The laboratory must demonstrate the ability to perform this method acceptably by performing an IPR test before analyzing any field samples.* Four reagent water samples for each coliphage type are required for the IPR test. IPR samples must be spiked in bulk to yield a target spike concentration of 80 PFU per sample. IPR samples must be spiked with enumerated sewage filtrate or equivalent. The relative standard deviation of the recovery (RSD_r) and the average percent recovery ($\bar{x}$) based on all four sample results for each coliphage type should meet the acceptance criteria in the QC acceptance table below.

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Quality control acceptance criteria for Method 1602

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

6. Sample Collection, Handling, and Preservation

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

6.1 Sample Collector

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

6.2 Sampling

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

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

6.3 Sample Icing

6.3.1 (Bacterial samples) Samplers are encouraged, but not required, to hold drinking water samples at <10°C during transit to the laboratory. Source water samples required by the Surface Water Treatment Rule (SWTR) must be held at <10°C during transit (see *Standard Methods*, Section 9060B). Laboratories should reject samples that have been frozen.

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

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

6.4 Sample Holding/Travel Time

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

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

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

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

6.5 Sample Information Form

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

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

6.6 Chain-of-Custody

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

7. Quality Assurance

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

7.2 States are encouraged to establish proficiency testing (PT) as part of their drinking water certification program for microbiology. A laboratory should successfully analyze at least one set of PT samples once every 12 months, for each method for which it is certified. For methods used to test the presence or absence of an organism in a sample, each PT set should contain ten samples, all shipped at the same time in either a lyophilized, dehydrated, or aqueous state. The set should include samples, in various combinations, that contain total coliforms, fecal coliforms, *E. coli*, non-coliforms, and at least one blank. Each set should be used only with a single analytical method. To be acceptable, a laboratory should correctly analyze a minimum of nine of the ten samples, with no false-negative result (i.e., a single false-positive result may be acceptable).

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

8. Records and Data Reporting

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

8.2 Maintenance of Records:
Public water systems are required to maintain records of microbiological analyses of compliance samples for five years (40 CFR 141.33). The laboratory should maintain easily accessible records for five years or until the next certification data audit is complete, whichever is longer. A change in ownership, merger, or closure of a laboratory does not cancel this requirement. The client water system should be notified before disposing of records so they may request copies if needed. This includes all raw data, calculations, and quality control data. These data files may be either hard copy, microfiche or electronic. Electronic data should always be backed up by protected tape or disk or hard copy. If the laboratory changes its computer hardware or software, it should make provisions for transferring old data to the new system so that it remains retrievable within the time frames specified above. Data which is expected to become part of a legal action will probably need to be maintained for a longer period of time. Check with your legal counsel. See *Good Automated Laboratory Practices*, EPA 2185, Office of Information Management, Research Triangle Park, NC 27711, 8/10/95.

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

8.3.1 Sample information form, from 6.5 above
8.3.2 Date and time of sample receipt by the laboratory
8.3.3 Name of laboratory person receiving the sample
8.3.4 Any deficiency in the condition of the sample. A sample should be invalidated for the following reasons:

- Time between sample collection and receipt by laboratory has been exceeded
- Presence of disinfectant in sample is noticed (e.g., odor)
- Evidence of freezing
- Use of a container not approved by the laboratory for the purpose intended
- Insufficient sample volume (e.g., <100 mL)
- Presence of interfering contaminant, if noticed (e.g., hydrocarbons, cleansers, heavy metals, etc.)
- Sample temperature exceeds the maximum allowable

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

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

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

9. Action Response to Laboratory Results

9.1 Testing Total Coliform-Positive Cultures

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

9.2 Notification of Positive Results

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

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

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

9.3 Notification of Total Coliform Interference

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

MICRO CHECKLISTS

General Audit Information

Laboratory:	
Mailing Address (mailing address of owner if different):	
Street	
City, State, Zip code	
Audit Location (if different):	
Telephone:	
Fax:	
E-mail:	
Other:	
Audit Organization:	
Auditors/Signatures:	
Date of Last On-Site:	**Report available:**
Date of Current PE Samples:	**Approximate Monthly Sample Load:**
Method(s) Lab is Requesting Evaluation for:	
Audit Date(s):	

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Laboratory Personnel

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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Element	Number	Yes	No	NA	Comments
1. PERSONNEL					
Supervisor/Consultant	**1.1**				
Does the supervisor of the microbiology laboratory have a bachelor's degree in microbiology, biology, or equivalent?					
Has a supervisor with a degree in a subject other than those listed above had at least one college-level microbiology laboratory course in which environmental microbiology was covered?					
In addition, has the supervisor had a minimum of two weeks training at a Federal or State agency or academic institution in microbiological analysis of drinking water or 80 hours of on-the-job training in water microbiology at a certified laboratory, or other training acceptable to the State or EPA?					
If a supervisor is not available, and a waiver has not been granted as per Section 1.3, is a consultant with the same qualifications substituted?					
Can the laboratory document that the consultant is acceptable to the State, and present on-site frequently enough to satisfactorily perform a supervisor's duties?					
Can the laboratory supervisor demonstrate that all laboratory personnel have the ability to satisfactorily perform the analyses to which they are assigned?					
Can the laboratory supervisor demonstrate that all data reported by the laboratory meets the required quality assurance and regulatory criteria?					
Analyst (or equivalent job title)	**1.2**				
Does the analyst have at least a high school education, a minimum of three months bench experience in water, milk or food microbiology, training in microbiological analysis of drinking water acceptable to the State (or EPA), and a minimum of 30 days on-the-job training under an experienced analyst?					
Has the analyst demonstrated acceptable results on unknown samples before analyzing compliance samples?					
Waiver of Academic Training	**1.3**				
Has the certification authority waived the need for the above specified academic training for highly experienced analysts in this laboratory?					
Has the certification authority waived the need for the above specified training for supervisors of laboratories associated with drinking water systems that only analyze samples from that system?					
If yes to either of the above, does the laboratory have a copy of that written and signed waiver available for inspection?					
Personnel Records	**1.4**				
Does the laboratory maintain personnel records on laboratory analysts that include academic background, specialized training courses completed, and types of microbiological analyses conducted?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

2. LABORATORY FACILITIES					
Does the laboratory have facilities that are clean and temperature and humidity controlled, and with adequate lighting at the bench tops?					
Does the laboratory maintain effective separation of incompatible testing areas?					
Does the laboratory control access where appropriate, and minimize traffic flow through the work areas?					
Does the laboratory ensure that contamination does not adversely affect data quality?					
Does the laboratory have bench tops and floors that are easily cleaned and disinfected?					
Does the laboratory have sufficient space for processing samples; storage space for media, glassware, and portable equipment; floor space for stationary equipment; and areas for cleaning glassware and sterilizing materials?					
Does the laboratory have provisions for disposal of microbiological wastes?					
3. LABORATORY EQUIPMENT AND SUPPLIES					
Does the laboratory have the equipment and supplies needed to perform the approved methods for which certification has been requested?					
pH meter	**3.1**				
Are accuracy and scale graduations within ±0.1 units?	**3.1.1**				
Are pH buffer aliquots used only once?	**3.1.2**				
Are electrodes maintained according to the manufacturer's recommendations?	**3.1.3**				
QC Are pH meters standardized before each use period with pH 7.0 and either 4.0 or 10.0 standard buffers, whichever covers the desired pH of the media or reagent?	**3.1.4**				
QC Are both the date and buffers used recorded in a logbook along with the analyst's initials?					
QC Is the pH slope recorded monthly, after calibration?	**3.1.5**				
QC If the pH meter does not have a feature to automatically calculate the slope, but can provide in the pH in millivolts, is the formula in Section 3.1.5.1 used to calculate the slope?	**3.1.5.1**				
QC If the slope is below 95% or above 105%, are the manufacturer's instructions followed for meter or electrode maintenance and general cleaning?	**3.1.5.2**				
Balance (top loader or pan)	**3.2**				
Does the balance have a readability of 0.1 g?	**3.2.1**				
Does the balance have a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less?	**3.2.2**				
QC Are the balances calibrated monthly using ASTM Class 1, 2, or 3 weights (minimum 3 traceable weights which bracket laboratory weighing needs, with a readability of 0.1 g)?	**3.2.3**				
QC Are non-reference weights calibrated every six months with reference weights?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

QC Are calibrations recorded in a logbook with the initials of the individual performing the calibration?					
QC Are correction values on file and used?					
QC Are reference weights re-certified every five years?					
QC Are damaged or corroded weights replaced?					
QC Are service contracts or internal maintenance protocols and maintenance records available?	**3.2.4**				
QC Is maintenance, calibration, and cleaning conducted at least annually by a qualified independent technician, unless the need is modified or waived by the certification officer?					
Temperature Monitoring Device	**3.3**				
Are glass, dial, or electronic thermometers graduated in 0.5°C increments (0.2°C increments for tests which are incubated at 44.5°C) or less, except as noted for hot air ovens (Section 3.6.1) and refrigerators (Section 3.9.1)?	**3.3.1**				
Does observation of glass thermometers indicate no separation in fluid columns?					
Are only dial thermometers which can be adjusted used?					
QC Are glass and electronic thermometers calibrated annually and dial thermometers quarterly at the temperature used, against a NIST-traceable reference thermometer or one that meets the requirements of NBS Monograph SP 250-23?	**3.3.2**				
QC Are both the calibration factor and calibration date indicated on the thermometer?					
QC Is the following calibration information recorded in a QC record book? - Serial number of the laboratory thermometer - Serial number of the NIST-traceable thermometer (or other reference thermometer) - Temperature of the laboratory thermometer - Temperature of the NIST-traceable thermometer (or other reference thermometer) - Correction (or calibration) factor - Date of check - Analyst's initials	______ ______ ______ ______ ______ ______ ______	___ ___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___ ___	
QC Is the thermometer discarded if it differs by more than 1°C from the reference thermometer?	**3.3.3**				
QC Are reference thermometers recalibrated at least every five years?					
QC Is reference thermometer calibration documentation maintained?					
QC Are continuous recording devices used to monitor incubator temperature recalibrated at least annually, using a reference thermometer that meets the specifications noted in Section 3.3.2?	**3.3.4**				
Incubator Unit	**3.4**				
Do incubator units have an internal temperature monitoring device and maintain a temperature specified by the method used, usually 35°±0.5°C and 44.5°±0.2°C?	**3.4.1**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

For non-portable incubators, are thermometers placed on top and bottom shelves of the use area and immersed in liquid as directed by the manufacturer (except for electronic thermometers)?					
When aluminum block incubators are used, do culture dishes and tubes fit snugly?					
QC Is the calibration-corrected temperature recorded for each thermometer being used at least twice per day during each day the incubator is in use?	**3.4.2**				
QC Are these readings separated by at least four hours?					
QC Does the documentation include the date and time of reading, temperature, and technician's initials?					
If a circulating water bath is used, is it equipped with a gable cover to ensure an incubation temperature of 44.5°±0.2°C?					
Autoclave	**3.5**				
Does the autoclave have an internal heat source, a temperature gauge with a sensor on the exhaust, a pressure gauge, and an operational safety valve?	**3.5.1**				
Can the autoclave maintain a sterilization temperature during the sterilizing cycle and complete an entire cycle within 45 minutes when a 12-15 minute sterilization period is used?					
Does the autoclave depressurize slowly enough to ensure that media will not boil over and bubbles will not form in inverted tubes?					
QC Is the following information recorded each time the autoclave is used? - Date - Contents - Sterilization time and temperature - Total time in the autoclave - Analyst's initials	**3.5.3**				
QC Are copies of the service contracts or internal maintenance protocols and maintenance records kept?					
QC Is maintenance conducted at least annually?					
QC Is a record of the most recent service performed on file and available for inspection?					
QC Is a maximum-temperature-registering thermometer, electronic temperature readout device, or continuous recording device used each autoclave cycle to ensure that the proper temperature was reached?	**3.5.4**				
QC Is the temperature recorded?					
QC Is overcrowding avoided?					
QC Are spore strips or spore ampules used monthly as bioindicators to confirm sterilization?					
QC Are automatic timing mechanisms checked quarterly with a stopwatch or other accurate timepiece or time signal, and the results recorded and initialed?	**3.5.5**				
Are autoclave door seals clean and free of caramelized media?	**3.5.6**				
Are autoclave drain screens cleaned frequently and debris removed?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Hot Air Oven	**3.6**				
Does the oven maintain a stable sterilization temperature of 170°-180°C for at least two hours?	**3.6.1**				
Is overcrowding avoided?					
Is the oven thermometer graduated in 10□C increments or less, with the bulb placed in sand during use?					
QC Is the following information recorded for each cycle? - Date - Contents - Sterilization time and temperature - Analyst's initials	**3.6.2**				
QC Are spore strips used monthly to confirm sterilization?	**3.6.3**				
Colony Counter	**3.7**				
Is a dark field colony counter used to count Heterotrophic Plate Count colonies?					
Conductivity Meter	**3.8**				
Are meters suitable for checking laboratory reagent-grade water and readable in units of either micromhos/cm or microsiemens/cm?	**3.8.1**				
QC Is the meter calibrated at least monthly, following the manufacturer's recommendations and using an appropriate certified and traceable low-level standard?	**3.8.2**				
QC If the meter cannot be calibrated as noted above, is the cell constant determined at monthly intervals using a method in *Standard Methods*, Section 2510?					
Is an in-line unit that cannot be calibrated used to check reagent-grade water?	**3.8.3**				
Refrigerator	**3.9**				
Does the refrigerator maintain a temperature of 1°-5°C?	**3.9.1**				
Is the refrigerator thermometer graduated in at least 1°C increments and the thermometer bulb immersed in liquid?					
QC On days the refrigerator is in use, and the laboratory is staffed, is the calibrated-corrected temperature recorded at least once per day	**3.9.2**				
Inoculating Equipment	**3.10**				
Are sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipet tips used?					
Are wood applicator sticks, if used, sterilized by dry heat?					
Are metal inoculating loops and/or needles made of nickel alloy or platinum?					
Membrane Filtration (MF) Equipment	**3.11**				
Are MF units made of stainless steel, glass, porcelain, or autoclavable plastic?	**3.11.1**				
Are they scratched, corroded, or leaking?					

Uncontrolled Copy

QC If graduations on clear or plastic funnels are used to measure sample volume, is their accuracy checked with a Class B graduated cylinder or better (or other Class B glassware) and a record of this calibration check retained?	**3.11.2**				
Is a 10x to 15x stereo microscope with a fluorescent light source used to count sheen colonies?	**3.11.3**				
Are the membrane filters approved by the manufacturer for total coliform water analysis?	**3.11.4**				
Are membrane filters to be used cellulose ester, white, gridmarked, 47 mm diameter, and 0.45 µm pore size?					
If alternate pore sizes are used, does the manufacturer provide performance data equal to or better than the 0.45 µm pore size?					
Are membrane filters and pads purchased presterilized or autoclaved for 10 minutes at 121°C before use?					
QC Is the lot number for membrane filters and the date received recorded?	**3.11.5**				
QC Are the membranes checked to see that they are not brittle or distorted?					
QC Are the manufacturer's specification/certification sheets available?					
Are the forceps blunt and smooth-tipped without corrugations on the inner sides of the tips?	**3.11.6**				
Culture Dishes (loose or tight lids)	**3.12**				
Are presterilized plastic or sterilizable glass culture dishes used?	**3.12.1**				
Is the sterility of the glass culture dishes maintained by placement in stainless steel or aluminum canisters or a wrap of heavy aluminum foil or char-resistant paper?					
Are loose-lid petri dishes incubated in a tight-fitting container with a moistened paper towel?	**3.12.2**				
Are opened packs of disposable culture dishes resealed between use periods?	**3.12.3**				
For membrane filter methods, are culture dishes of an appropriate size to allow the transfer of a single membrane per plate?	**3.12.4**				
Pipets	**3.13**				
Are glass pipets sterilized and maintained in stainless steel or aluminum canisters or wrapped individually in char-resistant paper or aluminum foil?	**3.13.1**				
Do pipets have legible markings and are they not chipped or etched?	**3.13.2**				
Are opened packs of disposable sterile pipets resealed between use periods?	**3.13.3**				
Are pipets delivering volumes of 10 mL or less accurate to within a 2.5% tolerance?	**3.13.4**				
Are calibrated micropipetters used with sterile tips?	**3.13.5**				
Are micropipetters calibrated annually and adjusted or replaced if the precision or accuracy is greater than 2.5%?					
Glassware and Plasticware	**3.14**				
Is the glassware made of borosilicate glass, or other corrosion-resistant glass, and free of chips and cracks?	**3.14.1**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Are markings on graduated cylinders and pipets legible?					
Are plastic items clear and nontoxic to microorganisms?					
QC Are the graduated cylinders used for measurement of sample volumes, or other precalibrated containers that have clearly marked volumes used in lieu of graduated cylinders, accurate to within a 2.5% tolerance?	**3.14.2**				
Are culture tubes and containers containing fermentation medium of sufficient size to contain medium plus sample without being more than three quarters full?	**3.14.3**				
Are tube closures made of stainless steel, plastic, aluminum, or screw caps with nontoxic liners?	**3.14.4**				
Are cotton or foam plugs used?					
Sample Containers	**3.15**				
Are sample containers wide-mouth plastic or non-corrosive glass bottles with non-leaking ground glass stoppers or caps with nontoxic liners, sterile plastic bags containing sodium thiosulfate, or other appropriate sample containers?	**3.15.1**				
Is sample container capacity at least 120 mL (4 oz) to allow at least a 1-inch head space?					
Are glass stoppers covered with aluminum foil or char-resistant paper for sterilization?	**3.15.2**				
Are unsterilized glass and plastic bottles sterilized by autoclaving or, alternatively, by dry oven for glass bottles?	**3.15.3**				
Are empty containers moistened with several drops of water before autoclaving to prevent an "air lock" sterilization failure?					
If chlorinated water is to be analyzed, is sufficient sodium thiosulfate added to the sample bottles before sterilization to neutralize any residual chlorine in the water sample?	**3.15.4**				
Ultraviolet Lamp (if used)	**3.16**				
Is the germicidal unit disconnected monthly and the lamp cleaned by wiping with soft cloth moistened with ethanol?	**3.16.1**				
Is the longwave unit used for fluorometric tests kept clean?					
QC Is the germicidal unit tested quarterly with a UV light meter or agar spread plate?	**3.16.2**				
QC Is the lamp replaced if it emits less than 70% of its initial output or if an agar spread plate containing 200 to 250 microorganisms, exposed to the UV light for two minutes, does not show a count reduction of 99%?					
Spectrophotometer or colorimeter (if used)	**3.17**				
Are wavelengths in the visible range?	**3.17.1**				
QC Is a calibration standard and a method-specific blank analyzed every day the instrument is used, prior to sample analysis?	**3.17.2**				
QC Is this calibration standard obtained from an outside source?					
QC Does the calibration standard give a reading in the desired absorbance range?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

4. GENERAL LABORATORY PRACTICES					
Are laboratory personnel aware of general and customary safety practices for laboratories?					
Does the laboratory have a safety plan available?					
Does the laboratory keep a copy, and follow the personal protection guidelines, of any material safety data sheet accompanying the receipt of a toxic material?					
Sterilization Procedures	**4.1**				
Does the laboratory follow the minimum times for autoclaving the materials listed below at 121°C? Membrane filters and pads 10 min Carbohydrate containing media 12-15 min[1] Contaminated test materials 30 min[2] Membrane filter assemblies 15 min Sample collection containers 15 min Individual glassware 15 min Dilution water blank 15 min Rinse water (0.5 - 1 L) 15-30 min[2] [1] except where otherwise specified by the manufacturer [2] time depends upon water volume per container and autoclave load	**4.1.1** _______ _______ _______ _______ _______ _______ _______ _______	 ___ ___ ___ ___ ___ ___ ___ ___	 ___ ___ ___ ___ ___ ___ ___ ___	 __ __ __ __ __ __ __ __	
Are autoclaved membrane filters and pads and all media removed immediately after completion of the sterilization cycle?	**4.1.2**				
Is membrane filter equipment autoclaved before the beginning of a filtration series?	**4.1.3**				
If a UV light (254 nm) is used to sanitize equipment after initial autoclaving for sterilization, are all supplies presterilized?	**4.1.4**				
Sample Containers	**4.2**				
QC Is at least one sample container selected at random from each batch of sterile sample bottles, or other containers (or lot of commercially available sample containers), and the sterility confirmed by adding 25 mL of a sterile non-selective broth, incubating at 35°±0.5°C, and checking for growth after 24 and 48 hours?					
QC Are these results recorded?					
QC If growth is detected, is the entire batch resterilized?					
Reagent-Grade Water	**4.3**				
Does the laboratory only use satisfactorily tested reagent water from stills or deionization units to prepare media, reagents, and dilution/rinse water for performing microbial bacteriological analyses?	**4.3.1**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

QC Is the quality of reagent water tested and does it meet the following criteria? Conductivity >0.5 megohms resistance or <2 micromhos/cm (microsiemens/cm) at 25°C Monthly*	**4.3.2**				
Pb, Cd, Cr, Not greater than 0.05 mg/L Annually					
Cu, Ni, Zn per contaminant. Collectively no greater than 0.1 mg/L Total chlorine <0.1 mg/L residual* Monthly					
Heterotrophic plate count* <500/mL CFU/mL* Monthly					
Bacteriological quality of reagent water* Ratio of growth rate 0.8 to 3.0 Annually					
*See Section 4.3.2 for footnotes					
Dilution/Rinse Water	**4.4**				
Is stock buffer solution or peptone water prepared as specified in *Standard Methods*, Section 9050C?	**4.4.1**				
Are stock buffers autoclaved or filter-sterilized?	**4.4.2**				
Are these containers labeled, dated, and refrigerated?					
Are stored stock buffers free from turbidity?					
QC Is each batch (or lot, if commercially prepared) of dilution/rinse water checked for sterility by adding 50 mL of water to 50 mL double strength non-selective broth, incubating at 35°± 0.5□C, and checking for growth after 24 hours and 48 hours?	**4.4.3**				
QC Are these results recorded?					
QC Is the batch/lot discarded if growth is detected?					
Glassware Washing	**4.5**				
Is distilled or deionized water used for the final rinse?	**4.5.1**				
Is laboratory glassware washed with a detergent designed for laboratory use?	**4.5.2**				
QC Is the glassware inhibitory residue test performed before the initial use of a washing compound and whenever a different formulation, or washing procedure is used?	**4.5.3**				
QC Are these results recorded?					
QC Is each batch of dry glassware used for microbial analysis spot-checked for pH reaction using 0.04% bromthymol blue (or equivalent pH indicator) and the color reaction recorded?	**4.5.4**				
5. ANALYTICAL METHODOLOGY					
General	**5.1**				
For compliance samples, does the laboratory use only the analytical methodologies specified in the Total Coliform Rule (TCR), the Surface Water Treatment Rule (SWTR), and the Groundwater Rule (GWR)?	**5.1.1**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Is the laboratory certified for all analytical methods it uses for compliance purposes?	**5.1.2**				
At a minimum, is the laboratory certified for one total coliform method and one fecal coliform or *E. coli* method?					
Is the laboratory certified for a second total coliform method if one method cannot be used for some drinking waters?					
For a laboratory that enumerates heterotrophic bacteria for compliance with the SWTR, is the laboratory certified for either the Pour Plate Method or the SimPlate method for heterotrophic bacteria?					
Are water samples shaken vigorously at least 25 times before analyzing?	**5.1.3**				
QC If dilution buffer is used, does the laboratory check the buffer volume in one dilution bottle of each batch or lot?	**5.1.4**				
QC For a 90-mL or 99-mL volume, is the tolerance ±2 mL?					
Does the laboratory analyze a 100-mL sample volume for total coliforms in drinking water?	**5.1.5**				
Media (or defined substrate)	**5.1.6**				
Are dehydrated media stored in a cool dry location and discarded by the manufacturer's expiration date?	**5.1.6.1**				
Is caked or discolored dehydrated media discarded?					
QC For media prepared in the laboratory is the following information recorded? - Date of preparation - Type of medium - Lot number - Sterilization time and temperature - Final pH (after sterilization) Technician's initials	**5.1.6.2** ____ ____ ____ ____ ____ ____	 __ __ __ __ __ __	 __ __ __ __ __ __	 __ __ __ __ __ __	
QC For media prepared commercially is the following recorded for each lot? - Date received - Type of medium - Lot number - pH verification	**5.1.6.3** ____ ____ ____ ____	 __ __ __ __	 __ __ __ __	 __ __ __ __	
QC Are media prepared commercially discarded by manufacturer's expiration date?					
QC Is each new lot of dehydrated or prepared commercial medium and each batch of laboratory-prepared medium checked before use for sterility and with positive and negative culture controls?	**5.1.6.4**				
QC Are these results recorded?					
QC For laboratories using commercially prepared media with manufacturer shelf-lives of greater than 90 days, are positive and negative controls run each quarter, in addition to that noted above?					
QC Are these results recorded?					
QC For control organisms, are stock cultures periodically checked for purity and the results recorded, or are commercially available disks impregnated with the organism used?					
If prepared medium is stored after sterilization, is it maintained in the dark as follows? poured plates 1°-5°C 2 weeks	**5.1.6.5**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

broth in containers with loose-fitting closures 1°-30°C 2 weeks					
broth in tightly closed containers 1°-30°C 3 months					
QC Does the laboratory perform parallel testing between a newly approved test and another EPA-approved procedure for enumerating total coliforms for at least several months and/or several seasons to assess the effectiveness of the new test for the wide variety of water types submitted for analysis? Recommended.	**5.1.7**				
Does the laboratory perform the approved methods listed in this section for the TCR, SWTR, and/or GWR?	**5.1.8**				
Fermentation broth methods	**5.2**				
General	**5.2.1**				
Is the water level of the water bath above the upper level of the medium in the culture tubes?	**5.2.1.1**				
If a Dri-bath incubator is used, is the specified temperature requirement maintained in all tube locations used?	**5.2.1.2**				
Multiple Tube Fermentation Technique (for detecting total coliforms in drinking water and enumerating total coliforms in source water)	**5.2.2**				
For drinking water samples, is the total sample volume of 100 mL used for each test?	**5.2.2.1**				
For source water samples, are at least 3 series of five tubes each with appropriate sample dilutions used?	**5.2.2.2**				
Media					
Is lauryl tryptose broth (LTB) used in the presumptive test and 2% brilliant green lactose bile broth (BGLBB) in the confirmed test?	**5.2.2.3.1**				
If lactose broth (LB) is used in lieu of LTB, has the laboratory conducted at least 25 parallel tests between this medium and LTB using the waters normally tested?					
Has this comparison demonstrated that the false-positive rate and false-negative rate for total coliforms, using LB, is less than 10%?					
Is this comparison information documented and the records retained?					
Is the final pH of LTB medium 6.8 ± 0.2?					
Is the final pH of 2% BGLBB 7.2 ± 0.2?					
Is the test medium concentration adjusted to compensate for the sample volume so that the resulting medium after sample addition is single strength?	**5.2.2.3.2**				
If a single 100-mL sample volume is used, is the inverted vial replaced with an acid indicator (bromcresol purple)?					
Is the medium autoclaved at 121°C for 12-15 minutes?					
Is the sterile medium in tubes examined to ensure that the inverted vials, if used, are free of air bubbles and are at least one-half to two-thirds covered after the water sample is added?	**5.2.2.3.3**				
Is the inoculated medium incubated at 35°±0.5°C for 24±2 hours?	**5.2.2.4**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

If no gas or acid detected, is the inoculated medium incubated for another 24 hours for a total incubation time of 48±3 hours?					
Is each 24- and 48-hour tube that has growth or is gas-positive or acid-positive confirmed using 2% BGLBB?	**5.2.2.5**				
For drinking water samples, is each total coliform-positive sample tested for the presence of either fecal coliforms or *E. coli*?	**5.2.2.6**				
Invalidation of total coliform-negative samples	**5.2.2.7**				
For drinking water samples, are all samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, invalidated?	**5.2.2.7.1**				
Does the laboratory then collect, or request that the system collect, another sample within 24 hours from the same location as the original invalidated sample?					
Although not required before invalidation, does the laboratory perform a confirmed test and/or a fecal coliform/*E. coli* test on the total coliform-negative culture to check for coliform suppression?					
And if the confirmed test is total coliform-positive or fecal coliform/*E. coli*-positive, does the laboratory report the sample as such?					
If the follow-up test is total coliform-negative, does the laboratory invalidate the sample?					
For source water samples are all samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production in LTB or LB invalidated?	**5.2.2.7.2**				
Does the laboratory collect, or request that the system collect, another sample from the same location as the original invalidated sample?					
Although not required before invalidation, does the laboratory perform a confirmed test on the total coliform-negative culture and, if the confirmed test is total coliform-positive, is the MPN reported?					
If the confirmed test is total coliform-negative, is the sample invalidated?					
Presence-Absence (P-A) Coliform Test (for detecting total coliforms in drinking water)	**5.2.3**				
Medium	**5.2.3.1**				
When six-times formulation strength medium is used, is it filter-sterilized rather than autoclaved?	**5.2.3.1.1**				
Is the medium autoclaved for 12 minutes at 121°C?	**5.2.3.1.2**				
Is the total time in the autoclave less than 30 minutes?					
Are the bottles placed in the autoclave with space between them?					
Is the final pH of the medium 6.8±0.2?					
If the prepared medium is stored, is it maintained in a culture bottle at 1°-30°C in the dark for no more than 3 months?	**5.2.3.1.3**				
Is the stored medium discarded if evaporation exceeds 10% of original volume?					
Is a 100-mL sample inoculated into a P-A culture bottle?	**5.2.3.2**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Is the sample/medium incubated at 35°±0.5°C and observed for yellow color (acid) after 24 and 48 hours?	**5.2.3.3**				
Are yellow cultures confirmed in BGLBB and a fecal coliform/*E. coli* test conducted?	**5.2.3.4**				
Are all samples which produce a non-yellow turbid culture in P-A medium invalidated?	**5.2.3.5**				
Does the laboratory collect, or request that the system collect, another sample from the same location as the original invalidated sample?					
Although not required before invalidation, does the laboratory perform a confirmed test on the total coliform-negative culture and/or a fecal coliform/*E. coli* test and, if the confirmed test is total coliform-positive, is the sample reported as such?					
If the confirmed test is total coliform-negative, is the sample invalidated?					
Fecal Coliform Test (using EC Medium for fecal coliforms in drinking or source water, or A-1 Medium for fecal coliforms in source water only)	**5.2.4**				
EC Medium	**5.2.4.1**				
Is EC medium used to test a total coliform-positive culture for fecal coliforms under the Total Coliform Rule?	**5.2.4.1.1**				
Is each total coliform-positive culture transferred from a presumptive tube/bottle, or each presumptive total coliform-positive colony (unless a cotton swab is used); to at least one tube containing EC Medium with an inverted vial?					
Is EC medium used to enumerate fecal coliforms in source water, in accordance with the SWTR?	**5.2.4.1.2**				
When conducting a MTF test, are three sample volumes of source water with five or ten tubes/sample volume used?					
Is a culture from each total coliform-positive tube transferred to a tube containing EC Medium with an inverted vial?					
Is EC Medium autoclaved at 121°C for 12-15 minutes?	**5.2.4.1.3**				
Is the final pH of EC medium 6.9±0.2?					
Are the inverted vials examined to ensure that they are free of air bubbles and at least one-half to two-thirds covered after the sample is added?	**5.2.4.1.4**				
Is EC Medium incubated at 44.5°±0.2°C for 24±2 hours?	**5.2.4.1.5**				
Is any amount of gas detected in the inverted vial of a tube that has turbid growth considered a fecal coliform-positive test, regardless of the result of any subsequent test on that culture?	**5.2.4.1.6**				
A-1 Medium	**5.2.4.2**				
If A-1 Medium is used, is it used to enumerate only fecal coliforms in source water, in accordance with SWTR, and not for drinking water samples?	**5.2.4.2.1**				
Are three sample volumes of source water used in a five- or ten-tube/sample volume format?					
Is A-1 Medium autoclaved at 121°C for 10 minutes?	**5.2.4.2.2**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

For A-1 Medium, is the final pH 6.9±0.1?					
Are inverted tubes examined to ensure that they are free of air bubbles?	**5.2.4.2.3**				
Is A-1 Medium incubated at 35°±0.5°C for three hours, and then incubated at 44.5°±0.2 °C for 21±2 hours?	**5.2.4.2.4**				
Are loose-cap tubes stored in the dark at room temperature for no longer than two weeks, or in tightly closed screw-cap tubes in the dark at <30°C for no longer than three months?	**5.2.4.2.5**				
Is any amount of gas detected in the inverted vial of a tube with turbid growth considered a fecal coliform-positive test?	**5.2.4.3**				
Azide dextrose medium (for detecting fecal streptococci in ground water)	**5.2.5**				
For testing 100-mL samples, is triple strength (3X) formulation in a culture bottle prepared and then autoclaved at 121°C for 15 minutes?	**5.2.5.1**				
Is medium final pH 7.2±0.2?					
Is a 100-mL water sample added to the sterilized medium and incubated at 35°±0.5°C?	**5.2.5.2**				
Is the culture checked for turbidity after 24±2 hours?	**5.2.5.3**				
If turbidity is not observed, is the culture reincubated and checked again after a total incubation period of 48±3 hours?					
Are turbid cultures confirmed as fecal streptococci by streaking a portion of the broth onto bile esculin agar (BEA) or bile esculin azide agar (BEAA)?	**5.2.5.4**				
Are BEA and BEAA autoclaved at 121°C for 15 minutes?	**5.2.5.5**				
Is the final pH 6.6±0.2 for BEA and 7.1±0.2 for BEAA?					
After streaking, are plates incubated at 35°±0.5°C for 48 hours?	**5.2.5.6**				
Are the brownish-black colonies with brown halos on BEA or BEAA used as confirming the presence of fecal streptococci?	**5.2.5.7**				
If required, does the laboratory perform an enterococci test by transferring one or more fecal streptococci colonies to brain heart infusion broth supplemented with 6.5% NaCl and incubating the culture at 35°±0.5°C for 48 hrs?					
Enzyme (chromogenic/fluorogenic) substrate tests	**5.3**				
General	**5.3.1**				
For detecting total coliforms and *E. coli* in drinking water by an enzyme substrate test, does the laboratory use one of the following: MMO-MUG test (Colilert), Colisure test, E*Colite test, Readycult Coliforms 100 Presence/Absence Test, Fluorocult LMX test, or Colitag test?	**5.3.1.1**				
For enumerating total coliforms in source waters by an enzyme substrate test, does the laboratory use the Colilert test?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

If a laboratory uses a fermentation method to detect total coliforms in drinking water, and the sample is total coliform-positive, does the laboratory transfer the positive culture to the EC+MUG test to detect *E. coli*, but not to any other enzyme substrate test medium in Section 5.3?					
Media	**5.3.1.2**				
Does the laboratory purchase media from a commercially available source only, and not prepare media from basic ingredients?	**5.3.1.2.1**				
Are media kept protected from light?	**5.3.1.2.2**				
Is each lot of medium checked for fluorescence before use with a 365-366-nm ultraviolet light with a six watt bulb?	**5.3.1.2.3**				
If medium exhibits faint fluorescence, is another lot used that does not fluoresce?					
If samples plus medium exhibit color changes before incubation, is the medium discarded and another lot of medium used?	**5.3.1.2.4**				
Are glass and plastic bottles and test tubes checked before use with a 365-366-nm ultraviolet light source with a 6-watt bulb to ensure that they do not fluoresce?	**5.3.1.3**				
If they fluoresce, does the laboratory use another lot of containers that does not fluoresce?					
If a Whirl-Pak® bag is used to incubate the Colilert or Colitag medium or any other medium which changes to a yellow color to indicate a positive result, is a type used that has a barrier (e.g., B01417) to prevent gaseous emissions to other Whirl-Pak® bags during incubation?	**5.3.1.4**				
QC If a small air-type incubator is used, are samples brought to room temperature before incubation?	**5.3.1.5**				
If a water bath is used, is the water level above the upper level of the medium?	**5.3.1.6**				
For E. coli testing, are all total coliform-positive samples placed under a UV lamp (365-366 nm) in a darkened area?	**5.3.1.7**				
Does the laboratory refrain from using the enzyme substrate test to confirm a presumptive total coliform-positive culture in a fermentation broth or on a membrane filter?	**5.3.1.8**				
Does the laboratory invalidate any sample that produces an atypical color change (in the absence of a yellow color) and then collect, or request that the system collect, another sample from the same location as the original invalidated sample?	**5.3.1.9**				
Does the laboratory use another method to test the second sample?					
Is the reference comparator provided by the manufacturer discarded by the manufacturer's expiration date?	**5.3.1.10**				
Criteria for specific media	**5.3.2**				
For the Colilert test, are samples incubated at 35°±0.5°C for 24 hours?	**5.3.2.1**				
Is a sample with a yellow color in the medium equal to or greater than reference comparator recorded as total coliform-positive?					
Is a sample with a yellow color lighter than comparator incubated for another four hours but no longer than 28 hours total?					
Is a sample with a yellow color lighter than the comparator after 28 hours of incubation recorded as total coliform-negative?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Are coliform-positive samples that fluoresce under a UV light marked as *E. coli*-positive?					
For the Colilert-18 test, are samples incubated for 18 hours (up to 22 hours if the sample after 18 hours is yellow, but lighter than the comparator)?					
For enumerating total coliforms in source waters, does the laboratory use the Colilert test, a 5- or 10-tube configuration, Quanti-Tray, or Quanti-Tray 2000 for each sample dilution tested?	**5.3.2.1.1**				
When dilution water is used, is it either sterile deionized or sterile distilled water, not buffered water?					
QC If the Quanti-Tray or Quanti-Tray 2000 test is used, is the sealer checked monthly by adding a dye to the water?	**5.3.2.1.2**				
For the Colisure test, are samples incubated at 35°±0.5°C for 24-48 hours?	**5.3.2.2**				
If the medium changes from a yellow color to a red/magenta color, is the sample noted as total coliform-positive?					
Is a coliform-positive sample that fluoresces under a UV light marked as *E. coli*-positive?					
For the E*Colite test, is the sample incubated at 35°±0.5°C for 28 hours?	**5.3.2.3**				
If the medium changes from a yellow color to a blue or blue-green color, or a blue color in the corners of the bag, is the sample marked as total coliform-positive?					
If the medium fluoresces under a UV light, is the sample considered as *E. coli*-positive?					
If fluorescence is not observed, is the sample reincubated for an additional 20 hours (for a total incubation time of 48 hours) and checked again for fluorescence?					
If the medium becomes red in color, is the sample discarded and another sample requested?					
For the Readycult Coliforms 100 Presence-Absence test, are the contents of a snap pack added to a 100-mL sample and then incubated at 35°±0.5°C for 24±1 hours?	**5.3.2.4**				
If the medium changes color from a slightly yellow color to blue-green, is the sample marked as coliform-positive?					
If the medium fluoresces a bright light-blue color when subjected to long wave UV (365-366 nm) light, is the sample marked as *E. coli*-positive?					
For the Fluorocult LMX test, is the medium added to purified water, mixed, and the mixture then boiled to dissolve the medium completely in the water?	**5.3.2.5**				
Are 100-mL aliquots transferred to 250-mL bottles and then autoclaved for 15 minutes?					
Are the autoclaved bottles cooled before adding the 100-mL water sample?					
Is the *E. coli*/Coliform Supplement not added to the medium?					
Is the sample then incubated at 35°±0.5°C for 24±1 hours?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

If the medium changes color from a slightly yellow color to blue-green, is the sample marked as coliform-positive?					
If the medium fluoresces a bright light-blue color when subjected to long wave UV (365-366 nm) light, is the sample marked as *E. coli*-positive?					
For the Colitag test, are samples incubated at 35°±0.5°C for 24±2 hours?	**5.3.2.6**				
If the medium changes to a yellow color, is the sample marked as coliform-positive?					
If the medium fluoresces under a UV light, is the sample marked as *E. coli*-positive?					
EC Medium + MUG (for detection of *E. coli*)	**5.3.3**				
If EC medium + MUG is used, is a total coliform-positive culture transferred from a presumptive tube/bottle or colony to this medium?	**5.3.3.1**				
Is the final pH of EC medium + MUG 6.9±0.2?	**5.3.3.2**				
Is the medium plus sample incubated at 44.5°±0.2°C for 24±2 hours and then tested for fluorescence?	**5.3.3.4**				
Enterolert test (for detection of enterococci in ground water)	**5.3.4**				
Is the medium stored in the dark at 4°-30°C until used?	**5.3.4.1**				
Is Enterolert reagent added to a 100-mL sample and the sample/medium incubated at 41°±0.5°C for 24-28 hours?	**5.3.4.2**				
Is fluorescence under a UV lamp used to indicate the presence of enterococci?					
Membrane Filter (MF) methods	**5.4**				
General	**5.4.1**				
For source water samples (SWTR), do dilutions yield 20 to 80 total coliform colonies or 20 to 60 fecal coliforms for at least one dilution or volume?	**5.4.1.1**				
QC Is at least one membrane filter and filtration unit sterility check conducted at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter and testing for growth?	**5.4.1.2**				
QC If the control indicates contamination, does the laboratory reject all data from affected samples and request an immediate resampling?					
QC Does the laboratory consider a filtration series as ended when 30 minutes or more has elapsed between sample filtrations?					
Are filtration funnels rinsed after each sample filtration with two or three 20-30 mL portions of sterile rinse water to ensure that the entire sample is rinsed off the funnel onto the filter?	**5.4.1.3**				
Are absorbent pads saturated with at least 2 mL of broth and the excess medium removed by "decanting" the plate?	**5.4.1.4**				
MF method for detecting total coliforms and *E. coli* in drinking water, enumerating total coliforms or fecal coliforms in source water, and detecting *E. coli* in ground water	**5.4.2**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Media for total coliforms, fecal coliforms, and *E. coli*	**5.4.2.1**				
If either M-Endo agar or broth or M-Endo agar LES is used to detect total coliforms in drinking water or enumerating total coliforms in source water, is either the single step or the enrichment technique used?	**5.4.2.1.1**				
Is denatured ethanol used in the rehydration procedure?					
Is the medium prepared in a sterile flask?					
Is a boiling water bath or a constantly attended hot plate with a stir bar used to bring the medium just to the boiling point but not boiled?					
Is the final for M-Endo medium pH 7.2±0.1 and the final pH for M-Endo agar LES 7.2±0.2?					
Is M-Endo medium or M-Endo agar LES incubated at 35°±0.5°C for 22-24 hrs?	**(5.4.2.2)**				
Are colonies with a metallic (golden) sheen recorded as presumptive total coliforms?	**(5.4.2.2)**				
If m-ColiBlue24 medium is used to detect total coliforms and *E. coli* in drinking water, are the ampules of broth inverted 2-3 times to mix contents before breaking and the contents then poured evenly over an absorbent pad?	**5.4.2.1.2**				
Are unopened refrigerated ampules stored in the dark?					
Are unopened ampules discarded before the expiration date, or earlier if contamination is observed?					
Is the medium final pH 7.0±0.2?					
Is m-ColiBlue24 incubated at 35°±0.5°C for 24 hrs?	**(5.4.2.2)**				
Are red colonies recorded as total coliforms, and blue to purple colonies recorded as *E. coli*?	**(5.4.2.2)**				
If MI medium (with or without agar) is used to detect total coliforms and *E. coli* in drinking water or enumerate total coliforms in source water, is commercially prepared presterilized bottled MI agar or broth not autoclaved?	**5.4.2.1.3**				
Is this presterilaized bottled agar medium melted in a boiling water bath (or by other processes recommended by the manufacturer), and care taken not to overheat the agar?					
Is the medium then cooled slightly and poured immediately into sterile plates?					
If dehydrated culture medium is used, is it prepared and autoclaved according to manufacturer's instructions?					
Is this agar medium cooled before adding freshly prepared, filter-sterilized cefsulodin, and then poured immediately into sterile plates?					
Is the final pH of MI agar 6.95±0.20 and the final pH of MI broth 7.05±0.20?					
Is MI medium incubated at 35°±0.5°C for 24±2 hrs?	**(5.4.2.2)**				
Are fluorescent colonies under UV light recorded as total coliforms, and blue colonies under normal light recorded as *E. coli*?	**(5.4.2.2)**				
If Chromocult® Coliform Agar is used to detect total coliforms and *E. coli* in drinking water, is the agar medium autoclaved or overheated?	**5.4.2.1.4**				
Is the final pH of this medium 6.8±0.2?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

If a heavy background of heterotrophic bacteria is expected, is cefsulodin solution added to 1L of cooled (45°-50°C) medium as a solution of 10 mg cefsulodin dissolved in 2-mL deionized or distilled water?					
Is Chromocult incubated at 36°±1°C for 24±1 hrs?	**(5.4.2.2)**				
Are salmon to red colonies recorded as total coliforms, and dark-blue to violet colonies recorded as *E. coli*?	**(5.4.2.2)**				
If Coliscan® is used to detect total coliforms and *E. coli* in drinking water or enumerate total coliforms in source water, is the manufacturer's protocol for reconstitution and antibiotic addition followed?	**5.4.2.1.5**				
Is the antibiotic, cefsulodin, overheated?					
Is the final pH of Coliscan agar 7.00±0.20?					
Is Coliscan incubated at 32°-37°C for 24-28 hrs?	**(5.4.2.2)**				
Are pink-magenta colonies recorded as total coliforms, and purple-blue colonies recorded as *E. coli*?	**(5.4.2.2)**				
If m-FC broth, with or without agar, is used to enumerate fecal coliforms in source water, is the medium autoclaved?	**.4.2.1.6**				
Is m-FC broth just brought to the boiling point?					
Is the final pH of m-FC medium 7.4±0.2?					
Is m-FC broth incubated at 44.5°±0.2°C for 24±2 hrs?	**(5.4.2.2)**				
Are blue colonies recorded as fecal coliforms?	**(5.4.2.2)**				
Is the prepared medium refrigerated when stored and brought to room temperature before use?	**5.4.2.1.7**				
Are petri dishes containing medium stored in a plastic bag or tightly closed container, and used within 2 weeks?					
Are plates with laboratory-prepared broth medium discarded after 96 hours, poured agar plates after 2 weeks, and ampuled broth discarded before the manufacturer's expiration date?					
Are the date and time of medium preparation recorded?					
For invalidation of a total coliform-negative drinking water sample, are all samples resulting in confluent growth or TNTC growth invalidated unless at least one total coliform colony is detected?	**5.4.2.3**				
If no coliforms are detected, is the sample recorded as "confluent growth" or "TNTC" and an additional sample requested from the same sampling site?					
Does the laboratory perform a verification test on the total coliform-negative culture before invalidation?					
If the verification test is total coliform-positive, does the laboratory report the sample as total coliform-positive?					
If the verification test is total coliform-negative, is the sample invalidated?					
For invalidation of source water samples (SWTR), where coliform density must be determined, does the laboratory invalidate any sample that results in confluent growth or TNTC, even when total coliform or fecal coliform colonies are present?	**5.4.2.4**				
For drinking water samples on M-Endo type media, are all sheen colonies, up to a maximum of five, verified by using either LB or LTB and then 2% BGLBB or, alternatively, by using a cytochrome oxidase and β-galactosidase procedure?	**5.4.2.5**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

If no sheen colonies are observed, are up to five red questionable sheen colonies and/or red non-sheen colonies representing different morphological types verified?					
For drinking water samples, are total coliform-positive colonies tested for *E. coli* or fecal coliforms?	**5.4.2.6**				
When EC Medium or EC + MUG is used, are colonies transferred by employing one of the options specified by the Total Coliform Rule?					
When the swab technique is used, is a single swab used to inoculate a presumptive total coliform-positive sample into EC or EC+MUG first, LTB second, and BGLBB third?					
For source water samples, are the initial total coliform counts adjusted based upon verified data?	**5.4.2.7**				
QC For source water samples when two or more analysts are available, does each analyst count the total coliform or fecal coliform colonies on the same membrane monthly and do the counts agree within 10%?	**5.4.2.8**				
Nutrient Agar + MUG Test (for detection of *E. coli* in drinking water or ground water)	**5.4.3**				
Is the medium autoclaved at 121°C for 15 minutes?	**5.4.3.1**				
Is the final MUG concentration 100 µg/L?					
Is the final pH of NA + MUG 6.8±0.2?					
QC Are positive and negative culture controls tested as stated in 5.1.6.4?	**5.4.3.2**				
QC Are culture controls filtered or spot-inoculated onto a membrane filter on M-Endo broth or agar, or M-Endo agar LES, and incubated at 35°±0.5°C for 24 hours?					
QC Is the filter then transferred to NA + MUG and incubated at 35°±0.5°C for another four hours?					
QC Are these results read and recorded?					
Is the membrane filter containing total coliform colonies transferred to the surface of the Nutrient Agar + MUG medium?	**5.4.3.3**				
Is the presence of each sheen colony marked on the petri dish lid with permanent marker, and the lid and base marked to realign the lid when removed?					
For the total coliform verification test, is a portion of each colony transferred with needle before the MF transfer or after the four-hour NA + MUG incubation time?					
Alternatively, is the membrane filter surface swabbed with a sterile cotton swab after the four-hour incubation time on NA + MUG and then transferred to a total coliform verification test?					
Is the inoculated NA + MUG medium incubated at 35°±0.5°C for four hours?	**5.4.3.4**				
Is fluorescence checked by using a UV lamp (365-366 nm) with a six-watt bulb in a darkened room and any fluorescence in the halo around a sheen colony considered positive for *E. coli*?	**5.4.3.5**				
MF method for detecting enterococci/fecal streptococci in ground water	**5.4.4**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Media	**5.4.4.1**				
When mE agar is used for the detection of enterococci, is basal mE agar prepared, autoclaved, and cooled before the addition of nalidixic acid (or its sodium salt) and triphenyl tetrazolium chloride, both of which are added separately to the medium and mixed?	**5.4.4.1.1**				
Is the final pH of mE agar 7.1±0.2?					
When m-Enterococcus agar is used for the detection of fecal streptococci (not enterococci), is the medium heated, not autoclaved, to dissolve the ingredients?	**5.4.4.1.2**				
Is the final pH of m-Enterococcus agar 7.2±0.2?					
When mEI agar is used for the detection of enterococci, is 0.75g indoxyl-β-D-glucoside added to 1L basal mE agar and then prepared according to 5.4.4.1.1 except that only 0.02 g/L triphenyl tetrazolium chloride is added?	**5.4.4.1.3**				
Is the final pH of mEI agar 7.1±0.2?					
Is a 100-mL sample filtered and the membrane placed on one of the agar media previously listed?	**5.4.4.2**				
If m-Enterococcus agar is used, are the plates incubated in an inverted position at 35°±0.5°C for 48 hours?	**5.4.4.3**				
Using magnification and a fluorescent lamp, are all light and dark red colonies counted as fecal streptococci?					
If mE agar is used, are the plates incubated in an inverted position for 48 hours at 41°±0.5°C?	**5.4.4.4**				
Is the membrane filter then transferred to EIA medium and incubated at 41°±0.5°C for 20-30 minutes?					
Using magnification and a fluorescent lamp, are all pink to red colonies on mE agar with a black or reddish brown precipitate on the underside of the filter on EIA agar counted as enterococci?					
If mEI agar is used, are plates incubated in an inverted position for 24 hours at 41°±0.5°C?	**5.4.4.5**				
Using magnification and a fluorescent lamp, is the plate examined, top and bottom, for colonies with a blue halo, and any colony with a blue halo (regardless of colony color) considered as positive for enterococci?					
Heterotrophic Plate Count (for enumerating heterotrophic bacteria in drinking water)	**5.5**				
Does the laboratory use the Pour Plate Method or the SimPlate Method for enumerating heterotrophic bacteria in drinking water and for testing reagent grade water?	**5.5.1**				
For systems granted a variance from the TCR's maximum contaminant level, does the laboratory use R2A medium with a method in *Standard Methods*, Section 9215 for enumerating heterotrophic bacteria in drinking water?					
Media	**5.5.2**				
Is the final pH recorded for plate count agar pH 7.0±0.2, R2A agar 7.2±0.2, and SimPlate 7.2±0.2?					
For the Pour Plate Method, is melted agar tempered at 44°-46°C in a water bath and maintained no more than 3 hours before pouring?	**5.5.3**				
Is this sterile medium melted only once?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

For the Spread Plate Method, is 15 mL of R2A medium (or other medium) poured into a sterile petri dish and allowed to solidify?	**5.5.4**				
Is refrigerated medium in bottles or screw-capped tubes stored for no longer than six months, or in petri dishes for no longer than 2 weeks (one week for prepared petri dishes with R2A medium)?	**5.5.5**				
For countable plates of most potable water samples, are 1.0 mL and/or 0.1 mL volumes of the undiluted sample plated?	**5.5.6**				
Are at least duplicate plates prepared per dilution tested?					
For the Pour Plate Method, is the sample pipetted aseptically onto the bottom of a sterile petri dish and then at least 10-12 mL tempered melted agar added?	**5.5.7**				
Is the sample and melted agar mixed, avoiding spillage?					
After the agar plates have solidified on a level surface, are they inverted and incubated at 35°±0.5°C for 48±3 hours?					
Are plates stacked no more than four high and arranged in the incubator to allow proper air circulation and to maintain a uniform incubation temperature?					
Does the laboratory ensure that incubator does not have excess humidity and that the plates do not lose more than 15% by weight during the 48 hours of incubation?					
For the Spread Plate Method, is 0.1 or 0.5 mL of the sample (or dilution) pipetted onto the surface of the predried agar in the plate and then spread over the entire surface using a sterile bent glass rod?	**5.5.8**				
Is the inoculum absorbed completely before incubating?					
Are the plates incubated in an inverted position at 20°-28°C for 5-7 days?					
For the Membrane Filter Technique, does the filtered volume yield between 20-200 colonies?	**5.5.9**				
Is the filter transferred to a petri dish containing 5 mL solidified R2A medium and then incubated at 20°-28°C for 5-7 days?					
Are plates with loose-fitting lids placed in a plastic box with a close-fitting lid and moistened paper towels, and rewetted as necessary?					
Are colonies counted using a stereoscopic microscope at 10-15X magnification?					
SimPlate Method	**5.5.10**				
For a single sample Unit Dose, is a 10-mL test sample added to a test tube containing dehydrated SimPlate medium and then poured onto the center of a plate containing 84 small wells?	**5.5.10.1**				
Alternatively, is 9-mL of sterile diluent added to the test tube containing the dehydrated medium, followed by a 1-mL sample, and the medium plus sample then poured onto the center of a plate containing 84 small wells?					
Is this mixture distributed evenly to the 84 wells and is the excess liquid drained into the absorbent pad on the plate?					
Is the plate inverted and incubated at 35°±0.5°C for 45-72 hours?					
Is bacterial density determined by counting the number of wells that fluoresce under a 365-366-nm UV light, and converting this value to a Most Probable Number/mL using the manufacturer's Unit Dose MPN table?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

If a 10-mL sample is used, is the Unit Dose MPN/mL read directly or, if a 1-mL sample is used, is the MPN/mL value corrected by multiplying it by 10?					
For the Multiple Dose for 10 samples of 1 mL each, is a 100-mL sterile diluent added to the dehydrated SimPlate medium and shaken to dissolve?	**5.5.10.2**				
Is a 1.0-mL test sample then pipetted to the center of a plate, followed by 9 mL of the reconstituted medium?					
Is the plate then gently swirled to mix and distribute the sample and medium mixture evenly to the 84 wells, with the excess liquid then being drained into the absorbent pad on the plate?					
Is the plate inverted and incubated at 35°±0.5°C for 45-72 hours?					
Is bacterial density determined by counting the number of wells that fluoresce under a 365-366-nm UV light, and converting this value to a Most Probable Number/mL using the manufacturer's Multi-Dose MPN table?					
If sample dilutions were made during sample preparation, is the MPN/mL value multiplied by the dilution factor?					
For the Pour Plate and Spread Plate Techniques, are colonies counted manually using a dark field colony counter?	**5.5.11**				
Are only plates having 30 to 300 colonies counted, except for plates inoculated with 1.0 mL of undiluted sample where counts of less than 30 are acceptable?					
QC Is each batch or flask of agar checked for sterility by pouring a final control plate?	**5.5.12**				
QC Does the laboratory reject data if the control is contaminated?					
Coliphage (Draft Method 1601 and 1602 for the GWR)	**5.6**				
EPA Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment	**5.6.1**				
Is the 100-mL (or 1-L) water sample supplemented with magnesium chloride, log-phase host bacteria, and tryptic soy broth (TSB) as an enrichment step for coliphage?					
After incubation overnight, are samples spotted onto a lawn of host bacteria, incubated, and then examined for circular lysis zones?					
Media	**5.6.1.1**				
For antibiotic stocks, are antibiotics always added to the medium *after* the medium has been autoclaved?	**5.6.1.1.1**				
Are antibiotic stocks stored frozen at -20°C for no longer than one year?					
Are antibiotic stocks thawed at room temperature or rapidly in a water bath up to 37°C and mixed well before using?					
Is 10X tryptic soy broth (TSB) stored at 1°-5°C until used?	**5.6.1.1.2**				
Are 1.5% tryptic soy agar (TSA) plates after antibiotic supplementation and solidification stored inverted at 1°-5°C for no longer than two weeks, if not used immediately?	**5.6.1.1.3**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Are 0.7% TSA top agar tubes with appropriate antibiotics, dispensed as 5-mL per sterile 10-mL tube, labeled, and kept at 45°-48°C until used?	**5.6.1.1.4**				
Are these tubes used the day of preparation?					
For spot plates that develop condensation during storage, are plates incubated for approximately 10 minutes to reduce condensation prior to inoculation?	**5.6.1.1.5**				
Are spot plates used that day or stored at 1°-5°C for up to four days?					
Coliphage stock	**5.6.1.2**				
Are MS2 (ATTC#15597-B1 male-specific) and phi-X 174 stock coliphage (ATTC#13706-B1, somatic) stored at 2°-8°C for up to five years?	**5.6.1.2.1**				
Does analysis of raw sewage filtrate begin within 24 hours of collection?	**5.6.1.2.2**				
Is at least 10 mL of filtered sewage obtained?	**5.6.1.2.4**				
If the filtrate is stored more than 24 hours, is it re-titered before use?	**5.6.1.2.5**				
Host bacteria stock cultures	**5.6.1.3**				
After preparation, are host bacteria stock cultures held at a temperature between -20°C and -70°C?	**5.6.1.3.1**				
Are bacteria stored no longer than two months at -20°C or no longer than one year at -70°C?					
Are prepared overnight host bacteria stock cultures chilled on wet ice or at 1°-5°C until ready to use?	**5.6.1.3.2**				
After preparation, are log-phase host bacteria stock cultures chilled on wet ice or at 1°-5°C to slow replication until ready for use?	**5.6.1.3.3**				
When stored, are these suspensions stored no more than 48 hours?					
Are remaining bacterial host cultures stored at 1°-5°C overnight to inoculate flasks for the preparation of new log-phase bacterial hosts?					
General QC	**5.6.1.4**				
Initial demonstration of capability (IDC)	**5.6.1.4.1**				
QC Did the laboratory demonstrated ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples?					
QC Did the IDC test consist of ten reagent water samples spiked with enumerated sewage filtrate or equivalent at 1-2 PFU mL per sample for every sample for each coliphage type used, according to the IDC Table?					
QC Were these IDC tests accompanied by a method blank for each coliphage type used?					
Method Blanks	**5.6.1.4.2**				
QC Is a method blank (reagent water sample containing no coliphage) analyzed to demonstrate freedom from contamination?					
For each coliphage type used, is a sterile reagent water sample prepared and analyzed using the same procedure used for analysis of field and QC samples?					
QC Is at least one method blank analyzed for every spot plate used for field samples?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Positive Controls	**5.6.1.4.3**				
QC Are positive controls analyzed to ensure that stock coliphage suspensions, host bacterial cultures, and growth media are performing properly?					
QC For each coliphage type used, is a 100 mL reagent water sample spiked with 20 PFU from sewage filtrate or 60 PFU from a pure coliphage stock culture?					
QC Is one positive control analyzed for every spot plate used for field samples?					
QC If multiple spot plates are inoculated with samples on the same day, is a single enriched positive control sample used to inoculate multiple spot plates that day?					
Matrix spikes (MS)	**5.6.1.4.4**				
QC Is method performance assessed by analyzing one set of MS samples for each coliphage type used when samples are first received from a new ground water source**?**					
QC For each coliphage type analyzed, are three field samples spiked with 1-2 PFU, with at least one out of the three MS samples being positive for each coliphage type?					
QC Is one set of MS samples analyzed on an ongoing basis after every 20th field sample for each ground water source?					
QC Are these MS samples collected at the same time as routine field samples?					
QC Are these samples spiked in "bulk" at the concentrations indicated in the MS and ODC Sample Spiking Table?					
Ongoing demonstration of capability (ODC)	**5.6.1.4.5**				
QC Does the laboratory demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples?					
QC Are three reagent water samples spiked with 1-2 PFU for each coliphage type used?					
QC Are ODC test samples analyzed exactly like field samples?					
QC Is a minimum of one out of three ODC samples positive for each coliphage type used?					
QC Is one set of ODC samples analyzed after every 20 field and MS samples or one per week, whichever occurs more frequently?					
QC Are samples spiked in "bulk" at the concentrations indicated in the MS and ODC Sample Spiking Table?					
Performance studies	**5.6.1.4.6**				
QC Does the laboratory periodically analyze external QC samples when available?					
QC Does the laboratory participate in available interlaboratory performance studies?					
QC Does the laboratory review results, correct unsatisfactory performance, and record corrective actions?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

EPA Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Layer Agar (SAL) Procedure	**5.6.2**				
Is the 100-mL ground water sample supplemented with magnesium chloride and host bacteria, and then added to 100-mL of double-strength molten tryptic soy agar containing the appropriate antibiotic?					
Is the sample thoroughly mixed and the total volume then poured into five to ten plates?					
After overnight incubation, are circular lysis zones recorded as coliphage?					
Media	**5.6.2.1**				
For antibiotic stocks, are antibiotics always added to the medium *after* the medium has been autoclaved?					
Are antibiotic stocks stored frozen at -20°C for up to one year?					
Are antibiotic stocks thawed at room temperature or rapidly in a water bath up to 37°C and then mixed well before using?					
Is 10X TSB stored at 1°-5°C until used?					
Are 1.5% TSA plates, after antibiotic supplementation and solidification, stored inverted at 1°-5°C for no longer than two weeks, if not used immediately?					
Are 0.7% TSA top agar tubes with appropriate antibiotics, dispensed as 5-mL per sterile 10-mL tube, labeled, and kept at 45°-48°C until used?					
Are these tubes used the day they are prepared?					
Are spot plates that develop condensation during storage, incubated for approximately 10 minutes to reduce condensation prior to inoculation?					
Are these plates used that day or stored at 1°-5°C for up to four days?					
Is 2X TSA, with appropriate antibiotics, kept molten at 45°-48°C in a water bath?	**5.6.2.1.2**				
Is this agar used only on the day of preparation?					
Coliphage Stock	**5.6.2.2**				
Are MS2 (ATTC#15597-B1 male-specific) and phi-X 174 stock coliphage (ATTC#13706-B1, somatic) stored at 2°-8°C for up to five years?					
Does analysis of raw sewage filtrate begin within 24 hours of collection?					
Is at least 10 mL of filtered sewage obtained?					
If the filtrate is stored more than 24 hours, is it re-titered before use?					
Host bacteria stock cultures	**5.6.2.3**				
After preparation, are host bacteria stock cultures held at a temperature between -20°C and -70°C?					
Are bacteria stored no longer than two months at -20°C, or no longer than one year at -70°C?					
After preparation, are overnight host bacteria stock cultures chilled on wet ice or at 1°-5°C until ready for use?					

After preparation, are log-phase host bacteria stock cultures chilled on wet ice or at 1°-5°C to slow replication until ready to use?					
When stored, are these suspensions stored no longer than 48 hours?					
Are remaining bacterial host cultures stored at 1°-5°C overnight to inoculate flasks for the preparation of new log-phase bacterial hosts?					
General QC	**5.6.2.4**				
Initial precision and recovery (IPR)	**5.6.2.4.1**				
QC Has the laboratory demonstrated the ability to perform this method acceptably by performing an IPR test before analyzing any field samples?					
QC Does the IPR Test consist of four reagent water samples tested for each coliphage type used, spiked with enumerated sewage filtrate or equivalent to yield a target spike concentration of 80 PFU per sample?					
QC Does the relative standard deviation of the recovery (RSD), and the average percent recovery ($\bar{X}$) based on all four sample results for each coliphage type used, meet the acceptance criteria in the QC Acceptance Criteria table?					
Method blanks	**5.6.2.4.2**				
QC Is one method blank (reagent water sample containing no coliphage) analyzed with each analytical batch to demonstrate freedom from contamination?					
QC For each coliphage type used, are sterile reagent water samples prepared and analyzed using the same procedures used for analysis of the field and QC samples?					
QC Is an analytical batch defined as all samples analyzed during a single day, up to a maximum of 20 samples (field samples and matrix spike samples) per coliphage type used?					
Matrix spikes (MS)	**5.6.2.4.3**				
QC Is method performance assessed by analyzing one set of MS samples for each coliphage type used when samples are first received from a new ground water source?					
QC Is one set of MS samples routinely analyzed after every 20th field sample for each ground water source?					
QC If the recovery for coliphage falls outside its limit (see the QC Acceptance Criteria table), is method performance considered unacceptable?					
QC If OPR results are not within control limits, is the problem identified and corrected and the data qualified?					
QC Does the laboratory maintain a control chart on recovery and update it on a regular basis?					
Ongoing precision and recovery (OPR)	**5.6.2.4.4**				
QC Does the laboratory demonstrate acceptable performance through analysis of an OPR sample on an ongoing basis?					
QC For each coliphage type used, is a reagent water sample spiked with approximately 80 PFU?					
QC Is the OPR sample analyzed exactly like a field sample?					
QC Is one OPR sample analyzed for each analytical batch?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

QC Does the laboratory compare the OPR percent recovery (R) with the corresponding limits in the QC Acceptance Criteria table?					
QC If R falls outside the range for recovery, is analysis stopped until the problem is identified, corrected, and another OPR test is successfully performed?					
Performance studies	**5.6.2.4.5**				
QC Does the laboratory periodically analyze an external QC sample when available?					
QC Does the laboratory participate in available interlaboratory performance studies?					
QC Does the laboratory review these results, correct unsatisfactory performance, and record corrective actions?					
6. SAMPLE COLLECTION, HANDLING, AND PRESERVATION					
Sample Collector	**6.1**				
Is the sample collector trained in aseptic sampling procedures and, if required, approved by the appropriate regulatory authority or its designated representative?					
Sampling	**6.2**				
Are the drinking water samples collected under the Total Coliform Rule representative of the water distribution system?	**6.2.1**				
Are the water taps used for sampling free of aerators, strainers, hose attachments, mixing type faucets, and purification devices?					
Are only cold water taps used?					
Are service lines cleared before sampling by maintaining a steady water flow for at least 2 minutes or until a steady water temperature is reached?					
Is at least a 100-mL sample volume collected, allowing at least a 1-inch air space in the container to facilitate mixing of the sample by shaking?					
Is a sample information form completed immediately after sample collection?					
If a sample bottle is filled too full to allow for proper mixing, is the entire sample poured into a larger sterile container and mixed before proceeding with the analysis?					
For the SWTR, are the source water samples representative of the source of supply and collected not too far from the intake point, but at a reasonable distance from the bank or shore?	**6.2.2**				
Is the sample volume sufficient to perform all the tests required?					
For the analysis of coliphage, *E. coli*, or enterococci under the GWR, is at least a 100-mL sample volume collected?	**6.2.3** **6.2.4**				
Sample Icing	**6.3**				
For drinking water bacterial samples, is the sampler encouraged to hold samples at <10□C during transit to the laboratory?	**6.3.1**				
For source water bacterial samples, are samples held at <10□C during transit to the laboratory?					
Does the laboratory reject samples that have been frozen?					
For coliphage analysis under the GWR, are samples shipped at <10°C, stored at 1°-5°C, and not frozen?	**6.3.2**				

Uncontrolled Copy

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

QC For SWTR samples and coliphage samples, does the laboratory record sample temperature upon receipt?	**6.3.3**				
QC Does the laboratory flag samples that have a temperature upon receipt of >10°C, whether iced or not, unless the time since the sample collection is less than two hours?					
Sample Holding Time	**6.4.**				
For the analysis of total coliforms in drinking water, does the time between sample collection and placement of the sample in the incubator not exceed 30 hours?	**6.4.1**				
Are all samples analyzed on the day of receipt?					
Are samples received late in the day refrigerated overnight only if analysis can begin within 30 hours of collection?					
For total coliforms and fecal coliforms in surface water sources, and for heterotrophic bacteria in drinking water, is the time from sample collection to placement in the incubator less than eight hours?	**6.4.2**				
For coliphage analysis, is the time from sample collection to placement of sample in the incubator less than 48 hours?	**6.4.3**				
For coliphage analysis, is the time from sewage sample collection to analysis of QC spiking suspension less than 24 hours, unless re-titered and the titer has not decreased by more than 50%?					
If the titer has not decreased by more than 50%, is the sample stored no longer than 72 hours?					
For ***E. coli*** and enterococci analysis under the GWR, is the time between sample collection and the placement of sample in the incubator less than 30 hours?	**6.4.4**				
Sample Information Form	**6.5**				
After collection, does the sampler enter the following information, in indelible ink, on sample information form? - Name of system (PWSS identification number if available) - Sample identification (if any) - Sample site location - Sample type (e.g., a routine distribution, repeat, raw or process, or other special purpose) - Date and time of collection - Analysis requested - Disinfectant residual - Name of sampler - Any remarks					
Chain-of-Custody	**6.6**				
Are applicable State regulations pertaining to chain-of-custody followed by sample collectors and the laboratory**?**					
7. QUALITY ASSURANCE					
Does the laboratory have a written QA Plan prepared and available for inspection?	**7.1**				
Does the laboratory follow the written QA Plan?					
Does the laboratory have a Standard Operating Procedure available for review pertaining to its own calibration of equipment or supplies?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Does the laboratory successfully analyze at least one set of PT samples once every 12 months for each method for which it is certified?	**7.2**				
For methods used to test the presence or absence of an organism in a sample, does the laboratory analyze each PT sample set using a single analytical method only?					
8. RECORDS AND DATA REPORTING					
Legal Defensibility	**8.1**				
Are compliance monitoring data being maintained by the laboratory both thorough and accurate, and thus legally defensible?					
Does the laboratory's QA plan and/or SOPs describe the policies and procedures used by the facility for record retention and storage?					
If samples are expected to become part of legal action, does the laboratory follow chain-of-custody procedures?					
Maintenance of Records	**8.2**				
Does the public water system maintain records of microbiological analyses for five years?					
Does the laboratory maintain easily accessible records for five years or until the next certification data audit is completed, whichever is longer?					
Does the laboratory notify the client water system before disposing of records so they may request copies if needed?					
Does the laboratory backup all electronic data by protected tape, disk, or hard copy?					
When the laboratory changes its computer hardware or software, are provisions in place for transferring old data to the new system so that data remain retrievable within the specified time frames?					
Sampling Records	**8.3**				
Are all data recorded in ink, with any changes lined through such that the original entry is visible?					
Are changes initialed and dated?					
Does the laboratory have the following sample information readily available? - Date and time of sample receipt by the laboratory - Name of the laboratory person receiving the sample - Information on any deficiency in the condition of the sample	**8.3.1-4**				
Are samples invalidated for the following reasons? - Time between sample collection and receipt by laboratory exceeded - Presence of disinfectant in sample noticed, e.g., odor - Evidence of freezing - Use of a container not approved by the laboratory for the purpose intended - Insufficient sample volume, e.g., <100 mL - Presence of interfering contaminants noticed, e.g., hydrocarbons, cleansers, heavy metals, etc. - Sample temperature exceeding the maximum allowable	**8.3.4**				

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Analytical Records	**8.4**				
Are all recorded data in ink with any changes lined through such that original entry is visible?					
Are these changes initialed and dated?					
Are the following readily available? - Laboratory sample identification information - Information concerning date and time analysis begins - Name of the laboratory and a signature or initials of the person(s) performing analysis - Information concerning the analytical technique or method used - Information concerning all items marked "QC" - Results of the analyses	**8.4.1-6**				
Preventive Maintenance	**8.5**				
Does the laboratory maintain preventive maintenance and repair records for all instruments and equipment?					
Are these records kept for five years in a manner that allows for easy inspection?					
9. ACTION RESPONSE TO LABORATORY RESULTS					
Testing Total Coliform-Positive Cultures	**9.1**				
For the Total Coliform Rule, does the laboratory test all total coliform-positive cultures for the presence of either fecal coliforms or *E. coli*?					
Notification of Positive Results	**9.2**				
For Total Coliform Rule, does the laboratory promptly notify the proper authority of a positive total coliform, fecal coliform, or *E. coli* result, so that appropriate follow-up actions can be conducted?	**9.2.1**				
For the Total Coliform Rule, if a sample is fecal coliform- or *E. coli*-positive, does the system notify the State as soon as it is notified of the test result, i.e., at the end of that day or, if the State office is closed, by the end of the next business day?	**9.2.2**				
Does the laboratory base a total coliform-positive result on the confirmed phase if the Multiple Tube Fermentation Technique or Presence-Absence Coliform Test is used, or the verified test for the Membrane Filtration Technique if M-Endo medium or M-Endo LES agar is used?	**9.2.3**				
If a presumptive total coliform-positive culture does not confirm/verify as such, but is found to be fecal coliform or *E. coli*-positive, is the sample considered total coliform-positive and fecal coliform/*E. coli*-positive?					
Notification of Total Coliform Interference	**9.3**				
For the Total Coliform Rule, does the laboratory promptly notify the proper authority when results indicate non-coliforms may have interfered with total coliform analysis?					

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

MICRO CHECKLISTS

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Appendix A

Chain-of-Custody Evaluations

A. Introduction
Written procedures for sample handling should be available and followed whenever samples are collected, transferred, stored, analyzed or destroyed. For the purposes of litigation, it is necessary to have an accurate written record to trace the possession and handling of samples from collection through reporting. The procedures defined here represent a means to satisfy this requirement.

A sample is in someone's "custody" if:

1. It is in one's actual physical possession;
2. It is in one's view, after being in one's physical possession;
3. It is one's physical possession and then locked up so that no one can tamper with it;
4. It is kept in a secured area, restricted to authorized personnel only.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

B. Sample Collection, Handling and Identification

1. It is important that a minimum number of persons be involved in sample collection and handling. Guidelines established in standard manuals for sample collection preservation and handling should be used (e.g., EPA NPDES Compliance Sampling Inspection Manual, MCD 51, *Standard Methods for Examination of Water and Wastewater*). Field records should be completed at the time the sample is collected and should be signed or initialed, including the date and time, by the sample collector(s). Field records should contain the following information:

   a. Unique sample or log number;

   b. Date and time;

   c. Source of sample (including name, location and sample type);

   d. Preservative used;

   e. Analyses required;

   f. Name of collector(s);

   g. Pertinent field data (pH, DO, Cl residual, etc.);

   h. Serial number on seals and transportation cases;

   i. Comments.

2. Each sample is identified by affixing a pressure sensitive gummed label or standardized tag on the container(s). This label should contain the sample number, source of sample, preservative used, and the collector(s') initials. The analysis required should be identified. Where a label is not available, the sample information should be written on the sample container with an indelible marking pen. An example of a sample identification tag is illustrated in Figure A-1.

3. The closed sample container should then be placed in a transportation case along with the chain-of-custody record form, pertinent field records, and analysis request form. The transportation case should then be sealed and labeled. All records should be filled out legibly in waterproof pen. The use of locked or sealed chests will eliminate the need for close control of individual sample containers. However, there will undoubtedly be occasions when the use of a

Uncontrolled Copy

chest will be inconvenient. On these occasions, the sampler should place a seal around the cap of the individual sample container which would indicate tampering if removed.

C. Transfer of Custody and Shipment

1. When transferring the possession of the samples, the transferee must sign and record the date and time on the chain-of-custody record. Custody transfers, if made to a sample custodian in the field, should account for each individual sample, although samples may be transferred as a group. Every person who takes custody must fill in the appropriate section of the chain-of-custody record.

2. The field custodian (or field sampler if a custodian has not been assigned) is responsible for properly packaging and dispatching samples to the appropriate laboratory for analysis. This responsibility includes filling out, dating, and signing the appropriate portion of the chain-of-custody record. A recommended chain-of-custody format is illustrated in Figure A-2.

3. All packages sent to the laboratory should be accompanied by the chain-of-custody record and other pertinent forms. A copy of these forms should be retained by the field custodian (either carbon or photocopy).

4. Mailed packages can be registered with return receipt requested. If packages are sent by common carrier, receipts should be retained as part of the permanent chain-of-custody documentation.

5. Samples to be transported must be packed to prevent breakage. If samples are shipped by mail or by other common carrier, the shipper must comply with any applicable Department of Transportation regulations. (Most water samples are exempt unless quantities of preservatives used are greater than certain levels.) The package must be sealed or locked to prevent tampering. Any evidence of tampering should be readily detected if adequate sealing devices are used.

6. If the field sampler delivers samples to the laboratory, custody may be relinquished to laboratory personnel. If appropriate personnel are not present to receive the samples, they should be locked in a designated area of the laboratory to prevent tampering. The person delivering the samples should make a log entry stating where and how the samples were delivered and secured. Laboratory personnel may then receive custody by noting in a logbook, the absence of evidence of tampering, unlocking the secured area, and signing the custody sheet.

D. Laboratory Sample Control Procedures

Sample control procedures are necessary in the laboratory from the time of sample receipt to the time the sample is discarded. The following procedures are recommended for the laboratory:

1. A specific person must be designated as custodian and an alternate designated to act as custodian in the custodian's absence. All incoming samples must be received by the custodian, who must indicate receipt by signing the accompanying custody/control forms and who must retain the signed forms as permanent records.

2. The custodian must maintain a permanent logbook to record, for each sample, the person delivering the sample, the person receiving the sample, date and time received, source of sample, date the sample was taken, sample identification log number, how transmitted to the laboratory, and condition received (sealed, unsealed, broken container, or other pertinent remarks). This log should also show the movement of each sample within the laboratory; i.e., who removed the sample from the custody area, when it was removed, when it was returned, and when it was destroyed. A standardized format should be established for logbook entries.

3. A clean, dry, isolated room, building, and/or refrigerated space that can be securely locked from the outside must be designated as a "custody room."
4. The custodian must ensure that heat-sensitive samples, light-sensitive samples, radioactive samples, or other sample materials having unusual physical characteristics, or requiring special handling, are properly stored and maintained prior to analysis.

5. Distribution of samples to the analyst performing the analysis must be made by the custodian.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

6. The laboratory area must be maintained as a secured area, restricted to authorized personnel only.

7. Laboratory personnel are responsible for the care and custody of the sample once it is received by them and must be prepared to testify that the sample was in their possession and view or secured in the laboratory at all times from the moment it was received from the custodian until the time that the analyses are completed.

8. Once the sample analyses are completed, the unused portion of the sample, together with all identifying labels, must be returned to the custodian. The returned tagged sample must be retained in the custody room until permission to destroy the sample is received by the custodian.

9. Samples will be destroyed only upon the order of the responsible laboratory official when it is certain that the information is no longer required or the samples have deteriorated. (For example, standard procedures should include discarding samples after the maximum holding time has elapsed.) The same procedure is true for sample tags. The logbook should show when each sample was discarded or if any sample tag was destroyed.

10. Procedures should be established for internal audits of sample control information. Records should be examined to determine traceability, completeness, and accuracy.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Figure A-1 Sample Identification Tag Examples

U.S. EPA REGION

GENERAL CHEMISTRY

Official Sample No.

SOURCE

Date and Time

Sampler's Signature   Office

Other Parameters:

PH	Acid
Cond	Alk
TS	SO_4
DS	Cl
SS	F
BOD_2	Cr. + 6
Turb	BOD_5
Color	

U.S. EPA REGION

MICROBIOLOGY

Official Sample No.

SOURCE

Date and Time

Sampler's Signature   Office

Tot. Colif.

Fecal Colif.

Fecal Strep.

Salmonella

U.S. EPA REGION

PESTICIDES, ORGANICS

Official Sample No.

SOURCE

Date and Time

Sampler's Signature   Office

Pesticides

PCB's:

Organics:

EPA

Station No.	Date	Time	Sequence No.
Station Location			____ Grab ____ Comp.

____ BOD   ____ Metals
____ Solids   ____ Oil and Grease
____ COD   ____ D.O.
____ Nutrients   ____ Bact.
____ Other

Remarks/Preservative:

Samplers:

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24
Authorised on: 22-Aug-2011. Authorised by: Paul Gray. SOP Unique Reference: 582-17895560. No review required.
Author(s): Paul Gray

Uncontrolled Copy

Figure A-2 Chain-of-Custody Record

Survey	**Samplers:** Signature

Station Number	Station Location	Date	Time	Sample Type			Seq. No.	No. Of Containers	Analysis Required
				Water		Air			
				Comp	Grab.				

Relinquished by: Signature	**Received by:** Signature	**Date/Time**
Relinquished by: Signature	**Received by:** Signature	**Date/Time**
Relinquished by: Signature	**Received by:** Signature	**Date/Time**
Relinquished by: Signature	**Received by Mobile Laboratory for Field analysis:** Signature	**Date/Time**

Dispatched by:	**Date/Time**	**Received for Laboratory by:** Signature	**Date/Time**

Method of Shipment:

Distribution: Orig. --Accompany Shipment, 1 Copy--Survey Coordinator Field Files

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24
Authorised on: 22-Aug-2011. Authorised by: Paul Gray. SOP Unique Reference: 582-17895560. No review required.
Author(s): Paul Gray

Uncontrolled Copy

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Appendix G

Analytical Methods for Microbiology

Note: Information in brackets [ ] is not yet included in the Code of Federal Regulations

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

(f) *Analytical methodology*.
(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 analytical methods in the following table.

Organisms	Methodology[12]	Citation1
Total	Total Coliform Fermentation Technique [3, 4, 5]	9221 A, B
Coliforms[2]	Total Coliform Membrane Filter Technique6	9222 A, B, C
	Presence-Absence (P-A) Coliform Test [5, 7]	9221 D
	ONPG-MUG Test[8]	9223
	Colisure Test[9]	
	E*Colite® Test[10]	
	m-ColiBlue24® Test[11]	
	Readycult® Coliforms 100[13]	
	Membrane Filter Technique using Chromocult® Coliform Agar[14]	
	Colitag Test[15]	

The procedures shall be done in accordance with the documents listed below. The incorporation by reference of the following documents listed in footnotes 1, 6, 8, 9, 10, 11, 13 and 14 was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR Part 51. Copies of the documents may be obtained from the sources listed below. Information regarding obtaining these documents can be obtained from the Safe Drinking Water Hotline at 800-426-4791. Documents may be inspected at EPA's Drinking Water Docket, 1301 Constitution Avenue, NW., EPA West, Room B102, Washington, DC 20460 (Telephone 202-566-2426) or at the Office of Federal Register, 800 North Capitol Street, NW, Suite 700, Washington, DC 20408.

1 *Standard Methods for the Examination of Water and Wastewater*, 18th edition (1992), 19th edition (1995), or 20th edition (1998). American Public Health Association, 1015 Fifteenth Street NW, Washington, D.C. 20005. The cited methods published in any of these three editions may be used.

2. The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

required to hold samples below 10 deg. C during transit.

3. Lactose broth, as commercially available, may be used in lieu of lauryl tryptose broth, if the system conducts at least 25 parallel tests between this medium and lauryl tryptose broth using the water normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliform[s], using lactose broth, is less than 10percent.

4. If inverted tubes are used to detect gas production, the media should cover these tubes at least one-half to two-thirds after the sample is added.

5. No requirement exists to run the completed phase on 10 percent of all total coliform-positive confirmed tubes.

6. MI agar also may be used. Preparation and use of MI agar is set forth in the article, ``New medium for the simultaneous detection of total coliform[s] and *Escherichia coli* in water'' by Brenner, K.P., et al., 1993, Appl. Environ. Microbiol. 59:3534-3544. Also available from the Office of Water Resource Center (RC-4100T), 1200 Pennsylvania Avenue, NW, Washington, DC 20460, EPA/600/J-99/225. Verification of colonies is not required.

7. Six-times formulation strength may be used if the medium is filter-sterilized rather than autoclaved.

8. The ONPG-MUG Test is also known as the Autoanalysis Colilert System.

9. A description of the Colisure Test, Feb 28, 1994, may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092. The Colisure Test may be read after an incubation time of 24 hours.

10. A description of the E*Colite® Test, ``Presence/Absence for Coliforms and *E. Coli* in Water,'' Dec 21, 1997, is available from Charm Sciences, Inc., 36 Franklin Street, Malden, MA 02148-4120.

11. A description of the m-ColiBlue24® Test, Aug 17, 1999, is available from the Hach Company, 100 Dayton Avenue, Ames, IA 50010.

12. 12EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the method(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for $-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

confirmation tests. False-positive and false- negative information is often available in published studies and/or from the manufacturer(s).

13. 13 The Readycult® Coliforms 100 Presence/Absence Test is described in the document, "Readycult® Coliforms 100 Presence/Absence Test for Detection and Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters," (November 2000, Version 1.0) and is available from EM Science [now EMD Chemicals, Inc.], an affiliate of Merck KGgA, Darmstadt Germany), 480 S. Democrat Road, Gibbstown, NJ 08027-1297. Telephone number is (800) 222-0342, E-Mail address is: adellenbusch@emscience.com. [E-Mail address is now adellenbusch@emdchemicals.com. Website is www.emdchemicals.com].

14. Membrane Filter Technique using Chromocult® Coliform Agar is described in the document, "Chromocult® Coliform Agar Presence/Absence Membrane Filter Test Method for Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters," November 2000, Version 1.0, available from EM Science [now EMD Chemicals, Inc.] (an affiliate of Merck KGgA, Darmstadt Germany), 480 S. Democrat Road, Gibbstown, NJ 08027-1297. Telephone number is (800) 222-0342, E-Mail address is: adellenbusch@emscience.com. [E-Mail address is now adellenbusch@emdchemicals.com. Website is www.emdchemicals.com].

15. Colitag® product for the determination of the presence/absence of total coliforms and *E. coli* is described in ``Colitag® Product as a Test for Detection and Identification of Coliforms and *E. coli* Bacteria in Drinking Water and Source Water as Required in National Primary Drinking Water Regulations,'' August 2001, available from CPI International, Inc., 5580 Skylane Blvd., Santa Rosa, CA, 95403, telephone (800) 878-7654, Fax (707) 545-7901, Internet address http://www.cpiinternational.com.

(4) [Reserved]

(5) Public water systems must conduct fecal coliform analysis in accordance with the following procedure. When the MTF Technique or Presence-Absence (PA) 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 a 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 waterbath 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 Method 9221E (paragraph 1a) in *Standard Methods for the Examination of Water and Wastewater*, 18th edition (1992), 19th edition (1995), and 20th edition (1998); the cited method in any one of these three editions may be used. Public water systems need only determine the presence or absence of fecal coliforms; a determination of fecal

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

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), as described in Method 9222G in Standard Methods for the Examination of Water and Wastewater, 19th edition (1995) and 20th edition (1998). Either edition may be used. Alternatively, the 18th edition (1992) may be used if at least 10 mL of EC medium, as described in paragraph (f) (5) of this section, is supplemented with 50 µg/mL of MUG before autoclaving. The inner inverted fermentation tube may be omitted. If the 18th edition is used, apply the procedure in paragraph (f)(5) of this section for transferring a total coliform-positive culture to EC medium supplemented with MUG, incubate the tube at 44.5 ± 0.2°C for 24 ± 2 hours, and then observe fluorescence with an ultraviolet light (366 nm) in the dark. If fluorescence is visible, *E. coli* are present.

(ii) Nutrient agar supplemented with 100 µg/mL 4-methylumbelliferyl-beta-D-glucuronide (MUG) (final concentration), as described in Method 9222G in Standard Methods for the Examination of Water and Wastewater, 19th edition (1995) and 20th edition (1998). Either edition
may be used for determining if a total coliform-positive sample, as determined by a membrane filter technique, contains *E. coli*. Alternatively, the 18th edition (1992) may be used if the membrane filter containing a total coliform-positive colony(ies) is transferred to nutrient agar, as described in Method 9221B (paragraph 3) of Standard Methods (18th edition), supplemented with100 µg/mL of MUG. If the 18th edition is used, incubate the agar plate at 35°C for 4 hours and then observe the colony(ies) under ultraviolet light (366 nm) in the dark for fluorescence. If fluorescence is visible, *E. coli* are present.

(iii) 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 Autoanalysis Colilert System is an MMO-MUG test).
If the MMO-MUG test is total coliform-positive after a 24-hour incubation, test the medium for fluorescence with a 366-nm ultraviolet light (preferably with a 6-watt lamp) in the dark. If fluorescence is observed, the sample is *E. coli*-positive. If fluorescence is questionable (cannot be
definitively read) after 24 hours incubation, incubate the culture for an additional four hours (but not to exceed 28 hours total), and again test the medium for fluorescence. The MMO-MUG Test with hepes buffer in lieu of phosphate buffer is the only approved formulation for the detection of *E. coli*.

(iv) The Colisure Test. A description of the Colisure Test may be obtained from the Millipore Corporation, Technical Services Department, 80 Ashby Road, Bedford, MA 01730. [Note: Manufacturer is now IDEXX Laboratories. See footnote 9 to the table in paragraph (f) (3) of this section.]

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

(v) The membrane filter method with MI agar, a description of which is cited in footnote 6 to the table in paragraph (f) (3) of this section.

(vi) E*Colite® Test, a description of which is cited in footnote 10 to the table at paragraph (f) (3) of this section.

(vii) m-ColiBlue24® Test, a description of which is cited in footnote 11 to the table in paragraph (f)(3) of this section.

(viii) Readycult® Coliforms 100 Presence/Absence Test, a description of which is cited in footnote 13 to the table at paragraph (f) (3) of this section.

(ix) Membrane Filter Technique using Chromocult® Coliform Agar, a description of which is cited in footnote 14 to the table at paragraph (f) (3) of this section.

(7) As an option to paragraph (f) (6) (iii) of this section, a system with a total coliform-positive, MUG-negative, MMO-MUG test may further analyze the culture for the presence of *E. coli* by transferring a 0.1 mL, 28-hour MMO-MUG culture to EC Medium + MUG with a pipet. The formulation and incubation conditions of EC Medium + MUG, and observation of the results are described in paragraph (f) (6) (i) of this section.

(8) The following materials are incorporated by reference in this section with the approval of the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies of
the analytical methods cited in *Standard Methods for the Examination of Water and Wastewater* (18th, 19th, and 20th editions) may be obtained from the American Public Health Association et al.; 1015 Fifteenth Street NW., Washington, DC 200052605. Copies of the MMO-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.*) may be obtained from the American Water Works Association Research Foundation, 6666 West Quincy Avenue, Denver, CO 80235. A description of the Colisure Test may be obtained from the Millipore Corp., Technical Services Department, 80 Ashby Road, Bedford, MA 01730 [Note: Now a description of the Colisure Test may now be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092.]. Copies may be inspected at EPA's Drinking Water Docket; 401 M St., SW.; Washington, DC 20460 [Note: current location of EPA's Drinking Water Docket is 1301 Constitution Avenue, NW., EPA West, Room B102, Washington, DC 20460], or at the Office of the Federal Register; 800 North Capitol Street, NW., Suite 700, Washington, DC.

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

(a) *Analytical requirements*. Only the analytical method(s) specified in this paragraph, or otherwise approved by EPA, may be used to demonstrate compliance with §§141.71, 141.72 and 141.73. Measurements for pH, turbidity, temperature and residual disinfectant concentrations must be conducted by a person approved by the State. Measurement 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

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

analysis of fecal coliforms and HPC, any laboratory certified for total coliforms analysis by the State or EPA is deemed certified for fecal coliforms and HPC analysis. The following procedures shall be conducted 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 St., SW., Washington, DC 20460 [Note: current location of EPA's Drinking Water Docket is 1301 Constitution Avenue, NW., EPA
West, Room B102, Washington, DC 20460;] or at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC. (1) Public water systems must conduct analysis of pH and temperature in accordance with one of the methods listed at §141.23(k) (1). Public water systems must conduct analysis of total coliforms, fecal coliforms, heterotrophic bacteria, and turbidity in accordance with one of the following analytical methods and by using analytical test procedures contained in *Technical Notes on Drinking Water Methods*, EPA-600/R-94-173, October 1994, which is available at NTIS PB95-104766.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Organism	Methodology	Citation1
Total Coliform [2].............	Total Coliform Fermentation Technique [3, 4, 5]	9221 A. B. C
	Total Coliform Membrane Filter Technique[6]	9222 A, B, C
	ONPG-MUG Test [7]	9223
Fecal Coliforms[2]...........	Fecal Coliform Procedure[8]	9221 E
	Fecal Coliform Filter Procedure	9222 D
Heterotrophic bacteria[2]...	Pour Plate Method	9215 B
	SimPlate[11]	
Turbidity	Nephelometric Method	2130 B
	Nephelometric Method	180.1[9]
	Great Lakes Instruments	Method 2[10]
	Hach FilterTrak	10133[12]

The procedures shall be done in accordance with the documents listed below. The incorporation by reference of the following documents listed in footnotes 1, 6, 7, 9-12 was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies of the documents may be obtained from the sources listed below. Information regarding obtaining these documents can be obtained from the Safe Drinking Water Hotline at 800-426-4791. Documents may be inspected at EPA's Drinking Water Docket, 1301 Constitution Avenue, NW., EPA West, Room B102, Washington, DC 20460 (Telephone: 202-566-2426); or at the Office of the Federal Register, 800 North Capitol Street, NW, Suite 700,Washington, D.C. 20408.

1 Except where noted, all methods refer to *Standard Methods for the Examination of Water and Wastewater*, 18th edition (1992), 19th edition (1995), or 20th edition (1998), American Public Health Association, 1015 Fifteenth Street, NW, Washington, D.C. 20005. The cited methods published in any of these three editions may be used.

2. The time from sample collection to initiation of analysis may not exceed 8 hours. Systems must hold samples below 10 deg. C during transit.

3. Lactose broth, as commercially available, may be used in lieu of lauryl tryptose broth, if the system conducts at least 25 parallel tests between this medium and lauryl tryptose broth using the water normally tested, and this comparison demonstrates that the false- positive rate and false-negative rate for total coliform, using lactose broth, is less than 10 percent.

4. Media should cover inverted tubes at least one-half to two-thirds after the sample is added.

5. No requirement exists to run the completed phase on 10 percent of all total coliform-positive confirmed tubes.

6. MI agar also may be used. Preparation and use of MI agar is set forth in the article, ``New medium for the simultaneous detection of total coliform[s] and *Escherichia coli* in water'' by Brenner, K.P., et al., 1993, Appl. Environ. Microbiol. 59:3534-3544. Also available from the Office of Water Resource Center (RC-4100T), 1200 Pennsylvania Ave., NW.,

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Washington, DC 20460, EPA 600/J-99/225. Verification of colonies is not required.

7 The ONPG-MUG Test is also known as the Autoanalysis Colilert System.

8 A-1 Broth may be held up to three months in a tightly closed screw cap tube at 4 deg. C.

9 ``Methods for the Determination of Inorganic Substances in Environmental Samples'', EPA/600/R-93/100, August 1993. Available at NTIS, PB94-121811.

10 GLI Method 2, ``Turbidity'', November 2, 1992, Great Lakes Instruments, Inc., 8855 North 55th Street, Milwaukee, Wisconsin 53223.

11 A description of the SimPlate method, "IDEXX SimPlate TM HPC Test Method for Heterotrophs in Water",
November 2000, can be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092,
telephone (800) 321-0207.

12 A description of the Hach FilterTrak Method 10133, "Determination of Turbidity by Laser Nephelometry", January 2000, Revision 2.0, can be obtained from Hach Co., P.O. Box 389, Loveland, Colorado 80539-0389. Phone: 800-227-4224.

3. Ground Water Rule (to be added after rule promulgation)

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

EPA 815-F-08-006
June 2008

Supplement 1 to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water

Supplement 1 to EPA 815-R-05-004
US Environmental Protection Agency
Office of Water
Office of Ground Water and Drinking Water
Technical Support Center
Cincinnati, Ohio 45268

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24
Authorised on: 22-Aug-2011. Authorised by: Paul Gray. SOP Unique Reference: 582-17895560. No review required.
Author(s): Paul Gray

Uncontrolled Copy

Author:
Judith A. Brisbin, Ph.D., OW/OGWDW/SRMD/TSC

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Table of Contents

Page

Quality Management System
Supplement to: Chapter III Implementation
2. Requirements for Certification of Laboratories 4
Certification Officer Fraud and Ethics Training

Supplement to: Chapter III Implementation
3. Individual(s) Responsible for the Certification Program 4
Laboratory Ethics and Fraud Detection/Deterrence

Supplement to: Chapter III Implementation
New Section 5
Radiochemistry Certification Officer Training

Supplement to: Chapter III Implementation
17. Training 6
Chemistry Sample Collection

Supplement to: Chapter IV Critical Elements for Chemistry
6. Sample Collection, Handling, and Preservation 6
Microbiology Methodology

Supplement to: Chapter V Critical Elements for Microbiology
5. Analytical Methodology 6
Microbiology Sample Collection

Supplement to: Chapter V Critical Elements for Microbiology
6. Sample Collection, Handling, and Preservation 7

Appendices
Supplement to: Appendix C: Definitions and Abbreviations 7

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Quality Management System
Supplement to: Chapter III Implementation
2. Requirements for Certification of Laboratories

Laboratories performing analysis of drinking water under the Safe Drinking Water Act (SDWA) are required to operate a formal Quality Control program. Laboratories should also have a formal Quality Management system documented and in place. Programs that operate in accordance with International Organization for Standardization (ISO) 9001, particularly ISO/IEC 17025 (*General Requirements for the Competence of Testing and Calibration Laboratories*), are encouraged. ISO/IEC 17025 includes both quality management requirements (based on ISO 9001) and a number of technical requirements specific for testing and calibration laboratories. ISO documents can be purchased from ISO (www.iso.org) or through other organizations, such as the American National Standards Institute (ANSI) (www.ansi.org). In the Unites States of America (USA), ANSI is the ISO member body and ANSI-ASQ National Accreditation Board (ANAB) is the accreditation body for management systems. Numerous organizations can issue third-party laboratory accreditation according to ISO 17025.

The NELAC Institute (TNI) (www.nelac-institute.org), formerly known as the National Environmental Laboratory Accreditation Conference (NELAC), implements an accreditation program with a Quality Management approach that is based on ISO/IEC 17025; the TNI program has also integrated SWDA-based requirements from the drinking water program into its standards.

Certification Officer Fraud and Ethics Training
Supplement to: Chapter III Implementation
3. Individual(s) Responsible for the Certification Program

All Certification Officers (COs) are encouraged to participate in fraud detection and ethics training, where available. As stated in *Promising Techniques Identified to Improve Drinking Water Laboratory Integrity and Reduce Public Health Risks* (Report No. 2006-P-00036, U.S. Environmental Protection Agency (EPA), Office of Inspector General (OIG), Washington, D.C., 2006) (www.epa.gov/oig/reports/2006/20060921-2006-P-00036.pdf), use of the following promising techniques is encouraged, as appropriate:

- Enhance on-site and follow-up audits to include techniques to identify and deter inappropriate procedures and fraud;
- Review raw electronic data and use electronic data analysis/tape audits;
- Review inventory of laboratory supplies; and
- Conduct data accuracy reviews.

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24
Authorised on: 22-Aug-2011. Authorised by: Paul Gray. SOP Unique Reference: 582-17895560. No review required.
Author(s): Paul Gray

Uncontrolled Copy

Laboratory Ethics and Fraud Detection/Deterrence
Supplement to: Chapter III Implementation
New Section

Laboratories are encouraged to have an ethics policy and implement a fraud detection and deterrence policy/program, including use of the following, as appropriate:

- Use data validation and verification techniques; and
- Use analyst notation and sign-off on manual integration changes to data.

Four key areas of concern were listed in the OIG report referenced above. These include:

1. **Inappropriate procedure:** A scientifically unsound or technically unjustified omission, manipulation, or alteration of procedures or data that bypasses the required quality control parameters, making the results appear acceptable.
2. **Laboratory fraud:** The deliberate falsification during reporting of analytical and quality assurance results that failed method and contractual requirements to make them appear to have passed requirements.
3. **Data quality:** The degree of acceptability or utility of data for a particular purpose – in this case, reporting public drinking water sample information.
4. **Laboratory integrity:** The laboratory's meeting general standards of objectivity, data quality, and ethical behavior, thus reporting accurate, complete, and valid information.

If a laboratory employee suspects that fraudulent behavior is occurring, they should follow reporting procedures established by their State; States should communicate these procedures to their laboratories. States should also advise laboratories as to the appropriate State point-of-contact should they have further questions related to suspected fraud. COs should familiarize themselves with their appropriate State and/or Regional reporting procedures and follow them upon becoming aware of suspected fraudulent behavior. EPA's Office of Enforcement and Compliance Assurance (OECA) may also be used as a resource (www.epa.gov/compliance/complaints/index.html) for questions and concerns related to suspected fraud. To the extent that suspected waste, fraud or abuse involves EPA staff, programs or contracts, EPA's OIG should be notified (www.epa.gov/oig/contactus.htm).
Additional information can be found in *Best Practices for the Detection and Deterrence of Laboratory Fraud* (California Military Environmental Coordination Committee, Chemical Data Quality/Cost Reduction Process Action Team, Version 1.0, March 1997) (www.epa.gov/region09/qa/pdfs/labfraud.pdf) and in the Department of Defense (DoD) *Policy and Guidelines for Acquisitions Involving Environmental Sampling or Testing* (November 2007) (www.navylabs.navy.mil/Archive/ProcPolicyGuideDec07.doc). Laboratories are particularly encouraged to become familiar with the prohibited practices identified in the DoD Guidelines. These include, but are not limited to:

- Fabrication, falsification, or misrepresentation of data;
- Improper clock setting (time traveling) or improper date/time recording;
- Unwarranted manipulation of samples, software, or analytical conditions;

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24
Authorised on: 22-Aug-2011. Authorised by: Paul Gray. SOP Unique Reference: 582-17895560. No review required.
Author(s): Paul Gray

Uncontrolled Copy

- Misrepresenting or misreporting QC samples;
- Improper calibrations;
- Concealing a known analytical or sample problem;
- Concealing a known improper or unethical behavior or action; and
- Failing to report the occurrence of a prohibited practice or known improper or unethical act to the appropriate laboratory or contract representative, or to an appropriate government official.

Radiochemistry Certification Officer Training
Supplement to: Chapter III Implementation
17. Training

Radiochemistry Certification Officers (COs) should complete the inorganic portion of the Chemistry COs Training Course and should also complete additional radiochemistry-specific training such as that offered by States, Universities, TNI, private organizations, EPA/Office of Radiation & Indoor Air (ORIA), or Association of Public Health Laboratories (APHL). Since EPA Method 200.8 is addressed during the inorganic portion of the Chemistry COs Training Course, and since this method includes uranium in its scope, completion of the inorganic portion of the Chemistry COs Training Course is sufficient to audit for uranium by EPA Method 200.8.

Chemistry Sample Collection
Supplement to: Chapter IV Critical Elements for Chemistry
6. Sample Collection, Handling, and Preservation

Sample temperatures should be noted upon receipt. Samples that arrive at the laboratory within 24 hours of sample collection, due to the close proximity of a public water system to the laboratory, may not yet have reached the appropriate temperature by the time they arrive at the laboratory. These samples should be considered acceptable ONLY if packed on ice or with frozen gel/ice packs immediately after sample collection and hence, delivered while the samples were in the process of reaching an appropriate equilibrium temperature.

Microbiology Methodology
Supplement to: Chapter V Critical Elements for Microbiology
5. Analytical Methodology

In section 5.2.4.2, A-1 Medium, section 5.2.4.2.5 should be replaced with:

A1 broth may be held up to 7 days in a tightly closed screw-cap tube at 4 ^{o}C.

Page 6

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy

Microbiology Sample Collection
Supplement to: Chapter V Critical Elements for Microbiology
6. Sample Collection, Handling, and Preservation

The time from sample collection to placement of the sample in the incubator (i.e. the 'holding time') for total coliforms and fecal coliforms in surface water sources, and heterotrophic bacteria in drinking water, must not exceed eight hours for samples being analyzed in compliance with the Surface Water Treatment Rule (40 CFR 141.74(a)(1)). Per 40 CFR 141.704, for surface water *E. coli* samples being analyzed in compliance with the Long Term 2 (LT2) rule, the holding time for the sample must not exceed 30 hours, unless an exception is granted by the State. The State may approve, on a case-by-case basis, the holding of an LT2 *E. coli* sample for up to 48 hours if the State determines that analyzing the sample within 30 hours is not feasible.

Appendices
Supplement to: Appendix C: Definitions and Abbreviations

The NELAC Institute (TNI) was created in November 2006 as an outgrowth of NELAC. References to "NELAC" are replaced with "TNI."

Page 7

Micro Handbook - Version: 3.1. Index: QSSEP 106. Printed: 22-Aug-2011 10:24

Uncontrolled Copy