WEST VIRGINIA
SECRETARY OF STATE
KEN HECHLER
ADMINISTRATIVE LAW DIVISION

Form #2

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OFFICE OF WEST VIRGINIA SECRETARY OF STATE

NOTICE OF A COMMENT PERIOD ON A PROPOSED RULE

AGENCY: Division of Health TITLE NUMBER: 64

RULE TYPE: Legislative ; CITE AUTHORITY W. Va. Code §16-1-9a

AMENDMENT TO AN EXISTING RULE: YES X NO

IF YES, SERIES NUMBER OF RULE BEING AMENDED: 3

TITLE OF RULE BEING AMENDED: Public Water Systems, Bottled Water, and Laboratory Certification

IF NO, SERIES NUMBER OF NEW RULE BEING PROPOSED:

TITLE OF RULE BEING PROPOSED:

IN LIEU OF A PUBLIC HEARING, A COMMENT PERIOD HAS BEEN ESTABLISHED DURING WHICH ANY INTERESTED PERSON MAY SEND COMMENTS CONCERNING THESE PROPOSED RULES. THIS COMMENT PERIOD WILL END ON March 4, 1993 AT 4:30 p.m.

ONLY WRITTEN COMMENTS WILL BE ACCEPTED AND ARE TO BE MAILED TO THE FOLLOWING ADDRESS. Comments may be hand delivered up to 4:30 p.m. or must be post marked no later than midnight.

Regulatory Development
State Capitol Complex
Building 3, Room 204
Charleston, WV 25305
ATTN: Kay Howard

THE ISSUES TO BE HEARD SHALL BE LIMITED TO THIS PROPOSED RULE.

Ruth Ann Panepinto, Ph.D.
Ruth Ann Panepinto, Ph.D., Secretary

5.60

ATTACH A **BRIEF** SUMMARY OF YOUR PROPOSAL

FISCAL NOTE FOR PROPOSED RULES

Rule Title: Public Water Systems, 64 CSR 3

Type of Rule: X Legislative _____ Interpretive _____ Procedural

Agency Department of Health and Human Resources

Address Building 3, Capitol Complex Charleston, W. Va. 25305

1. Effect of Proposed Rule	ANNUAL		FISCAL YEAR		
	Increase	Decrease	Current	Next	Thereafter
Estimated Total Cost	$	$	$	$198,000	$187,000
Personal Services				125,000	125,000
Current Expense				61,000	61,000
Repairs and Alterations					
Equipment				12,000	1,000
Other					

2. Explanation of above estimates.

The Department estimates that additional staff consisting of one engineer, two data management personnel and two technicians will be required to handle the increased technical and data management responsibilities specified by the new federal requirements. Current expenses includes employee benefits, travel and routine office operational costs. Equipment includes office furniture and computer and printing equipment.

3. Objectives of these rules:

The proposed changes revise and retitle Public Water Systems, Bottled Water, and Laboratory Certification, 64 CSR 3. Some changes adopt recent National Primary Drinking Water Regulations and National Secondary Drinking Water Regulations. Adoption of these standards will allow the State to retain primacy for the national "Safe Drinking Water Act" which regulates the State's 2,500 public water supply systems. Retaining primacy means that the State, rather than the Federal government, will enforce compliance with mandatory Federal standards.

Other proposed amendments adopt new State standards, correct errors, adopt by reference the newest federal standards for drinking water laboratory certification, and make a few stylistic changes.

4. Explanation of Overall Economic Impact of Proposed Rule.

A. Economic Impact on State Government.

The added enforcement responsibilities will require additional general revenue funds. Failure to adopt the revised rule will result in the loss of approximately $560,000 in federal funding annually and federal, rather than State, administration of drinking water standards.

B. Economic Impact on Political Subdivisions; Specific Industries; Specific Groups of Citizens.

Some public water treatment plants may be required to upgrade facilities to meet the new federal treatment requirements. Cost increases required by the new federal standards will vary widely, depending on the individual system. Data to provide specific estimates for individual systems is not available. The Division of Health will assist individual systems to minimize costs, but many of West Virginia's marginal systems will have difficulty complying. Larger systems are not likely to incur any appreciable new costs. Availability of water treated to meet current national standards may encourage location of new businesses in West Virginia.

It should be emphasized that these upgrade costs will be incurred regardless of whether or not the State adopts the new federal standards. If the proposed amendments are not adopted, the standards will be enforced by the federal government.

C. Economic Impact on Citizens/Public at Large.

The economic impact for the public at large will be negligible. Those systems which are required to upgrade facilities will likely increase rates to their customers (if approved by the Public Service Commission). The economic outlook may be improved in those ares in which water systems are upgraded.

Date January 22, 1992

Signature of Agency Head or Authorized Representative

Ruth Ann Panepinto, Ph.D., Secretary
Department of Health and Human Resources

RULE ABSTRACT

Date: February 1, 1993

Agency: Department of Health and Human Resources

Rule Title: **Public Water Systems**

CSR Title and Series: 64 CSR 3 **Type:** Legislative

Summary: The proposed changes revise and retitle **Public Water Systems, Bottled Water, and Laboratory Certification, 64 CSR 3.** Some changes adopt recent National Primary Drinking Water Regulations and National Secondary Drinking Water Regulations. Adoption of these standards will allow the State to retain primacy for the national "Safe Drinking Water Act" which regulates the State's 2,500 public water supply systems. Retaining primacy means that the State, rather than the Federal government, will enforce compliance with mandatory Federal standards.

Other proposed amendments adopt new State standards, correct errors, adopt by reference the newest federal standards for drinking water laboratory certification, and make a few stylistic changes.

For further information contact: William Herold, Office of Environmental Health, Bureau of Public Health, 815 Quarrier Street, Suite 418, Charleston, WV 25301, telephone 558-2981 or the Regulatory Development Section, Office of Legislation and Regulation, Department of Health and Human Resources, Bldg. 3, Capitol Complex, Charleston, WV 25305, telephone 558-3223.

Copies of the federal drinking water standards are available from the U.S. Environmental Protection Agency, Region III, 841 Chestnut Building, Philadelphia, PA 19107, Telephone (215) 597-8227 or can be viewed at the Office of Environmental Health or in any public library which is a Government Documents Depository Library. Copies of the Manual for the Certification of Laboratories Analyzing Drinking Water adopted by reference are available from the Office of Laboratory Services, Division of Health, 167 Eleventh Avenue, South Charleston, WV 25303-1137.

[PROPOSED]

TITLE 64

WEST VIRGINIA ADMINISTRATIVE RULES
DIVISION OF HEALTH

PUBLIC WATER SYSTEMS

SERIES 3

199__

For Public Comment
Period Ending March 4, 1993

[PROPOSED]
WEST VIRGINIA ADMINISTRATIVE RULES
DIVISION OF HEALTH
PUBLIC WATER SYSTEMS
64 CSR 3

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[PROPOSED]
TITLE 64
WEST VIRGINIA ADMINISTRATIVE RULES
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PUBLIC WATER SYSTEMS~~,-BOTTLED-WATER,~~
~~AND-LABORATORY-CERTIFICATION~~

§64-3-1. General.

1.1. Scope - This legislative rule establishes State standards and procedures and adopts national drinking water standards for public water systems. It establishes standards for the production and distribution of bottled drinking water, and also adopts federal standards for the certification of laboratories performing analyses of drinking water.

1.2. Authority - W.Va. Code §16-1-9a.

1.3. Filing Date -

1.4. Effective Date -

1.5. Supersession or Repeal of Former Regulations - ~~This rule supersedes and repeals the following West Virginia Board of Health Legislative rules: Public Water Supply Regulations, 64 CSR 3, 1982; Volatile Synthetic Organic Chemicals, 64 CSR 61, 1989; and Plumbing Requirements, 64 CSR 57, 1989.~~ This rule amends and reenacts Public Water Systems, Bottled Water and Laboratory Certification, 64 CSR 3, 1991.

§64-3-2. Application and Enforcement.

2.1. Application - This rule applies to public drinking water systems, to bottled water treatment plants and distributors and to laboratories desiring certification to perform analytic tests of drinking water.

2.2. Enforcement - ~~Enforcement of this rule is vested with~~ This rule is enforced by the director of the division of health.

§64-3-3. Definitions.

3.1. Bottled Water - Any natural or artificial mineral, spring, well, distilled or other water bottled or containerized for use primarily as drinking water.

3.2. Bottled Water Distributor - A person who buys and sells bottled water on a wholesale basis.

3.3 Community Water System - A public water system which serves at least fifteen (15) service connections used by year-round residents or regularly serves at least twenty-five (25) year-round residents.

3.4. **Director** - Director of the division of health or his or her designee.

3.5. **Non-Community Water System** - Any public water system that is not a community water system.

3.6. **Person** - Individual, partnership, association, syndicate, company, firm, trust, corporation, government corporation, institution, department, division, bureau, agency, federal agency or any other entity recognized by law.

3.7. **Public Water System** - Any water system or supply which regularly supplies or offers to supply, piped water to the public for human consumption, if serving at least an average of twenty-five (25) individuals per day for at least sixty (60) days per year, or which has at least fifteen (15) service connections and includes:

(1) Any collection, treatment, storage, and distribution facilities under the control of the owner or operator of such system and used primarily in connection with such system, and

(2) Any collection or pretreatment storage facilities not under such control which are used primarily in connection with such system.

A public water system does not include a system which meets all of the following conditions:

(1) which consists only of distribution and storage facilities (and does not have any collection and treatment facilities);

(2) which obtains all of its water from, but is not owned or operated by a public water system which otherwise meets the definition;

(3) which does not sell water to any person;

(4) which is not a carrier conveying passengers in interstate commerce.

3.8. **Sanitary Survey** - An on-site review of the water source, facilities, equipment, operation and maintenance of a public water system for the purpose of evaluating the adequacy of ~~such~~ the source, design, facilities, equipment, operation and maintenance for producing and distributing drinking water, as described in the federal regulations adopted herein.

§64-3-4. Public Water System Construction, Alteration or Renovation; Standards; Exceptions.

4.1. No person may construct, alter, renovate or award a contract for any construction, alteration or renovation of a

public water system without obtaining a permit from the director.

4.2. Application for a permit to construct, alter or renovate shall be made to the director on forms prescribed by the director at least forty-five (45) days prior to the date on which approval by the director is desired. The application shall be accompanied by an engineering report, maps, and detailed plans and specifications of the proposed construction, alteration or renovation prepared by or under the direction of a registered professional engineer.

4.3. A permit to construct, alter or renovate may be revoked by the director for failure of the public water system to comply with this rule.

4.4. A permit to construct, alter or renovate shall be valid for two (2) years from the date of issuance.

4.5. The public water system shall be constructed, altered or renovated in accordance with the plans and specifications approved by the director in accordance with **Design Standards for Public Water Supply Systems**, 64 CSR 42.

4.6. To the extent practical, all new or expanded facilities shall be located outside of the one-hundred-year flood plain.

4.7. The director has the authority to issue an order requiring a change in the source of the water supply for the system or in the manner of collection, treatment, storage, or distribution facilities of the system before delivery to the consumer as may be necessary to safeguard the public health.

4.8. A permit to construct, alter or renovate is not required for any minor addition to, or alteration or renovation of an existing public water system which will not significantly affect the quality or quantity of water supply service rendered. ~~Provided, That~~ The work shall be done in accordance with the provisions of **Design Standards for Public Water Supply Systems**, 64 CSR 47.

A written description of the proposed additions, alterations or renovations shall be submitted to the director no less than ten (10) working days prior to implementing ~~such~~ the additions, alterations or renovations under this provision. The director shall notify the system whether or not the proposed additions, alterations or renovations qualify under this provision within five (5) days of receipt of the description.

4.9. All public water supply systems using a raw water source which is open to the atmosphere or subject to surface runoff shall, at a minimum, provide filtration treatment.

§64-3-5. Permit to Operate a Public Water System.

5.1. A public water system shall be operated in accordance with this rule and the federal regulations adopted herein.

5.2. The director shall have the authority to develop a program for the issuing of a permit to operate a public water system. ~~Such~~ The permit shall be renewable annually and may be revoked for failure to comply with the requirements of this rule or the federal standards adopted herein. ~~Such~~ The permit program shall be administered uniformly. No permit shall be granted until after the director has completed a sanitary survey.

5.3. In the event of a proposed change in the ownership of a public water system, a written application to transfer the permit to operate shall be made to the director by the new owner at least fifteen (15) days before the proposed change.

5.4. The current permit to operate shall be posted in a conspicuous place at the public water system's treatment plant or main office.

§64-3-6. Inspections and Sanitary Surveys of Public Water Systems.

6.1. Public water systems shall be inspected as scheduled by the director and sanitary surveys shall be conducted by the director in accordance with the federal regulations adopted herein.

6.2. The director has the right of access to all parts of a public water system and shall be furnished access to all information and records required to be kept by this rule and the federal regulations adopted herein.

§64-3-7. Public Water System Disinfection Requirements.

7.1. Disinfection with chlorine, chlorine dioxide, chloramine or ozone is required of all public water systems, provided the requirements of Section 7.6 of this rule are met.

7.2. The disinfectant shall be applied during treatment at a point before entering the distribution system which will provide effective contact time.

7.3. The minimum chlorine contact time for groundwater systems not influenced by surface waters is thirty (30) minutes from the point of application to the point of delivery to the first consumer or as stipulated in Design Standards for Public Water Supply Systems, 64 CSR 42. At the end of the chlorine contact time, minimum free chlorine residuals shall comply with the requirements of Table 64-3A found at the end of this rule. For ~~such~~ these systems, the amount of residual disinfectant in

the drinking water at the treatment plant and in the distribution system shall be determined at least once per day, or more often if considered necessary by the director.

7.4. Surface water systems and groundwater systems under the direct influence of surface waters shall meet the disinfection requirements of the federal regulations adopted herein.

7.5. Chlorine residual testing equipment shall enable measurement of free and total chlorine residuals to the nearest 0.2 milligrams per liter in the range of 0.0 milligrams per liter to 2.0 milligrams per liter.

7.6. For all public water systems, at least 0.2 milligrams per liter of total chlorine residual shall be maintained throughout the distribution system at all times.

7.7. The director ~~shall have~~ has the authority to authorize variances in the chlorine disinfection parameters specified in this section.

§64-3-8. Public Water System Fluoridation.

8.1. Except for water systems operated by public schools, average concentrations of fluoride present in the drinking water of a public water system, which artificially adjusts fluoride concentrations, shall be no less than the minimum and no higher than the maximum concentrations shown in Table 64-3B found at the end of this rule.

8.2. Average concentrations of fluoride present in a public school drinking water system shall be no less than three (3.0) milligrams per liter and no higher than six (6.0) milligrams per liter, with an optimum concentration of four and one-half (4.5) milligrams per liter.

8.3. The drinking water of fluoridated or defluoridated public water systems shall be monitored once per day for fluoride concentration. Records of ~~such~~ the monitoring shall be maintained in accordance with ~~Section 10~~ Sections 9 and 10 of this rule.

8.4. At least once a month, a sample of drinking water shall be submitted by the public water system to the director or to a certified laboratory for fluoride analysis.

8.5. The requirements of Section 8.2 of this rule supersede the requirements of the National Secondary Drinking Water Regulations, 40 CFR Part 143, as applicable to fluoridation of public school drinking water.

§64-3-9. Public Water System Control Tests and Record Maintenance

9.1. Records of microbiological, turbidity, radiological and chemical analyses, or a summary thereof, shall be retained at a convenient location on or near the premises of the public water system. ~~Microbiological, turbidity and~~ Turbidity, radiological and chemical analytical records shall be kept for ten (10) years. Control ~~tests~~ test, microbiological and operational records shall be kept for five (5) years. All tests and analyses required by this rule or the federal regulations adopted herein, with the exception of turbidity and chlorine residual analyses, shall be conducted by a laboratory certified by the director.

9.2. The records shall include the date, place and time of sampling; the name of the person who collected the sample; identification as to whether it was a routine distribution system sample, resample, raw or drinking water sample, or other special purpose sample; the date of the analysis; the laboratory and person responsible for performing the analysis; the analytical technique or method used for microbiological testing; and the results of the analysis.

9.3. Records of action taken by the system to correct violations of this rule or the federal regulations adopted herein shall be kept for three (3) years after the correction is completed.

9.4. Copies of written reports relating to sanitary surveys of the system shall be kept for ten (10) years.

9.5. Records concerning a variance or exemption from this rule or the federal regulations adopted herein shall be kept for at least five (5) years following the expiration of ~~such~~ the variance or exemption.

§64-3-10. Adoption of National Regulations.

~~The National Primary Drinking Water Regulations, 40 CFR Parts 141 and 142 subparts E, F, G, as amended in the Federal Register June 29, 1989 and June 19, 1990 and effective as of December 31, 1990 and the National Secondary Drinking Water Regulations, 40 CFR Part 143, in effect as of October 30, 1990, are hereby adopted by reference. The director shall use the provisions of 40 CFR 142, Subparts E, F and G, as applicable, in granting variances.~~

10.1. The following National Primary Drinking Water Regulations and National Secondary Drinking Water Regulations, promulgated and published prior to January 1, 1993, as final rules are hereby adopted by reference:

10.1.1. 40 C.F.R. Part 141, except for Section 141.21(d)(2) and Subpart I (the lead and copper rule);

10.1.2. Subparts F and G of 40 C.F.R. Part 142, except for

Sections 142.57, 142.60, 142.61, and 142.62(c)-142.62(h); and

10.1.3. 40 C.F.R. Part 143.

10.2. The Director shall use the provisions of 40 C.F.R. Part 142, Subparts F and G, as adopted in this rule as applicable in granting exemptions.

10.3. In the event of a conflict between a federal standard adopted in this rule and a state standard adopted in this rule, the more stringent standard shall apply, except as stated in Section 8.5 of this rule.

10.4. Copies of these regulations are available from:

U.S. Environmental Protection Agency
Region III
841 Chestnut Building
Philadelphia, PA 19107

§64-3-11. Bottled Water Treatment Plants and Distributors.

11.1. No person may operate a bottled water treatment plant in this State without receiving a permit to bottle and distribute water from the director.

11.2. No person may distribute bottled water in this State without receiving a permit to distribute bottled water from the director.

11.3. Application for a permit to bottle and distribute water shall be made to the director on forms prescribed by the director. Four (4) sets of completed applications, and plans and specifications for the treatment plant shall be submitted to the director for approval at least forty-five (45) days prior to the date on which a permit from the director is desired.

11.4. The source of the water to be bottled and the bottled water shall comply with the requirements of ~~**Design Standards for Public Water Supply Systems**, 64 CSR 47,~~ this rule and the requirements of the federal regulations adopted herein pertaining to primary and secondary contaminants, sodium, fluoridation, maximum contaminant levels, sampling techniques and monitoring frequencies, for a community water system, except that the monitoring frequency for microbiological contaminants shall be no less than once each week.

11.5. A bottled water treatment plant shall be operated in accordance with the provisions of the federal standards, **Current Good Manufacturing Practice in Manufacturing, Packaging or Holding Human Food**, 21 CFR Part 110, in effect as of October 30, 1990, and ~~such~~ these standards are hereby adopted by reference.

11.6. Each in-State bottled water treatment plant shall be inspected every twelve (12) months or as otherwise determined by the director.

11.7. An out-of-State bottled water treatment plant desiring to distribute bottled water in West Virginia shall apply for a permit to bottle and distribute bottled water on forms approved by the director. Four (4) copies of all materials shall be submitted. The out-of-State treatment plant shall comply with the requirements of this rule and the federal regulations adopted herein for in-State bottled water treatment plants. Subsequent to the initial evaluation, monitoring of the treatment plant by the regulatory agency of the state wherein the treatment plant is located ~~will be~~ is considered acceptable for the purposes of this rule. The out-of-State treatment plant shall notify the director of any corrective action it is required to take by its state regulatory authority and shall notify the director of any change in ownership or in the event that it closes.

11.8. A person wishing to distribute bottled water in the State who does not operate a bottled water treatment plant shall apply for a permit to distribute bottled water on a form approved by the director. The applicant shall identify the location of the plants from which the bottled water is obtained and any distributor other than the bottled water plant from which the bottled water is obtained and shall provide other information required by the director. The director shall grant a permit to distribute bottled water if the bottled water complies with the requirements of this rule.

11.9. A permit issued by the director may be revoked for failure to comply with provisions of this rule.

§64-3-12. Public Water System Reporting Requirements.

12.1. Unless otherwise specified in this rule or the federal regulations adopted herein, the results of any test, measurement or analysis required to be made by this rule or the federal regulations adopted herein shall be reported to the director within forty (40) days of the system's receipt of the test, measurement or analysis.

12.2. Analytical results for total trihalomethane (TTHM) analyses shall be reported to the director within thirty (30) days of the system's receipt of ~~such~~ the results.

12.3. Failure to comply with this rule or the federal regulations adopted herein shall be reported to the director within forty-eight (48) hours of the discovery of the violation.

12.4. Analytical results of tests performed by the laboratory of the division of health are not required to be reported.

12.5. A written summary of the public water system operation, test data, and ~~such~~ other information as may be required by the director shall be submitted to the director at least once per month. The director may require more frequent reports in cases where there are public health concerns.

12.6. All reports and summaries required by this rule or the federal regulations adopted herein shall be submitted in a manner or form approved by the director.

12.7. The water supply system shall submit to the director a representative copy of each type of notice distributed, posted or made available to the public or media within seven (7) days following any notification of the public of a violation of this rule or of the federal regulations adopted herein.

§64-3-13. Certification of Laboratories to Conduct Drinking Water Tests.

13.1. All laboratories providing drinking water testing results for purposes of this rule or the federal regulations adopted herein shall be certified by the director or by the Federal Environmental Protection Agency.

13.2. A certified laboratory shall comply with the requirements of this rule and with the requirements and criteria contained in the sections titled "Local Laboratories," "Other Considerations for Laboratory Certification," "Requirements for Maintaining Certification Status," "Criteria and Procedures for Downgrading/Revoking Certification Status," and "Training," of Chapter III, and in Chapters IV, V and VI of the federal Environmental Protection Agency's Manual for the Certification of Laboratories Analyzing Drinking Water, Third Edition, April, 1990, Change 1, October, 1991, and Change 2, September, 1992 and ~~such~~ these parts of ~~said~~ the manual are hereby adopted by reference.

13.3. An in-State laboratory shall submit an application form when seeking initial approval sixty (60) days prior to the date certification is desired.

13.4. A laboratory located outside the boundaries of this State ~~will~~ shall be certified by the director if:

13.4.1. It has been certified by the Federal Environmental Protection Agency; or

13.4.2. It has been certified by a program for the certification of laboratories equivalent to the program of this State as determined by the director. If the program of the state in which the laboratory is located is not judged equivalent, the laboratory may request an on-site evaluation and full certification review by the director.

13.5. An out-of-state laboratory shall submit an application form when seeking initial approval and shall include with its application evidence of compliance with Section 13.4.1 or 13.4.2 of this rule. The out-of-state laboratory shall notify the director immediately of any change in its certification status under Section 13.4.1 or 13.4.2.

13.6. ~~On-site--Inspection~~-- An on-site inspection of in-State laboratories to determine compliance with this rule and the federal standards adopted herein shall be conducted initially prior to certification, and at least every three (3) years thereafter. The division shall have the right of entry upon proper identification at ~~such~~ any times as considered necessary during operating hours in order to conduct ~~such~~ the inspections.

13.7. Certificates of approval shall be issued upon initial approval and shall be renewable on an annual basis thereafter pursuant to the conditions listed herein. Certificates issued will contain the name and location of the laboratory, a laboratory code number, the name of the laboratory director and the date of expiration of the certificate.

13.8. Certified laboratories shall notify the director when there is a change in ownership, laboratory director, technical personnel or location of the laboratory.

13.9. The director shall administer and use the criteria and procedures of the Section titled "Criteria and Procedures for Downgrading/Revoking Certification Status" of the **Manual for the Certification of Laboratories Analyzing Drinking Water** referenced in Section 13.2 of this rule.

§64-3-14. Penalties.

Any person who violates any provision of this rule or orders issued hereunder, shall be guilty of a misdemeanor, and upon conviction thereof, shall be fined not less than twenty-five dollars ($25) nor more than two hundred dollars ($200) and each day's violation shall constitute a separate offense. In addition, thereto, the director of health or his or her authorized representative may seek injunctive relief in the circuit court of the county in which all or part of the public water system is situated for threatened or continuing violations. For a willful violation of this rule or orders issued hereunder, a person, upon a finding thereof by the circuit court of the county in which the violation occurs, shall be subject to a civil penalty of not more than five thousand dollars ($5,000), and each day's violation shall be grounds for a separate penalty.

§64-3-15. Administrative Due Process.

Those persons adversely affected by the enforcement of this rule desiring a contested case hearing to determine any rights,

duties, interests or privileges shall do so in a manner prescribed in the Rules of Procedure for Contested Case Hearings and Declaratory Rulings, 64 CSR 1.

§64-3-16. Severability.

The provisions of this rule are declared to be severable. If any provision of this rule ~~shall be~~ is held invalid, the remaining provisions ~~shall~~ remain in effect.

TABLE 64-3A. Minimum Levels of Free Chlorine Residual at Various Water Sample pH Levels

pH VALUE	FREE CHLORINE RESIDUAL
Up to 7.0	0.4 mg/l
7.1 to 8.0	0.6 mg/l
8.1 to 9.0	1.0 mg/l

TABLE 64-3B. Average Acceptable Range of Fluoride Concentration at Various Annual Average Maximum Daily Air Temperatures

ANNUAL AVERAGE MAXIMUM DAILY AIR TEMPERATURE	FLUORIDE CONCENTRATION IN MILLIGRAMS PER LITER		
	Lower	Optimum	Upper
53.8 - 58.3° F 12.1 - 14.6° C	0.8	1.1	1.5
58.4 - 63.8° F 14.7 - 17.7° C	0.8	1.0	1.3
63.9 - 70.6° F 17.7 - 21.4° C	0.7	0.9	1.2

United States
Environmental Protection
Agency

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

EPA/570/9-90/008
April 1990

Manual for the Certification of Laboratories Analyzing Drinking Water

Criteria and Procedures Quality Assurance

Third Edition

Prepared by
The Laboratory Certification Program Revision Committee

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

Notice

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

Acknowledgments

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

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Preface

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

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

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

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

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

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

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

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

Contents

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Chapter I
Introduction

Public water systems serving at least 25 persons or having at least 15 service connections must comply with the Safe Drinking Water Act and the requirements of the National Primary Drinking Water Regulations (40 CFR Part 141). Section 1401(1)(D) of the Act defines a National Primary Drinking Water Regulation to include "criteria and procedures ... [for] quality control and testing procedures to insure compliance ...." 40 CFR Part 142 sets out implementation requirements.

The regulations at 40 CFR 142.10(b)(4) require a State that has primary enforcement responsibility (primacy) to have laboratory facilities available which have been certified by EPA (see Table I-1). The regulations at 40 CFR 141.28 require that all testing for compliance purposes, except for turbidity, free chlorine residual, temperature, and pH, be performed by laboratories certified by the State. This manual is intended to assist EPA in implementing 40 CFR 142.10(b)(4) by specifying procedures for certifying principal State laboratories. States with primacy may also choose to use equivalent, nonidentical criteria and procedures to those in this manual for their own certification programs.

Table I-1. Primacy Requirements for States

To obtain and maintain primary enforcement responsibility ("primacy"), a State must comply with 40 CFR 142.10, which includes the following two provisions:

"The establishment and maintenance of a State program for the certification of laboratories conducting analytical measurements of drinking water contaminants pursuant to the requirements of the State primary drinking water regulations including the designation by the State of a laboratory officer, or officers, certified by the Administrator, as the official(s) responsible for the State's certification program. The requirements of this paragraph may be waived by the Administrator for any State where all analytical measurements required by the State's primary drinking water regulations are conducted at laboratories operated by the State and certified by the Agency." (40 CFR 142.10(b)(3(i))

"Assurance of the availability to the State of laboratory facilities certified by the Administrator and capable of performing analytical measurements of all contaminants specified in the State primary drinking water regulations ...." (40 CFR 142.10(b)(4))

The EPA laboratory certification program extends to its Regional laboratories, principal State laboratories in primacy States, and laboratories that perform analyses under the Safe Drinking Water Act in States without primacy. Primacy States must have a certification program for local laboratories if all analyses are not performed in principal State laboratories (See Table I-1). The State certification program may involve a third party certifier (see Appendix D).

EPA's Environmental Monitoring Systems Laboratory in Cincinnati, Ohio (EMSL-CI), is responsible for determining what certification status is warranted for EPA Regional laboratories in microbiology and chemistry. The Environmental Monitoring Systems Laboratory in Las Vegas (EMSL-LV) has this responsibility for radiochemistry. Regional certification officers are responsible for the certification of the principal State laboratory in each primacy State and are also responsible for all laboratories in non-primacy States. Evaluations of all laboratories for radiochemistry are conducted by EMSL-LV, except where the Regions have this capability.

Primacy States with certification programs are responsible for certifying local laboratories, i.e., laboratories other than the principal State laboratory. Under EPA's program, principal State laboratories are expected to successfully analyze a complete set of unknown performance evaluation (PE) samples from EMSL-CI (or EMSL-LV, where applicable) at least annually and pass an on-site evaluation every three years. Regional laboratories must successfully analyze a set of PE samples at least annually for all regulated contaminants for which they conduct analyses and pass an on-site evaluation at least every three years. The criteria in this manual will be used for the on-site evaluation.

Chapter II describes the responsibilities of each of the EPA organizations for this certification program. Chapter III describes how the program operates. Chapters IV, V and VI cover the technical criteria for chemistry, microbiology, and radiochemistry, respectively, used during an on-site evaluation of a laboratory. Evaluation forms are also included in Chapters IV, V and VI.

The appendices include: recommended chain-of-custody procedures; a recommended protocol and format for conducting on-site laboratory evaluations, which may be used by the evaluators; abbreviations; EPA's policy on third-party certification; a list of contaminants a principal State laboratory must have the capability to analyze; a list of not yet regulated contaminants which EPA is scheduled to regulate; and a list of unregulated chemicals which systems must monitor under §1445 of the Safe Drinking Water Act.

Chapter II
Responsibilities

The success of the laboratory certification program depends upon cooperation among the organizations responsible for its implementation. Within the Agency, primary responsibilities for laboratory certification are shared by the Office of Drinking Water (ODW), the Office of Research and Development (ORD), and the Regional Offices. The Drinking Water Laboratory Certification Work Group (DWLC) is a standing group that reviews problems and provides guidance.

Office of Drinking Water (ODW)

ODW is responsible for developing and implementing the national certification program for laboratories that analyze drinking water samples and for implementing the Safe Drinking Water Act, including the preparation of regulations and standards.

Office of Research and Development (ORD)

EMSL-CI and EMSL-LV share responsibility with ODW for developing and implementing the laboratory certification program.

EMSL-CI is the lead organization for managing the national certification program for laboratories performing chemical and microbiological analyses. Its responsibilities include:

- Reviewing EPA Regional certification programs and conducting on-site evaluations of each Regional laboratory every three years to determine whether a change in the certification status is warranted;
- Preparing and distributing PE samples and quality control (QC) samples for regulated chemical and microbiological contaminants (when available) and calibration standards for organic contaminants, as appropriate;
- Conducting water supply performance evaluation studies at least annually for all Regional and principal State laboratories. Other laboratories may participate in these studies, if EPA resources allow, by submitting their requests to the State laboratory officer(s) for forwarding to EPA;
- Evaluating the resources and personnel available in each EPA Region to carry out the certification program;
- Developing and participating in training courses to support the certification program; and
- Providing technical assistance to EPA and the States, as required, and participating in DWLC Work Group activities.

EMSL-LV is the lead organization for managing the certification program for laboratories performing radiochemical analyses. Its duties correspond to those described for EMSL-CI. In addition, at the request of a Region, EMSL-LV is responsible for conducting on-site evaluations for radiochemistry of principal State laboratory systems and, if resources are available, other laboratories. In these cases, EMSL-LV will report the results of its inspections to the responsible Regional Administrator, who will have final authority to determine certification status.

EPA Regions

The ten Regions oversee progress of the certification program in the States. The Regions are responsible for:

- Determining what certification status is warranted for the principal State laboratory in each primacy State and the local laboratories in non-primacy States, including an on-site evaluation of each such laboratory at least once every three years (the Regional Administrator or designee is the certifying authority). Regions will provide the laboratory with an evaluation report within 45 days of the on-site evaluation;
- Coordinating EMSL water supply performance evaluation studies with laboratories in the Region;
- Performing an annual review of State certification programs and performance evaluation reports and monitoring the adequacy of State programs for certifying laboratories, as described below;

- Providing technical assistance to EPA-certified drinking water laboratories, as needed;

- Operating the certification program in non-primacy States; and

- Insuring that the Regional laboratory, if one exists, is certified and meets the criteria in this manual.

Regions are to monitor the adequacy of State programs for certifying laboratories by periodically assessing each program's scope, staffing, policy, procedures, and effectiveness. The adequacy of these essential program elements are to be monitored by:

- Evaluating and acting as approval authority for the State's certification program. The Region must review the program plan/regulation (including program description), responsibilities, organizational structure, staff (including educational background and experience), scope and description of the certification process and certification downgrading criteria and procedures, and use of PE samples;

- Requesting States to submit an annual program report that includes program highlights, training and continuing education efforts, number of on-site evaluations performed, listing of laboratories certified by discipline or contaminant, and any certification downgrading or upgrading actions along with reasons for those actions;

- Observing selected State on-site evaluations of local laboratories to allow Regional certification specialists to evaluate specific elements of the State certification program;

- Allowing State evaluators to participate in Regional on-site evaluations of the principal State laboratory to provide experience for State evaluators; and

- Hosting annual meetings of State certification officers to discuss program issues, policies, and problems. Key Regional, EMSL, and Headquarters personnel should be invited to participate.

In addition to its laboratory certification duties, the Region has administrative, enforcement, and local laboratory certification responsibilities in non-primacy States. Some of these duties may be performed by the State, but the Region must retain responsibility for the on-site evaluation of the designated principal State laboratory. Local laboratories may be evaluated by the Region, or under a Region-approved program carried out by a designated principal State laboratory. In either case, this manual will be the basis for the on-site evaluations of State and local laboratories conducted by the EPA Region in non-primacy States.

Drinking Water Laboratory Certification Work Group

The Drinking Water Laboratory Certification Work Group is responsible for overseeing the operation of the national certification program for drinking water laboratories. This group advises ODW and includes representatives from ODW, ORD (EMSL-CI, EMSL-LV, Risk Reduction Engineering Laboratory, and QAMS), Office of Water Enforcement and Permits, Regional Offices and States. The Work Group's responsibilities include:

- Monitoring the certification program and recommending technical and administrative revisions to ODW as dictated by experience or updated information;

- Developing guidance and responding to questions and comments from the Regions;

- Developing technical and administrative criteria to support additional certification needs imposed by future regulations;

- Ascertaining laboratory availability and capability for future regulatory activities; and

- Making recommendations to ODW on resources needed to implement the certification program.

Chapter III
Implementation

EPA Regional Laboratories and Programs

EMSL-CI is responsible for certifying the Regional laboratory to perform microbiological and chemical analyses. It also approves the Regional program for certifying other laboratories to perform these same analyses. EMSL-LV has similar responsibilities for Regions that have radiochemistry capabilities. EMSL-CI (or EMSL-LV for radiochemistry) must approve the Regional certification program before a Region can exercise its authority to certify other laboratories. The certifying authority resides with the Director, EMSL-CI, for microbiology and chemistry or with the Director, EMSL-LV, for radiochemistry, or with their respective designees.

Certification of Regional Laboratories

In order to be eligible to analyze compliance samples under the Safe Drinking Water Act, EPA Regional laboratories must meet the minimum criteria specified in the manual, pass an on-site inspection at least once every three years, and satisfactorily analyze an annual set of PE samples or other unknown test samples, as specified by regulations or this guidance. For those Regions certified for radiochemistry, satisfactory performance on two intercomparison samples per year is also necessary. EMSL-LV currently provides intercomparison samples to laboratories without charge, but this may change in the future. The EMSLs will use the same criteria and procedures for certifying Regional laboratories as the Regions use for principal State laboratories.

Individual(s) Responsible for Certification Program

Each EPA Regional Administrator or designee will appoint an individual(s) to coordinate drinking water certification activities. This individual(s) must be experienced in quality assurance; hold an advanced degree or have equivalent experience in microbiology, chemistry, or radiochemistry; and have sufficient administrative and technical stature to be considered a peer of the director of the principal State laboratory.

On-Site Evaluation Team

One or more teams must be established by the Region to evaluate a laboratory in microbiology and chemistry. Team members must be experienced professionals and hold at least a bachelor's degree, (or equivalent education and experience) in the specific discipline being evaluated. Team members must complete a laboratory certification course presented by EMSL-CI and pass the course requirements.

Development of Regional Plans for Certifying Local Laboratories in Non-Primacy States

Regions are required to develop plans for certifying local drinking water laboratories in non-primacy States. Written plans should include the following:

- Designation of certification official;
- Types and numbers of laboratories to be evaluated;
- Specific types of analyses to be examined;
- Schedule for on-site evaluations; and
- Plans for providing technical assistance to laboratories in need of upgrading.

Principal State Laboratories

The principal state laboratory system must have the capability to analyze every contaminant included in the drinking water regulations (40 CFR 142.10(b)(4)); however, an individual laboratory that is part of a principal State laboratory system may be certified for only one, several, or all the cited analyses. If a principal State laboratory contracts with another laboratory, including a laboratory outside the State, to assume the lead role in analyzing a regulated parameter (e.g., radiochemical contaminants), that contract laboratory will, for the purposes of this manual, be considered part of the principal State laboratory system. In this case, the contract laboratory must be certified by EPA, unless the contract laboratory is in another State, and that State has certified the laboratory for the contaminants of interest, with the concurrences of the two affected EPA Regions.

The certification process for a principal State laboratory will begin when the laboratory director or State certification officer makes a formal request to the Region. The Regional certification officer may also initiate a request for certification. This application may result from the following:

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

The Region should respond to a formal application for any of the requests within 30 days, and a mutually agreeable date and time should be set for the on-site laboratory evaluation. The recommended protocol for conducting these evaluations is given in Appendix B. EPA will only certify laboratories that pass an on-site inspection (see Chapters IV, V, and VI for inspection checklists) and satisfactorily analyze performance evaluation samples (or other unknown test samples for those contaminants for which it requests certification).

After the on-site visit and the review of PE sample results, the Region can classify the laboratory for each type of analysis according to the following rating scheme:

- *Certified* — a laboratory that meets the minimum requirements of this manual and all applicable regulatory requirements. The certification shall be valid for up to three years;
- "*Provisionally Certified*"—a laboratory that has deficiencies but demonstrates its ability to consistently produce valid data; and
- *Not Certified*—a laboratory that possesses major deficiencies and, in the opinion of the Regional Administrator, cannot consistently produce valid data within specified acceptance limits.

A "provisionally certified" laboratory may analyze drinking water samples for compliance purposes. However, in no case should provisional certification be given if the evaluation team believes that the laboratory cannot perform an analysis within acceptance limits. Furthermore, neither "certified" nor "provisionally certified" status may be granted to any laboratory that has not met the performance criteria specified in any National Primary Drinking Water Regulation.

For laboratories requesting first-time certification or certification to analyze additional or newly regulated contaminants, the Region may administratively grant a laboratory "provisionally certified" status, as specified in a drinking water regulation, pending an on-site evaluation. "Provisionally certified" status is granted only when the Region judges that the laboratory has both the appropriate instrumentation and trained personnel to perform the analyses, and that the laboratory has satisfactorily analyzed PE samples for the contaminants in question. Regions should perform an on-site evaluation as soon as possible, but in no case later than seven months after it has granted the laboratory "provisionally certified" status.

For those Regions lacking the expertise required to certify laboratories in radiochemistry, ESML-LV will conduct on-site inspections.

Local Laboratories

For the purposes of this document, local laboratories include any State, county, municipal, utility, Federal, or commercial laboratory, but exclude principal State laboratories and EPA Regional laboratories. In non-primacy States, the Regions will certify local laboratories using the criteria and policies in this manual.

Only those primacy States where not all drinking water analyses are conducted at State-operated laboratories are required to establish a certification program for local laboratories (see 40 CFR 1422.10(b), Table I-1.). All States, however, are encouraged to develop such programs. Certification can be based either upon criteria contained in this manual or upon State-developed equivalents that are in accordance with this manual, as determined by EPA. In addition, all State certification programs must require compliance with all related provisions of any National Primary Drinking Water Regulation. Those States required by regulation to develop a certification program must appoint a laboratory certification officer(s), certified by EPA, as the official(s) responsible for the State program.

The principal State laboratory system must have the technical capability to analyze for all regulated contaminants. If a principal State laboratory system has the intent and resources to perform 100% of the analyses for some contaminants, it need not include certification criteria for those contaminants. But, if the principal State laboratory system does not perform 100% of the analyses for other contaminants (e.g., it only analyzes 20% of all total coliform samples), then the State certification program must include those contaminants.

For the purpose of certification, Federal laboratories that analyze compliance samples, and other laboratories that analyze compliance samples for Federal facilities, are local laboratories and must, therefore, be certified by the State or EPA. If

requested by the State, the Region may carry out certification activities for Federal laboratories in that State.

EPA will certify individual laboratories on Federal Indian lands, if requested by the tribal chairperson, as resources allow.

EPA operates the certification program for local laboratories in non-primacy States. The criteria, procedures, and mechanism EPA uses to certify local laboratories are the same as those for principal State laboratories, except that a local laboratory does not have to possess the capability to analyze every regulated contaminant.

Other Considerations for Laboratory Certification

Laboratory Quality Assurance Plan

It is essential that all laboratories analyzing drinking water compliance samples adhere to defined quality assurance procedures. This is to insure 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 should prepare a written description of its quality assurance activities (a QA plan). The following items should be addressed in each QA plan:

1. Sampling procedures;
2. Sample handling procedures;
   - specify procedures used to maintain integrity of all samples, i.e., tracking samples from receipt by laboratory through analysis to disposal;
   - samples likely to be the basis for an enforcement action may require special safeguards (see Chain-of-Custody procedures).
3. Instrument or equipment calibration procedures and frequency of their use;
4. Analytical procedures;
5. Data reduction, validation and reporting;
   - data reduction: conversion of raw data to mg/L, picocuries/L, coliforms/100mL, etc.
   - validation: includes insuring accuracy of data transcription and calculations.
   - reporting: includes procedures and format for reporting data to utilities, State officials, and EPA.
6. Types of quality control (QC) checks and frequency of their use;
   - may include preparation of calibration curves, instrument calibrations, replicate analyses, use of EMSL-provided QC samples or calibration standards and use of QC charts[1].
7. Preventive maintenance procedures and schedules;
8. Specific routine procedures used to determine data precision and accuracy for each contaminant measured;
   - precision is based on the results of replicate analyses.
   - accuracy is normally determined by comparison of results with "known" concentrations in reagent water standards and by analyses of water matrix samples before and after adding a known contaminant "spike."
9. Corrective action contingencies;
   - response to obtaining unacceptable results from analysis of PE samples and from internal QC checks.
10. Laboratory organization and responsibility;
    - include a chart or table showing the laboratory organization and line authority.
    - 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).

The QA plan may be a separately prepared QA document or may incorporate, by reference, already available standard operating procedures (SOPs) that are approved by the laboratory director and that address the listed items. Documentation for many of the listed QA plan items can be made by reference to appropriate sections of this manual, to the laboratory's SOPs, or to other literature (e.g.,

[1]QC chart for chemistry is explained in *Standard Methods for the Examination of Water and Wastewater*, 16th ed., 1985, pp. 25-32. QC chart for radiochemistry is explained in *Handbook for Analytical Quality Control and Radioactivity Analytical Laboratories*, EPA-600/7-77-088, August 1977.

Standard Methods for the Examination of Water and Wastewater).

If a particular listed item is not relevant, the QA plan should state this and provide a brief explanation (e.g., some laboratories do not collect samples and thus are not required to describe sampling procedures). A laboratory QA plan should be concise but responsive to the above-listed items (a maximum of five pages is suggested). Minimizing paperwork while improving dependability and quality of data are the intended goals.

Performance on Routine Water Samples

Each EPA Region will develop a strategy to assess laboratory performance on routine water samples as part of its certification program for principal State laboratories in primacy States, and for local laboratories in non-primacy States. This strategy may include one or more of the following approaches or some other approach: (1) send the laboratory a blind audit sample, (2) perform an unannounced on-site evaluation, (3) require laboratory to analyze an unknown sample during the on-site evaluation, or (4) arrange a split sample program with the laboratory. Each Region should develop a written plan, approved by EMSL-CI and concurred in by ODW, that addresses this issue.

Chain-of-Custody Procedures

Certified laboratories, when requested to process a sample for possible legal action against a supplier, must use an adequate chain-of-custody procedure. An example of such a procedure is found in Appendix A.

Requirements for Maintaining Certification Status

Periodic Performance Evaluation (PE) Samples and Other Unknown Test Samples

Certified drinking water laboratories must satisfactorily analyze PE samples (all concentration levels provided) or other unknown test samples at least once annually for each chemical, radiochemical, or microbiological analyte (when available) for which certification has been granted. However, in some cases, EPA will permit certification of a group of related analytes (e.g., volatile organic chemicals) on the basis of a limited number of analytes in that group. If the laboratory does not analyze an analyte in the PE sample, or other unknown test sample, within the acceptance limits established by EPA, the certifying authority must follow the procedure discussed in the section entitled, "Criteria and Procedures for Downgrading/Revoking Certification Status." To maintain certification in radiochemistry, the laboratory must satisfactorily analyze two intercomparison samples per year in addition to the annual set of PE samples. The laboratory should be able to provide evidence that the person(s) analyzing any PE sample is a laboratory employee who routinely analyzes drinking water compliance samples.

Methodology

Laboratories must use methodologies specified by the drinking water regulations (40 CFR 141.21 - 141.30, 141.41, 141.42).

Notification of Certifying Authority (CA) for Major Changes

Laboratories certified by EPA must notify the appropriate CA (Regional Administrator, or designee, or the appropriate EMSL), in writing, within 30 days of major changes in personnel, equipment, or laboratory location which might impair analytical capability. A major change in personnel is defined as the loss or replacement of the laboratory supervisor or a situation in which a trained and experienced analyst is no longer available to analyze a particular parameter for which certification has been granted. The CA will discuss the situation with the laboratory supervisor and establish a schedule for the laboratory to rectify deficiencies. If the CA determines that the laboratory can no longer produce valid data, the CA must begin certification downgrading actions, including revoking certification, when warranted.

On-Site Evaluation

The CA must be satisfied that a laboratory is maintaining the required standard of quality for certification. Normally, this will be based upon recommendation of an EPA on-site evaluation conducted at least every three years. If the laboratory undergoes a major change, however, or if it fails a PE sample or other unknown test sample, the CA should consider an evaluation sooner.

Criteria and Procedures for Downgrading/Revoking Certification Status

Criteria for Downgrading Certification Status

A laboratory will be downgraded to "provisionally certified" status for a particular contaminant analysis for any of the following reasons:

1. Failure to analyze a PE sample (or an EMSL-LV intercomparison sample or any other unknown test sample) within the acceptance limits established by EPA. Failure on a PE sample is defined as a failure on any concentration provided, unless otherwise specified by ODW or EMSL-CI for a particular PE study;

2. Failure of a certified laboratory to notify the CA within 30 days of major changes which might

impair analytical capability (e.g., in personnel, equipment, or laboratory location);

3. Failure to satisfy the CA that the laboratory is maintaining the required standard of quality, based upon an EPA on-site evaluation; or

4. Failure to notify the State and/or the public water system in a timely manner of unsatisfactory results on water samples, thereby preventing compliance with Federal and/or State reporting requirements.

Procedures for Downgrading to "Provisionally Certified" Status

If a laboratory is subject to downgrading on the basis of the indicated criteria, the CA will notify the laboratory director or owner, in writing (by registered or certified mail), within 14 days. The laboratory director will review the problems cited and, within 30 days of receipt of the letter, send a letter to the CA specifying what corrective actions are being taken. The CA will consider the adequacy of the response and notify the laboratory by mail, within 14 days of receipt, of its certification status. The CA will follow up to insure that corrective actions have been taken.

If a laboratory fails to analyze an unknown test sample within the acceptance limits established by EPA, the CA will not downgrade certification if the laboratory identifies and corrects the problem to the CA's satisfaction within 30 days of being notified of the failure. If, after review of the submitted information, the CA determines that the laboratory need not be downgraded, then within two months of this decision, the CA will send the laboratory another unknown sample containing the failed contaminant (see Figure III-1). If the laboratory analyzes this second unknown sample within the acceptance limits established by EPA (using the most recent PE summary statistical compilations from EMSL), the laboratory will not be downgraded. If the laboratory fails to analyze this second unknown sample within the established limits, the CA will downgrade the laboratory to "provisionally certified" status and notify the laboratory, in writing, by registered or certified mail. Laboratories should be downgraded only for the analyte failed, except where EPA certifies a group of related analytes based on a limited number of analytes in that group.

During any phase of this procedure, a laboratory may request that EPA provide technical assistance to help identify and resolve any problem.

Once the CA notifies a laboratory, in writing, that it has been downgraded to "provisionally certified" status, the laboratory must correct its problem within 3 months for a procedural or administrative deficiency and 6 months for an equipment deficiency. If the laboratory was downgraded to "provisionally certified" status because of a failure to analyze a PE sample (or other unknown test sample) within the acceptance limits specified by EPA, the laboratory must correct its problems and satisfactorily analyze another PE sample (or other unknown sample) within 2 months of being notified. A "provisionally certified" laboratory may continue to analyze samples for compliance purposes, but must immediately notify its clients of its downgraded status and provide that information, in writing, on any report.

Criteria for Revoking Certification Status

A laboratory will be downgraded immediately from "certified" or "provisionally certified" status to "not certified" for a particular contaminant analysis for the following reasons:

1. (For "provisionally certified" laboratories) Failure to analyze a PE sample (or EMSL-LV intercomparison sample or any other unknown test sample) for a particular contaminant within the acceptance limits established by EPA (see Figure III-1);

2. Failure to satisfy the CA that the laboratory has corrected deviations identified during the on-site evaluations within 3 months for a procedural or administrative deficiency or 6 months for an equipment deficiency;

3. Submission of a PE sample to another laboratory for analysis and reporting data as its own;

4. Falsification of data or other deceptive practices; or

5. Failure to use analytical methodology specified in the regulations.

Procedures for Revocation

The CA will notify the laboratory, in writing (by registered or certified mail), of the intent to revoke certification. If the laboratory wishes to challenge this decision, a notice of appeal must be submitted in writing to the CA within 30 days of receipt of the notice of intent to revoke certification. If no notice of appeal is so filed, certification will be revoked.

The notice of appeal must be supported with an explanation of the reasons for the challenge and must be signed by a responsible official from the laboratory such as the president/owner for a commercial laboratory, or the laboratory supervisor in the case of a municipal laboratory.

Within 60 days of receipt of the appeal, the CA will make a decision and notify the laboratory in writing. Denial of the appeal will result in immediate

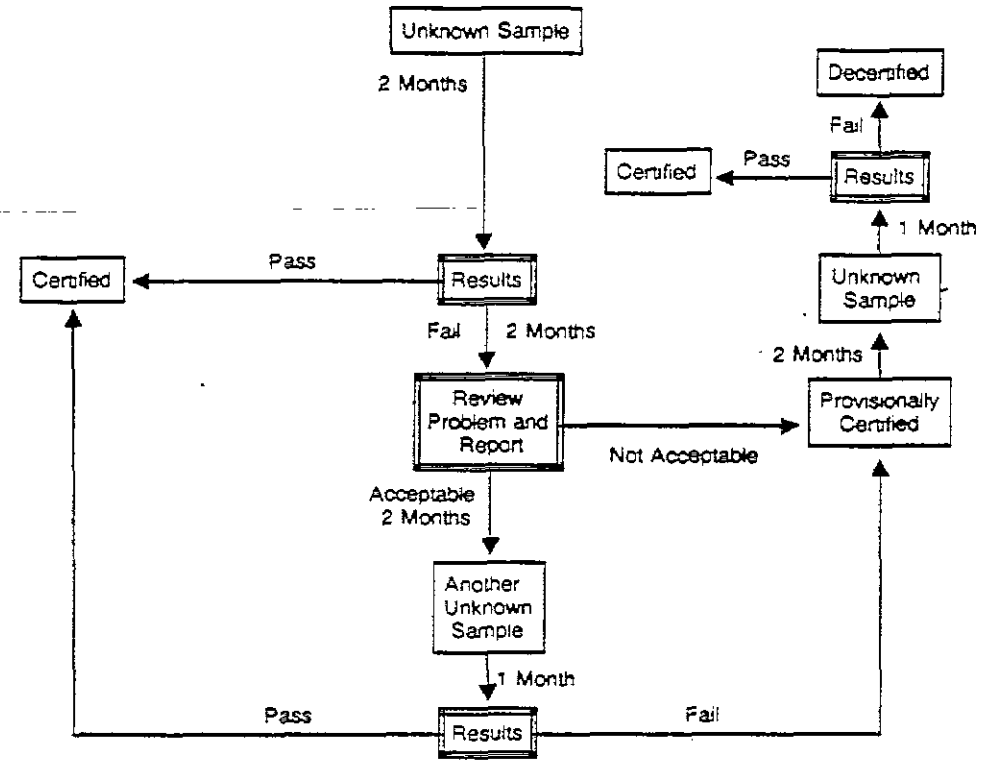

Figure III-1. Criteria and procedures for certification downgrading under the EPA Program on basis of unsatisfactory PE samples.

revocation of the laboratory's certification. Once certification is revoked, a laboratory may not analyze drinking water samples for compliance until its certification has been reinstated.

If the appeal is determined to be valid, the CA will take appropriate measures to reevaluate the facility and notify the laboratory, in writing, of its decision within 60 days of the reevaluation.

Reinstatement of Certification

Certification will be reinstated when and if the laboratory can demonstrate to the CA's satisfaction that the deficiencies which produced "provisionally certified" status or revocation have been corrected. This may include an on-site evaluation, a successful analysis of samples on the next regularly scheduled EMSL water supply performance evaluation study, or any other measure the CA deems appropriate.

Reciprocity

Reciprocity, which is defined as mutually acceptable certification among primacy States, is strongly endorsed by EPA as a highly desirable element in the certification program for drinking water laboratories. The new, more specific certification process should instill greater confidence of comparable performance by laboratories in different jurisdictions. EPA also believes that a third party certifying agent used by more than one State should promote reciprocity. (EPA's policy on third party certification is described in Appendix D.)

States are encouraged to adopt provisions in their laws and regulations to permit reciprocity. Even though ultimate responsibility for reciprocal certification resides with the primacy States, the States may ask for the assistance of EPA in cases involving reciprocity. Such requests should be submitted to ODW through the Region.

Training

Training is an integral part of the laboratory certification process for:

1. Personnel conducting on-site evaluations of laboratories on behalf of either the Regional Office or a primacy State, and
2. Laboratory analysts and samplers responsible for microbiological, chemical and radiochemical measurements.

Each Regional laboratory certification evaluator must initially pass the laboratory certification training course for chemistry or microbiology conducted by EMSL-Cl.

State and third party evaluators (see Appendix D) are encouraged to take these courses. Mechanisms for providing periodic upgrade training for both evaluators and analysts should be examined by the Regions and States. EMSL-CI will notify previous course participants of major updates to their course manual.

Technical Services

Reference Samples

There are four types of EMSL reference samples: calibration standards, quality control (QC), performance evaluation (PE), and intercomparison cross-check samples. EMSL-CI provides QC and PE samples for all regulated chemical and microbiological contaminants and residual chlorine and in addition, provides calibration standards for trace organic chemicals. EMSL-LV provides calibration standards, PE, and intercomparison samples for all regulated radiochemical contaminants. EMSL-CI and EMSL-LV currently provide these samples without charge, but this practice may change in the future.

QC samples and standards are provided on request as part of a laboratory's own quality assurance activities (see section on laboratory quality assurance plans). Contaminant concentrations are furnished with the samples. They serve as independent checks on reagents, instruments, and analytical techniques; as an aid for testing or training analysts; or for determining precision and accuracy within the laboratory. Although no certification or other formal EPA evaluation functions result from using these samples, their routine use is considered fundamental to a proper laboratory QA plan.

EMSL-CI and EMSL-LV conduct periodic water supply performance evaluation studies using PE samples as a requirement for certification. In contrast to QC samples and calibration standards, contaminant concentrations are not furnished before analysis.

At the conclusion of each study, the EMSLs prepare individual reports for each laboratory (indicating data acceptable) on an analyte-by-analyte and sample-by-sample basis and send them to the participants. The certifying authority reviews the data with the laboratory to identify and resolve problems (QC samples and calibration standards are useful for this purpose), and to determine certification status.

In addition to the annual PE sample requirement, EMSL-LV also requires satisfactory performance in two intercomparison studies per year. Intercomparison samples differ from PE samples in that the former contain only one or two radionuclides (e.g., radium-226 and radium-228), while PE samples for radiochemistry are complex mixtures of alpha, beta, and photon-emitting radionuclides. (The one exception is the mixed gamma intercomparison sample, which may contain up to 5 radionuclides.) In neither case are contaminant concentrations furnished to the laboratory until after completion of the study.

Early Warning System for Problems with Test Supplies and Equipment

A voluntary national system has been established to (1) identify potential problems with chemical and microbiological test materials and equipment; (2) notify the EPA, manufacturers, and users of these problems; and (3) encourage improvements and tighter quality control over the products. The problems are concerned with performance, QA, specification, design, and labeling of microbiological media and membrane filters, chemical reagents, and other supplies, equipment, and instrumentation used in microbiological and chemical analyses of drinking water. EMSL-CI has the responsibility for maintaining a QA program on methodologies and test materials, and serves as the focal point for identifying and reporting to the users and the manufacturers significant problems with such materials. The following protocol is used:

1. State and local drinking water laboratories or Regional staff members should report microbiological and chemical problems by phone or in writing to the Microbiology Section (513-569-7319) or the Chemistry Research Division (513-569-7309), respectively, of EMSL-CI, EPA, 26 West Martin Luther King Drive, Cincinnati, Ohio 45268. Forms for written reports are provided in Figures III-2 and III-3. A copy of the report should be sent to the QA officer in the appropriate Region. For radiochemistry problems, send Figure III-3 to the Radioanalysis Branch, EMSL-LV, P.O. Box 93478, Las Vegas, NV 89193-3478; or phone 702-798-2136.

2. EMSL-CI/EMSL-LV will record the details of the problem, including name and location of the reporting laboratory; product type, manufacturers, lot/catalog/model numbers and date received; description of the problem; specific observations; method of preparation, and length and conditions of storage for media or reagents; and data documenting unacceptable test results.

3. EMSL-CI/EMSL-LV will then describe the reported problem to the manufacturer, obtain manufacturing and QA data, and discuss its significance. Corrections or changes by the manufacturer will be encouraged.

4. Based on the results of discussions with the reporter(s) of the problem and manufacturer, EMSL-CI/EMSL-LV will alert the Regional QA Officers of possible problems with the product.

Product* _____ Date _____

Manufacturer _____

Address _____

Date Received _____ Expiration Date _____

Lot No. _____ Cat. No. _____ Model No. _____

Description of Problem:** _____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Name _____ Phone No. _____
(Person Reporting)

Laboratory/Facility _____

Address _____

_____

*Membrane filters, microbiological media, reagents, portable incubators, waterbaths, etc.

**Information should include the length and condition of storage, and the method of preparation for media and reagents. Specific observations, quality control checks, and data that document unacceptable results are useful in describing the problem.

Send to: Microbiology Section, EMSL-CI, U.S. EPA, 26 W. Martin Luther King Drive, Cincinnati, OH 45268, or phone (513) 569-7319.

Figure III-2. Report of problem with microbiological supplies or equipment.

Product* _____ Date _____

Manufacturer _____

Address _____

Date Received _____ Expiration Date _____

Lot No. _____ Cat. No. _____ Model No. _____

Description of Problem:** _____

_____

_____

_____

_____

_____

_____

_____

_____

Name _____ Phone No. _____
(Person Reporting)

Laboratory/Facility _____

Address _____

_____

*Chemicals, prepared reagents, instruments, etc.

**Information should include the length and condition of storage, and the method of preparation for reagents. Specific observations, quality control checks, and data that document unacceptable results are useful in describing the problem.

Send to: Chemistry Research Division, EMSL-CI, U.S. EPA, 26 W. Martin Luther King Drive, Cincinnati, OH 45268, or phone (513) 569-7309.

Figure III-3. Report of problem with chemical supplies or equipment.

The QA Officers will alert the appropriate EPA and State personnel. This system is not intended to label the media, reagents, or other materials as unacceptable, but rather to alert water laboratories that a problem may exist and to determine if similar problems have been observed elsewhere.

5. If multiple reports of the same problem are received, EMSL-CI/EMSL-LV will inform the manufacturer of a potentially broad-scope problem and request samples from reporting laboratories for testing.

6. If the product is unsatisfactory in these tests, EMSL-CI/EMSL-LV will notify the manufacturer and the Regional QA Officers who, in turn, will notify the Regional, State, and local authorities.

Alternate Analytical Techniques

Although the drinking water regulations at 40 CFR 141.27 currently describe approval of limited alternate analytical techniques, EPA no longer uses this procedure and will propose to repeal this regulation. In its place, the Agency is establishing a two-tiered system for rapidly adopting new and revised analytical technology for use by all laboratories. The first tier is for new methods, significantly revised methods, or new applications of currently approved methods. These will be evaluated for equivalency by EMSL and become candidates for accelerated regulation development. Through formal proposal, public comment, and promulgation in the *Federal Register*, the list of methods approved for use by the National Primary Drinking Water Regulations will be amended accordingly, thus making the changes available to all laboratories.

The second tier covers improvements to existing methods which are optional and do not substantially alter the method. These will be evaluated by EMSL and become candidates for inclusion in a *Federal Register* notice which EPA will periodically issue. Rather than formally amending the regulations, this notice will interpret the existing regulatory methods to include minor optional changes. Analysts may use these minor changes or continue to use the method as originally promulgated.

This two-tiered process provides an avenue to evaluate all methodology changes which would have been handled under the old limited alternate test procedures program. The new system makes changes available to all laboratories and provides for a more uniform system for compliance determination.

The process and requirements for obtaining EPA approval for new or revised methods is described in the document, "Requirements for Nationwide Approval of New and Optionally Revised Methods for Drinking Water Monitoring." N. S. Ulmer, Environmental Monitoring Systems Laboratory, Cincinnati, OH 45268. To obtain more specific information, contact EMSL-CI at (513) 569-7453.

Chapter IV
Chemistry

1. Personnel

1.1 Director

A laboratory's volume and scope of services may not require this position. However, there should be a person either in this position or an individual available for consultation meeting the same requirements as the Director. If the Director is also a supervisor, the requirements of paragraph 1.2 are also to be met.

1.1.1 Academic training: Minimum bachelor's degree in science is required. If bachelor's degree is in a field other than chemistry, the individual should have the number of credit hours in chemistry equivalent to a minor in chemistry.

1.1.2 Experience: Minimum of 2 years of experience in a water laboratory is required.

1.2 Supervisor

Minimum requirements for the supervisor position are listed below. If the supervisor is also an instrument operator, the requirements of paragraph 1.3 are also to be met.

1.2.1 Academic training: Bachelor's degree in science that includes the number of credit hours in chemistry courses required for a major in chemistry.

1.2.2 Experience: Minimum of 1 year experience in chemical analysis of water is required.

1.3 Instrument Operators

Operators for the following instruments are needed: Atomic Absorption (AA), Ion Chromatograph (IC), Gas Chromatograph (GC), Gas Chromatograph/Mass Spectrometer (GC/MS), Inductively Coupled Plasma-Atomic Emission Spectrophotometer (ICP-AES), Transmission Electron Microscope (TEM). The following are minimum standards for these analyses.

1.3.1 Academic training: Bachelor's degree in chemistry or related field. The analyst need not have a bachelor's degree if the immediate supervisor has a bachelor's degree in chemistry or related field or if the analyst has the number of credit hours in chemistry courses required for a major in chemistry.

1.3.2 Specialized training: Satisfactory completion of a short course in GC/MS, ICP or TEM offered by equipment manufacturer, professional organization, university, or other qualified training facility is essential for these operators. Specialized training for other instruments is recommended.

1.3.3 Experience: Minimum of six months experience in the operation of either AA, IC, GC, ICP or TEM. Minimum of 12 months experience in the operation of the GC/MS. (See paragraph 1.5.)

1.3.4 Initial qualification: After appropriate training, it is essential that the analyst demonstrate acceptable results in the analysis of an applicable QC or PE sample.

1.4 Other Analysts

The following are required minimum standards for the analyst position.

1.4.1 Academic training: Minimum of a high school diploma or equivalent.

1.4.2 Initial qualification: After being trained in a methods training course or by any qualified analyst, the person being trained shall demonstrate acceptable results in the analysis of an applicable QC or PE sample.

1.5 Analysts and Operators in Training

Data produced by analysts and instrument operators while in the process of obtaining the required training or experience are acceptable when reviewed and validated by a fully qualified analyst or the laboratory supervisor.

1.6 Waiver of Academic Training Requirement

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

2. Laboratory Facilities

The laboratory facilities should be clean, have temperature and humidity adequately controlled in the instrument areas and have adequate lighting at the bench top. It is important for the laboratory to have provisions for the proper storage and disposal of chemical wastes. Exhaust hoods are required for preparation, extraction and analysis where applicable.

It is recommended that a minimum of 150 to 200 square feet/laboratory person be available. The laboratory should contain at least 15 linear feet of usable bench space per analyst. Workbench space should be convenient to sink, water, gas, vacuum and electrical sources free of surges. It is recommended that the organic and inorganic facilities be separate rooms. The analytical and sample storage area is to be isolated from all potential sources of contamination.

3. Laboratory Equipment and Instrumentation

The laboratory is only required to have those instruments that are needed to perform the approved methods for which certification has been requested. Those instruments must meet the specifications in the checklist entitled "Required Equipment and Instruments for Inorganic and Organic Contaminants".

4. General Laboratory Practices

4.1 General

4.1.1 Chemicals/reagents: "Analytical reagent grade" (AR) chemicals or better are to be used for analyses. Consult *Standard Methods for the Examination of Water and Wastewater*, 16th ed., part 102, pp. 4-6 for more detailed information on reagent grades. Individual analytical methods in the approved reference may specify additional requirements for the reagents to be used.

4.1.2 Laboratory safety: While specific safety criteria are not an aspect of laboratory certification, laboratory personnel should apply general and customary safety practices as a part of good laboratory procedure. Each laboratory is strongly encouraged to have a safety plan as part of their standard operating procedure. Where safety practices are included in an approved method, they must be strictly followed.

4.2 Inorganic Contaminants

4.2.1 Reagent water: The laboratory is to have a source of reagent water having a sensitivity value of at least 0.5 megohms (less than 2.0 micromhos/cm) at 25°C. High quality water meeting such specifications may be purchased from commercial suppliers. Quality of reagent water is best maintained by sealing it from the atmosphere. Quality checks to meet specifications above should be made and documented at planned intervals based on use. This planned interval should not exceed one month.

4.2.2 Glassware preparation: Glassware should be washed in a warm detergent solution and thoroughly rinsed first with tap water and then with reagent water. This cleaning procedure is sufficient for general analytical needs, but the individual procedures must be referred to for precautions to be taken against contamination of glassware. It is advantageous to maintain separate sets of suitably prepared glassware for the nitrate, mercury, and lead procedures due to the potential for contamination from the laboratory environment.

4.3 Organic Contaminants

4.3.1 Reagent water: Reagent water for organic analysis is to be free of interferences for the analytes being measured. It may be necessary to treat water with activated carbon to eliminate all interferences.

4.3.2 Glassware preparation: Glassware and sample bottles should be washed in a detergent solution and thoroughly rinsed first in tap water and then in reagent water. Glassware should have a final organic solvent rinse or must be baked at 400°C for 30 minutes and then dried or cooled in an area free of organic contamination. Glassware should be covered with organic-free aluminum foil during storage. Bottles and cap liners, used for collection of samples for determination of volatile organic chemicals (VOCs), should be dried at 105°C for 1 hr, sealed, and stored in an area free of volatile organics.

5. Analytical Methodology

5.1 General

A list of approved methodology for inorganic and organic contaminants can be found in Tables IV-1 and IV-2, respectively. In general, all procedural steps in these methods are considered requirements. Other methods cannot be used unless approved by the Agency. Contact the appropriate certifying authority for an alternate test procedure application. Application for the use of an alternate method may require acceptable comparability data. Prepackaged test kits other than the U.S. EPA-approved DPD and the FACTS Colorimetric Test Kits are not approved for use. Recommended methods for inorganic contaminants that do not require the use of an approved method are listed in Table IV-3.

5.2 *Free Chlorine Residual, Turbidity, pH and Temperature*

Free chlorine residual, turbidity, pH and temperature measurements need not be made in certified laboratories, but may be performed by any persons acceptable to the State. The State should institute a quality assurance program to assure validity of data from these measurements.

5.2.1 Methodology: Only the EPA-approved methodology listed in Table IV-1 can be used for free chlorine residual and turbidity. Recommended procedures for pH and temperature are in Table IV-3.

5.2.2 Sealed liquid turbidity standards purchased from the instrument manufacturer must be calibrated against properly prepared and diluted formazin or styrene divinylbenzene polymer standards at least every 4 months in order to monitor for any eventual deterioration. This calibration is to be documented. These standards are to be replaced when they do not meet the criteria listed in Table IV-6. Solid turbidity standards composed of plastic, glass, or other materials are not reliable and should not be used.

5.2.3 If visual comparison devices such as color wheels or sealed ampules are used for determining free chlorine residual, the standards incorporated into such devices should be calibrated at least every six months. These calibrations are to be documented. Directions for preparing temporary and permanent type visual standards can be found in Method 408E, *Standard Methods*, 16th ed., 1985. By comparing standards and plotting such a comparison on graph paper, a corrective factor can be derived and applied to future results obtained on the now calibrated apparatus.

6. Sample Collection, Handling, and Preservation

The manner in which samples are collected and handled is critical for obtaining valid data. It is essential that a written sampling protocol with specific sampling instructions be available to sample collectors and for inspection by the certification officer (see Appendix A, Chain-of-Custody).

6.1 *Rejection of Samples*

The laboratory is to reject any sample taken for compliance purposes not meeting the criteria in paragraphs 6.2 through 6.6 below and notify the system/individual requesting the analyses.

6.2 *Sample Containers and Preservation*

The type of sample container and the required preservative for each inorganic and organic chemical contaminant are listed in Tables IV-4 and IV-5, respectively.

6.3 *Maximum Holding Times*

Samples must be analyzed within the maximum holding times listed in Tables IV-4 and IV-5.

6.4 *Sample Collection and Transport*

When the laboratory has responsibility for sample collection, handling, and preservation, there needs to be strict adherence to correct sampling procedures, complete identification of the sample, and prompt transfer of the sample to the laboratory.

6.5 *Sample Collector*

The collector should be trained in sampling procedures and approved by the State regulatory authority or its delegated representative.

6.6 *Sample Report Form*

The sample report form should contain the location, date and time of collection, collector's name, preservative added, and any other special remarks concerning the sample. Indelible ink should be used.

7. Quality Assurance

7.1 *General Requirements:*

7.1.1 All quality control information is to be available for inspection by the certification officer.

7.1.2 A manual of analytical methods and the laboratory's QA plan are to be available to the analysts (see Chapter III's discussion of the QA Plan).

7.1.3 Class S Weights or better should be available to make periodic checks on balances. A record of these checks is to be available for inspection. The specific checks and their frequency are to be as prescribed in the laboratory's QA plan and the laboratory's operations manual, if appropriate. This frequency should not exceed one month.

7.1.4 Color standards or their equivalent such as built-in internal standards are to be available to verify wavelength settings on spectrophotometers. A record of these checks should be available for inspection. The specific checks and their frequency are to be as prescribed in the laboratory's QA plan and the laboratory's operations manual, if appropriate. The frequency of these checks should not exceed 6 months.

7.2 *Analytical Quality Control*

The following are necessary for each analyte for which a laboratory is certified:

7.2.1 The laboratory must analyze PE samples (when available) at least annually.

7.2.2 At least once each quarter, the laboratory should analyze a QC sample (EPA QC sample or equivalent). If errors exceed limits specified, corrective action is to be taken and documented, and a follow-up quality control standard analyzed as soon as possible to demonstrate the problem has been corrected.

7.2.3 At the beginning of each day that samples are to be analyzed, a standard curve composed of at least a reagent blank and three standards covering the sample concentration range are to be prepared. These standards should be from a source different than the quality control standard used for paragraph 7.2.2.

7.2.4 Calibration for some methods is so time-consuming that paragraph 7.2.3 is impractical. For these methods, the standard curve is to be initially developed as specified in paragraph 7.2.3. Thereafter, at the beginning of each day on which analyses are performed, this curve is to be verified by analysis of at least a reagent blank and one standard in the expected concentration range of the samples analyzed that day. All checks should be within the control limits specified in paragraph 7.2.7 or the system recalibrated as specified in paragraph 7.2.3.

7.2.5 If the reagent blank specified in paragraph 7.2.3 (or paragraph 7.2.4) is not carried through the full analytical procedure, then some other blank (at least one per day) is to be carried through the entire analytical procedure. Results from reagent blanks should not exceed the laboratory's method detection limit (MDL); see paragraph 7.2.8.

7.2.6 The laboratory should add a known spike to a minimum of 10% of the routine samples (except when the method specifies a different percentage, i.e., furnace methods) to determine if the entire analytical system is in control. The spike concentration should not be substantially less than the background concentration of the sample selected for spiking. These checks should be evenly spaced and one check should be at the end of the day's analyses. Over time, samples from all routine sample sources should be spiked. If any of these checks are not within the control limits specified in paragraph 7.2.7, a standard should be analyzed to determine if the "out of control" condition was due to sample matrix or system operation. This standard is to be analyzed through the complete analytical system. Corrective action is to be taken in accordance with the laboratory's QA plan.

7.2.7 Until sufficient data are available from the laboratory, usually a minimum of 15 to 25 test results on a specific analysis, the laboratory is to use the control limits, if available, developed from the mean (X) and standard deviation (S) relationships in Table IV-6. This Table was derived from EPA's PE sample data. After inserting the analytical concentration (c), including the background concentration (B) wherever appropriate, into the proper pair of relationships, compute control limits for standards as $X \pm 3(S)$ and for spike recoveries as $(X-B) \pm 3(S)$. As sufficient data become available, the laboratory should develop traditional QC chart criteria for the various QC checks specified above (see Chapter 6 of the *Handbook for Analytical QA in Water and Wastewater Laboratories*, EPA-600/4-79-019, or similar QC reference texts for further information). Since percent recovery may not be a constant, the percent recovery data may have to be separated into concentration intervals before control limits are calculated for each interval. If any of these control limits are tighter than the matching control limits developed from the relationships in Table IV-6, the laboratory shall use the tighter criteria. Otherwise, control limits calculated from the relationships in Table IV-6 are required. The laboratory should continue to calculate traditional control limits for each analyte as additional results become available.

7.2.8 It is further recommended that the laboratory periodically determine the MDL in accordance with the procedure given in 40 CFR Part 136, Appendix B. This procedure is available from EPA, Environmental Monitoring Systems Laboratory, 26 W. Martin Luther King Drive, Cincinnati, Ohio 45268.

8. Records and Data Reporting

8.1 Laboratory Records

Records of chemical analyses are to be kept by the laboratory for a minimum of 3 years. This includes all raw data, calculations, and quality control data. These data files may be either manual or computer based. The following information may be available as a sample data report or summary record:

8.1.1 Date, place, time of sampling, preservative added and name of person who collected the sample.

8.1.2 Identification of sample as to whether it is a routine distribution system sample, check

sample, raw or finished water sample, or other special purpose sample.

8.1.3 Date of receipt of sample and date of analysis.

8.1.4 Laboratory and person(s) responsible for performing analysis.

8.1.5 Analytical technique/method used, and quality control data.

8.1.6 Result of analysis.

9. Action Response to Laboratory Results

When the action response is a designated laboratory responsibility, the laboratory must notify the proper authority of noncompliance sample results and request resampling from the same sampling point immediately.

Table IV-1. Approved Methodology for Inorganic Contaminants

Contaminant	MCL mg/L	Methodology[5]	Reference (Method Number) EPA[1]	ASTM[2]	SM[3]	Other
Arsenic	0.05	Atomic Absorption: furnace	206.2			
		: gaseous hydride	206.3	D2972-78B	301A VII	I-1062-78[4]
		Spectrophotometric: Silver Diethyl-dithiocarbamate	206.4	D2972-78A	404A after B(4)	
		Inductively Coupled Plasma	200.7A			
Barium	1	Atomic Absorption: direct aspiration	208.1		301A-IV	
		: furnace	208.2			
		Inductively Coupled Plasma	200.7A			
Cadmium	0.01	Atomic Absorption: direct aspiration	213.1	D3557-78A or B	301A-II or III	
		: furnace	213.2			
		Inductively Coupled Plasma	200.7A			
Chromium	0.05	Atomic Absorption: direct aspiration	218.1	D1687-77D	301A-II or III	
		: furnace	218.2			
		Inductively Coupled Plasma	200.7A			
Fluoride	4	Colorimetric SPADNS, with distillation	340.1	D1179-72A	413C and A[6]	
		Potentiometric ion selective electrode	340.2	D1179-72B	413B[6]	
		Automated Alizarin fluoride blue, with distillation	340.3		413E[6]	129-71W[7]
		Automated ion selective electrode				380-75WE[8]
Lead	0.05	Atomic Absorption: direct aspiration	239.1	D3559-78A or B	301A-II or III	
		: furnace	239.2			
		Inductively Coupled Plasma	200.7A			
Mercury	0.002	Manual cold vapor technique	245.1	D3223-79	301A-VI	
		Automated cold vapor technique	245.2			
Nitrate-N	10.0	Manual cadmium reduction	353.3	D3867-79B	419C	
		Automated hydrazine reduction	353.1			
		Automated cadmium reduction	353.2	D3867-79A	605	
		Ion selective electrode				WeWWG/5880[9]
		Colorimetric Brucine	352.1	D992-71	419D	
		Ion Chromatography	300.0			B1011[10]
Residual Disinfectant						
Chlorine		Amperometric Titration			408C[6]	
		Ferrous Titrimetric Method			408D[6]	
		DPD Colorimetric Method			408E[6]	
		Leuco Crystal Violet Method			408F[6]	
Ozone		Indigo Method				Note [11]
Chlorine Dioxide		Amperometric Method			410B[6]	
		DPD Colorimetric Method			410C[6]	
Selenium	0.01	Atomic Absorption: furnace	270.2			
		: gaseous hydride	270.3	D3859-79	301A-VII	I-1667-78[4]
Silver	0.05	Atomic Absorption: direct aspiration	272.1		301A-II	
		: furnace	272.2			
		Inductively Coupled Plasma	200.7A			
Sodium		Atomic Absorption: direct aspiration	273.1			
		: furnace	273.2			
		Flame Photometric		D1428-64A	320A	
Turbidity		Nephelometric	180.1		214A[6]	

[1] "Methods of Chemical Analysis of Water and Wastes," EPA Environmental Monitoring and Systems Laboratory, Cincinnati, Ohio 45268 (EPA-600/4-79-020). March 1979. Available from ORD Publications, CERI, EPA, Cincinnati, Ohio 45268.

[2] "Annual Book of ASTM Standards," Part 31 Water, American Society for Testing and Materials. 1978, 1916 Race Street, Philadelphia, PA 19103.

[3] "Standard Methods for the Examination of Water and Wastewater," 14th Ed., American Public Health Association; American Water Works Association; Water Pollution Control Federation; 1975.

[4] "Techniques of Water Resources Investigation of the United States Geological Survey, "Chapter A-1, "Methods for the Determination of Inorganics Substances in Water and Fluvial Sediments," Book 5 (1979, Stock #024-001-03177-9). Available from the Superintendent of Documents, US Government Printing Office, Washington, DC 20402.

[5] For approved analytical procedures for metals the technique applicable to total metals must be used.

[6] "Standard Methods for the Examination of Water and Wastewater," American Public Health Association et al., 16th Ed., 1985

[7] "Fluoride in Water and Wastewater," Industrial Method 129-71W, "Technicon Industrial Systems, Tarrytown, NY 10591, December 1972.

[8] "Fluoride in Water and Wastewater," Technicon Industrial Systems, Tarrytown, NY 10591, February 1976.

[9] "Orion Guide to Water and Wastewater Analysis." Form WeWWG/5880, pp 5, 1985. Orion Research Inc., Boston, MA 02129.

[10] "The Determination of Nitrite and Nitrate in Water Using Single Column Ion Chromatography," method B-1011, Millipore Corp., Waters Chromatography Division, 34 Maple Street, Milford, MA 01754.

[11] "Determination of Ozone in Water by the Indigo Method," A Submitted Standard Method; Ozone Science and Engineering, Vol. 4, pp 169-176. Pergamon Press Ltd., 1982.

Table IV-2. Approved Methodology for Organic Contaminants

Contaminant	MCL ug/L	Methodology	Reference (Method Number or Page Numbers) EPA[1]	ASTM[2]	SM[3]	USGS[4]
Chlorinated hydrocarbons[5]		Solvent extraction, gas chromatography	pp. 1-19	D3086-85	509A	0-3104-83
endrin	0.2					
lindane	.4					
methoxychlor	100					
toxaphene	5					
Chlorophenoxys		Solvent extraction, derivatization gas chromatography	pp. 20-35	D3478-85	509B	0-3105-83
2,4-D	100					
2,4,5-TP	10					
Total Trihalomethanes (TTHM)	100	Purge and trap, gas chromatography	6			
		Solvent extraction, gas chromatography	7			
		Gas chromatography/mass spectrometry	8,9			
Maximum Trihalomethane Potential (MTP)		TTHM after incubation	10			
Volatile Organic Contaminants (VOC)		Purge and trap, gas chromatography	502.1[11]			
			502.2[11]			
			503.1[11]			
			524.1[11]			
Regulated						
benzene	5	Gas chromatography/mass spectrometry	524.2[11]			
carbon tetrachloride	5					
p-dichlorobenzene	75					
1,2-dichloroethane	5					
1,1-dichloroethylene	7					
1,1,1-trichloroethane	200					
trichloroethylene	5					
vinyl chloride	2					
Unregulated[12]		Solvent extraction	504[11]			
		Purge and trap, gas chromatography	502.1[11]			
			502.2[11]			
			503.1[11]			
		Gas chromatography/mass spectrometry	524.1[11]			
			524.2[11]			

[1] "Methods for Organochlorine Pesticides and Chlorophenoxy Acid Herbicides in Drinking Water and Raw Source Water," Available from ORD Publications, CERI, EPA, Cincinnati, Ohio 45268.

[2] "Annual Book of ASTM Standards," Volume 11.02, American Society for Testing and Materials. 1916 Race Street, Philadelphia, PA 19103.

[3] "Standard Methods for the Examination of Water and Wastewater," 14th Ed., American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1975.

[4] U.S. Geological Survey Techniques of Water—Resources Investigations, Chapter A3, "Methods for the Determination of Organic Substances in Water and Fluvial Sediments," Book 5, 1983. Available from: Open File Service Section, Western Distribution Branch, Box 25425, Federal Center, Denver, CO 80225.

[5] These analytes may be extracted using Bakers Solid Phase Extraction procedure as referenced in the Nation Wide Approval in FR 2-19-88, Vol. 53, No. 33, pp. 5142.

[6] "The Analysis of Trihalomethanes in Finished Waters by the Purge and Trap Method," Method 501.1, EMSL, EPA, Cincinnati, Ohio 45268.

[7] "The Analysis of Trihalomethanes in Drinking Water by Liquid/Liquid Extraction," Method 501.2, EMSL, EPA, Cincinnati, Ohio 45268.

[8] "Measurement of Trihalomethanes in Drinking Water by Gas Chromatography/Mass Spectrometry and Selected Ion Monitoring," Method 501.3, EMSL, EPA, Cincinnati, Ohio 45268.

[9] "Measurement of Purgeable Organic Compounds in Drinking Water by Gas Chromatography/Mass Spectrometry," Method 524, EMSL, EPA, Cincinnati, Ohio 45268.

[10] 40 CFR 141.30(e)(2).

[11] "Methods for the Determination of Organic Compounds in Finished Drinking Water and Raw Source Water," September, 1986, EMSL, EPA, Cincinnati, Ohio 45268.

[12] The complete list of unregulated volatile organic chemicals can be found in 40 CFR part 141.40.

Table IV-3. Recommended Methods for Inorganic Contaminants

Contaminant	Methodology	Reference (Method Number) EPA[1]	ASTM[2]	SM[3]	Others
Alkalinity	Titrimetric or Potentiometric	310.1	D1067-70B	403	I-1030-84[5]
Calcium[4]	EDTA titrimetric	215.2	D511-84A	311C	
	Atomic absorption: direct aspiration	215.1	D511-84B	303A	
	Inductively coupled plasma	200.7A			
Chloride	Potentiometric			407C	
	Ion chromatography	300.0	D4327	429	A-1000[6]
Copper	Atomic absorption: furnace technique	220.2		304	
	:direct aspiration	220.1	D1688-84D or E	303A or B	
	Inductively coupled plasma	200.7A			
Corrosivity	Langelier Index			203[7]	
	Aggressive Index				C400-80[8]
Nitrite	Spectrophotometric	354.1			
	Automated cadmium reduction	353.2	D3867-85A	418F	
	Manual cadmium reduction	353.3	D3867-85B	418C	
	Ion chromatography	300.0			B-1011[9]
pH	Potentiometric	150.1	D1293-78A or B	423	
Residue, total dissolved	Gravimetric	160.1		209B	I-1750-84[5]
Sulfate	Turbidimetric	375.4	D516-82A		
	Ion chromatography	300.0	D4327	429	A-1000[6]
Temperature	Thermometric			212	

[1] "Methods of Chemical Analysis of Water and Wastes," EPA, Environmental Monitoring and Systems Laboratory, Cincinnati, Ohio 45268 (EPA-600/4-79-020) March 1979. Available from ORD Publications, CERI, EPA, Cincinnati, Ohio 45268.

[2] "Annual Book of ASTM Standards," Volume 11.01, American Society for Testing and Materials. 1916 Race Street, Philadelphia, PA 19103.

[3] "Standard Methods for the Examination of Water and Wastewater," 16th Ed., American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1985.

[4] For approved analytical procedures for metals, the technique applicable to total metals must be used..

[5] "Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments," Techniques of Water-Resources Investigation of the United States Geological Survey Books, Chapter A1, 1985, Open file report 85-495. Available from Open-File Services Section, Western Distribution Branch, US Geological Survey, MS 306, Box 24525, Denver, CO 80225.

[6] "Conductivity Detection of Anions Using Single Column Chromatography," Method A-1000, Millipore Corp., Waters Chromatography Division, 34 Maple Street, Milford, MA 01754.

[7] "Standard Methods for the Examination of Water and Wastewater," 14th Ed., American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1975.

[8] "AWWA Standard for Asbestos-Cement Pipe, 4 in. through 16 in. for Water and Other Liquids," AWWA C400-80, Revision of C400-77, AWWA, Denver, CO.

[9] "The Determination of Nitrite and Nitrate in Water Using Single Column Ion Chromatography," Method B1011, Millipore Corp., Waters Chromatography Division, Milford, MA 01754.

Table IV-4. Sample Collection, Containers, and Preservation for Inorganic Contaminants[1,2]

Contaminant	Preservative[3]	Container[4]	Maximum Holding Time[5]
Alkalinity	Cool, 4 °C	P or G	14 days
Arsenic	Conc HNO_3 to pH < 2	P or G	6 months
Asbestos	Cool 4 °C[6]	P or G	
Barium	Conc HNO_3 to pH < 2	P or G	6 months
Cadmium	Conc HNO_3 to pH < 2	P or G	6 months
Calcium	Conc HNO_3 to pH < 2	P or G	6 months
Chloride	None	P or G	28 days
Chromium	Conc HNO_3 to pH < 2	P or G	6 months
Copper	Conc HNO_3 to pH < 2	P or G	6 months
Fluoride	None	P	28 days
Free Chlorine Residual	None	P or G	Analyze immediately[7]
Lead	Conc HNO_3 to pH < 2	P or G	6 months
Mercury	Conc HNO_3 to pH < 2	P or G	28 days
Nitrate			
Chlorinated	Cool 4 °C	P or G	28 days
Non-chlorinated	Conc H_2SO_4 to pH < 2	P or G	14 days[8]
Nitrite	Cool 4 °C	P or G	48 hours
pH	None	P or G	Analyze immediately[7]
Selenium	Conc HNO_3 to pH < 2	P or G	6 months
Silver	Conc HNO_3 to pH < 2	P or G	6 months
Sodium	Conc HNO_3 to pH < 2	P or G	6 months
Sulfate	Cool 4 °C	P or G	28 days
Temperature	None	P or G	Analyze immediately[7]
Total Dissolved Residue	Cool 4 °C	P or G	7 days
Turbidity	Cool 4 °C	P or G	48 hours

[1] The laboratory director must reject any samples, taken for compliance purposes, not meeting these criteria and notify the authority requesting the analysis.

[2] Other holding times can be obtained through alternate approval.

[3] If HNO_3 cannot be used because of shipping restrictions, sample for analysis of metals may be initially preserved by icing and immediately shipping it to the laboratory. Upon receipt in the laboratory, the sample must be acidified with conc. HNO_3 to pH < 2. At the time of analysis, the sample container should be thoroughly rinsed with 1:1 HNO_3; washings should be added to the sample. A volume correction for these washings must be made.

[4] P = plastic, hard or soft; G = glass, hard or soft.

[5] In all cases, samples should be analyzed as soon after collection as possible.

[6] These samples should never be frozen.

[7] "Analyze immediately" generally means within 15 minutes of sample collection.

[8] Ion chromatographic methods using conductivity as the detector cannot be used.

Table IV-5. Sample Collection, Containers, and Preservation for Organic Contaminants[1]

Contaminants	Preservative	Container	Maximum Holding Time[2]
Chlorinated hydrocarbons	Refrigerate at 4°C as soon as possible after collection	Glass with foil or Teflon-lined cap	14 days
Chlorophenoxys	Refrigerate at 4°C as soon as possible after collection.	Glass with foil or Teflon-lined cap.	7 days[3]
TTHMs	Ascorbic acid and 6N HCl	Glass with Teflon-lined septum	14 days
VOCs	HCL to pH < 2, Cool 4°C	Glass with Teflon-lined septum	14 days

[1] If a laboratory has no control over these factors, the laboratory director must reject any samples not meeting these criteria and notify the authority requesting the analyses.
[2] In all cases, samples should be analyzed as soon after collection as possible.
[3] Well-stoppered and refrigerated extracts can be held up to 30 days.

Table IV-6. Background for Development of Control Limits for the Required Quality Control Program (See 7.2.7)

Analyte	Units	Application Concentration Range	Estimate of Concentration c: Mean	Estimate of Concentration c: Standard Deviation
Arsenic	ug/L	3.56 to 106	0.982(c)-0.10	0.0693(c) + 0.28
Barium	ug/L	41 to 938	0.974(c) + 0.52	0.0504(c) + 1.93
Cadmium	ug/L	1.6 to 42	0.972(c) + 0.14	0.0682(c) + 0.12
Chromium	ug/L	12.7 to 127	0.997(c) + 0.11	0.0567(c) + 0.63
Lead	ug/L	3.2 to 109	0.999(c) + 0.24	0.0647(c) + 0.59
Mercury	ug/L	0.72 to 7.5	0.972(c)	0.0858(c) + 0.06
Selenium	ug/L	9.71 to 86.9	0.993(c)-0.11	0.0985(c) + 0.15
Silver	ug/L	3.42 to 103	0.994(c) + 0.20	0.0585(c) + 0.29
Nitrate-N	mg/L	0.35 to 8.5	1.008(c) + 0.01	0.0810(c) + 0.03
Fluoride	mg/L	0.18 to 2.0	0.988(c) + 0.01	0.0290(c) + 0.01
Endrin	ug/L	0.13 to 6.7	0.971(c)	0.138(c)
Lindane	ug/L	0.12 to 5.8	0.949(c)	0.163(c) + 0.01
Methoxychlor	ug/L	1.96 to 95	0.927(c) + 0.14	0.149(c) + 0.03
Toxaphene	ug/L	1.42 to 12.8	0.968(c)-0.05	0.152(c) + 0.15
2,4-D	ug/L	1.79 to 89.6	0.874(c) + 0.14	0.230(c) + 0.13
2,4,5-TP	ug/L	1.20 to 73.1	0.862(c) + 0.01	0.238(c) - 0.05
Chloroform	ug/L	9.06 to 81.5	0.980(c) + 0.30	0.0814(c) + 0.55
Bromoform	ug/L	12.3 to 84.3	1.008(c) + 0.49	0.109(c) + 0.33
Bromodichloromethane	ug/L	11.1 to 75.1	1.000(c)-0.23	0.106(c) + 0.03
Dibromochloromethane	ug/L	7.66 to 80.5	1.004(c)-0.17	0.111(c) + 0.16
Residual Free Chlorine[1]	mg/L	0.38 to 1.8	0.974(c) + 0.02	0.0295(c) + 0.09
Turbidity[1]	NTU	0.35 to 5.0	0.946(c) + 0.07	0.0517(c) + 0.05
Total Dissolved Residue	mg/L	100 to 610	1.027(c)-1.79	0.0874(c) + 4.03
Calcium, as $CaCO_3$	mg/L	0.90 to 103	1.002(c) + 0.32	0.0443(c) + 0.16
pH[1]	units	4.00 to 9.2	0.987(c) + 0.07	0.0147(c) - 0.04
Alkalinity, as $CaCO_3$	mg/L	4.97 to 110	0.976(c) + 0.84	0.0133(c) + 1.10
Langelier Index, 20°C[1]	units	0.74 to 1.0	1.045(c)-0.04	0.0036(c) + 0.15
Sodium	mg/L	7.58 to 95	0.988(c) + 0.20	0.0396(c) + 0.15

[1]Not amenable to spiking procedure

Sample Forms for On-Site Evaluation of Laboratories Involved in Analysis of Public Water Supplies—Chemistry

Laboratory _____

Street _____

City _____ State _____

Telephone Number _____

Survey by _____

Affiliation _____

Date _____

Codes for Marking On-Site Evaluation Forms

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

Laboratory _____ Evaluator _____

Location _____ Date _____

Personnel _____

Position/Title	Name	Education Level Degree—Major*	Specialized Training	Present Speciality	Experience
Lab Director Manager					
Supervisor					
Instrument Operator					
AA					
TEM					
GC					
ICP					
GC/MS					
IC					
Other Analysts					

*If the major is not in chemistry, list hours of college level courses in chemistry.

Laboratory_____ Evaluator_____

Location_____ Date_____

Laboratory Equipment and Instruments for Inorganic and Organic Contaminants

Item	No. of Units	EPA Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
-ANALYTICAL BALANCE: 0.1 mg sensitivity Stable base Class S weights Service contracts						
MAGNETIC STIRRER: Variable speed TFE coated stir bar						
pH METER: ± 0.05 units Readability ± 0.1 units Line or battery Usable with specific ion electrodes						
CONDUCTIVITY METER: Readable in ohms or mhos Range of 2 ohms to 2 megohms Line or battery						
HOT PLATE: Temp. control						
CENTRIFUGE: To 3000 rpm Option of 4 x 50 mL						
COLOR STANDARDS: To verify wavelengths on photometers Should cover 200 to 800 nm						
REFRIGERATOR: Standard laboratory Explosion proof for organic storage						
DRYING OVEN: Gravity or convection Controlled from room to 180°C or higher (±2°C) To 400°C for cleaning organic glass						

Laboratory Equipment and Instruments for Inorganic and Organic Contaminants (Continued)

Item	No. of Units	EPA Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
THERMOMETER: Mercury-filled celsius 1°C or finer subdivision To 180°C Certified by or traceable to NBS						
GLASSWARE: Borosilicate Class A volumetric						
SPECTROPHOTOMETER: Range 400 to 700 nm Band width—not greater than 20 nm Use several size and shape cells Path length 1 to 5 cm		206.4-340.1-340.3 245.1-254.2-352.1 353.3-353.2-353.1 409E or F-408G&E 375.4-410B&C				
FILTER PHOTOMETER: Range 400 to 700 nm Band width 10 to 70 nm Use several size and shape cells Path length 1 to 5 cm		Same as above				
SPECIFIC ION METER: Readable & accurate to ± 1 mV ELECTRODES: As needed		340.2				
INDUCTIVELY COUPLED PLASMA: Computer control Background coordination Radio frequency generator Argon gas supply		200.7-200.7A				
WATER BATH: Electric or steamed heat Heat to 100°C Controllable within 5°C		245.1-352.1 Pesticides				
ION CHROMATOGRAPH: Conductivity detector Suppressor column Separator column U.V. detector		300.0 300.0 300.0-B1011 B-1011				

Laboratory Equipment and Instruments for Inorganic and Organic Contaminants (Continued)

Item	No. of Units	EPA Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
AMPEROMETRIC TITRATOR		408-C				
ATOMIC ABSORPTION SPECTROPHOTOMETER: Single channel Single or double beam Grating monochrometer Photomultiplier detector Adjustable slits Range 190 to 800 nm		208.1 206.2 213.1 208.2 218.1 213.2 239.1 218.2 272.1 239.2 215.1 270.2 273.1 272.2 206.3 273.2 270.3				
Readout system: Response time compatible with AA Able to detect positive interference for furnace Chart recorder, CRT, or hardcopy printer		Same as above				
Fuel and oxidant: Commercial grade Acetylene Air Reagent grade nitrous oxide		208.1-239.1 213.1 272.1 215.1 273.1 218.1				
Commercial grade argon or nitrogen (furnace) Hydrogen (hydride)		206.2 218.2 272.2 208.2 239.2 273.2 213.2 270.2 206.3 270.3				
Burner: Recommended by manufacturer for the above gases		See Atomic Absorption				
Hollow cathode lamps: Single element preferred Multiple element acceptable EDLs acceptable Graphite furnace: Any that will reach temps required		See Atomic Absorption 206.2 208.2 213.2 218.2 239.2 270.2 272.2 273.2				
Background corrector: Required for furnace Provision for off-line analysis Hydride generator		See Atomic Absorption 206.3 270.3				

Laboratory Equipment and Instruments for Inorganic and Organic Contaminants (Continued)

Item	No. of Units	EPA Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
AUTOMATED ANALYSES SYSTEM:		340.3-353.1-353.2 380-75WE				
Sampler		340.3-353.1-353.2				
Proportioning pump		380-75WB				
Manifold or cartridge		340.3-353.1-353.2 380-75WE				
Heating bath		353.1				
Bath with distilling head		413E (Std Methds)				
Continuous filter		340.3-353.1				
Colorimeter		340.3-353.1-353.2				
ISE detector		380-75WE				
Recorder		340.3-353.1-353.2 380-75WE				
MERCURY ANALYZER: Spectrophotometer Dedicated mercury analyzer acceptable Having a mercury hollow cathode lamp		245.1-245.2				
Absorption Cell: 10 cm quartz cell with quartz end windows or 11.5 cm plexiglass cell with I.D. of 2.5 cm		245.1-245.2				
Air Pump: To deliver flow of at least 1 L per minute		245.1-245.2				
Aeration tube: With coarse glass frit		245.1-245.2				
Flowmeter: To measure air flow of 1 L per minute		245.1-245.2				
Drying Unit: 6-inch tube with 20 g magnesium Perchlorate or Heating device		245.1-245.2				

Laboratory Equipment and Instruments for Inorganic and Organic Contaminants (Continued)

Item	No. of Units	EPA Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
PIPETS AND TIPS: Microliter capacity with disposable tips Sizes—5 to 100 microliters Tips should be metal-free		See graphite furnace method list				
GLASSWARE: Separatory Funnels Kuderna Danish (K-D) concentrators Water bath for K-D		Organochlorine Pesticides Chlorophenoxys				
ARSINE GENERATOR: A Gutzeit generator or equivalent		206.4				
GAS CHROMATOGRAPH: ±0.2°C oven Temperature control Recorder, hardcopy Oven temperature programmer		All All 501.1 502.1 502.2 503.1 504 524.1 524.2				
GC Detectors Linearized electron capture or equivalent Electrolytic conductivity Photoionization		Pesticides Chlorophenoxys 501.2 501.1 502.1 502.2 503.1				
Mass Spectrometer: Electron-impact ionization (70eV nominal) All-glass enrichment device All-glass transfer line		501.3 524 524.1 524.2				
Software to acquire and manipulate data for only a few ions		501.3 524 524.1 524.2				
Purge and trap system		501.1 501.3 502.1 502.2 503.1 524 524.1 524.2				

Laboratory _____ Evaluator _____

Location _____ Date _____

Methodology

Contaminant	Name or Description of Method	Reference (Cite Source and Method by Number or Page and Year)	Sample Load Per Month	Satisfactory	
				Yes	No
Inorganic Arsenic					
Barium					
Cadmium					
Chromium					
Fluoride					
Lead					
Mercury					
Nitrate					
Selenium					
Silver					
Organic Chlorinated Hydrocarbons					
Chlorophenoxys					
TTHM					
MTP					
VOC					

Laboratory _____ Evaluator _____

Location _____ Date _____

Sample Handling and Preservation

Contaminant	Container Used (Material and Size)	Preservative Used	Maximum Holding Time	Satisfactory	
				Yes	No
Inorganic Arsenic					
Barium					
Cadmium					
Chromium					
Fluoride					
Lead					
Mercury					
Nitrate					
Selenium					
Silver					
Organic Chlorinated Hydrocarbons					
Chlorophenoxys					
TTHM					
MTP					
VOC					

Laboratory_____ Evaluator_____

Location_____ Date_____

Sample Collection

Item	Comments	Satisfactory Yes	Satisfactory No
General Trained Sample Collector			
Representative sampling			
Complete sample form			
Inorganic Appropriate sampling and preservation			
Overaged samples discarded			
Organic Appropriate sampling and preservation			
TTHM Stabilizer added to same bottle in laboratory prior to shipment to site or at time of sample collection.			
TTHM Hermetic seal			
Overaged samples discarded			

Laboratory _____ Evaluator _____

Location _____ Date _____

Quality Assurance and Data Reporting

Item	Comments	Satisfactory Yes	Satisfactory No
QA plan and data			
Annual performance samples analyzed			
Methods manual available			
Records kept 3 years			
pH meter calibration			
10% spiked samples			
Check sample with each group of 20 samples			
Daily method blank			
Daily Calibration			
Quarterly QC samples or Daily calibration check			
Organic TTHM/VOCs field blanks			
10% TTHM/VOCs in duplicate			
TTHM/VOCs control standards			
TTHM/VOCs startup test			
Source water blank check			
BFB tuning check			

Chapter V
Microbiology

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

1. Personnel

1.1 Supervisor/Consultant

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

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

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

1.2 Analyst (or equivalent job title)

The analyst performs microbiological tests with minimal supervision.

1.2.1 Academic training: Minimum of high school education.

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

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

2. Laboratory Facilities

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

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

3. Laboratory Equipment and Supplies

A laboratory may request or contract with another certified laboratory to conduct specified quality control testing, e.g., testing the quality of laboratory pure water (paragraph 4.3.2 in this chapter); calibration of non-reference weights (paragraph 3.2.2 in this chapter); and calibration of temperature monitoring devices (paragraph 3.3.2 in this chapter). The laboratory conducting the actual quality control test(s) is to be certified for microbiology and provide copies of quality control data to the requesting laboratory. Therefore, the requesting laboratory is not necessarily required to have equipment, supplies, and materials to conduct specified quality control tests.

3.1 pH Meter

3.1.1 Accuracy and scale graduations within ±0.1 units.

3.1.2 Use pH buffer aliquot only once.

3.1.3 Maintain electrodes according to manufacturer's recommendations.

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

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

3.2 *Balance (top loader or pan)*

3.2.1 Balance detects 100 mg at a 150 gram load.

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

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

3.3 *Temperature Monitoring Device*

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

QC **3.3.2** Check calibration of in-use glass/mercury thermometers annually and in-use dial thermometer quarterly, at the temperature used, against a reference National Institute of Standards and Technology (formerly National Bureau of Standards) (NBS) thermometer or one that meets the requirements of NBS Monograph 150.

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

3.4 *Incubator Unit*

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

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

3.5 *Autoclave*

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

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

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

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

QC **3.5.5** Check automatic timing mechanism with stopwatch quarterly.

3.6 *Hot Air Oven*

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

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

3.7 *Colony Counter*

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

3.8 *Conductivity Meter*

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

QC **3.8.1** Conductivity meter is calibrated monthly with a 0.01 M KCl solution (See Method 120.1 in *Methods for Chemical Analyses of Water and Wastes*, 1979, EPA 600/4-79-020 (revised 1983); or Section 205, "Conductvity", pp. 76-80, in *Standard Methods for the Examination of Water and Wastewater* (16th ed.), 1985).

3.9 *Refrigerator*

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

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

3.10 *Inoculating Equipment*

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

3.11 Membrane Filtration Equipment (if MF procedure is used)

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

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

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

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

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

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

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

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

QC **3.11.5** Check sterility of each lot number of membranes by placing one membrane in 50 mL volume of non-selective broth medium (e.g., tryptic soy broth) and check for growth after 24 hours incubation at 35° ± 0.5°C.

3.12 Culture Dishes (loose or tight lid)

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

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

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

3.13 Pipets

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

3.13.2 Pipets have legible markings and are not chipped nor etched.

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

3.14 Culture Tubes and Closures

3.14.1 Tubes are made of borosilicate glass or other corrosion-resistant glass.

3.14.2 Culture tubes used for Presumptive Test in the Multiple Tube Fermentation Technique (MPN) are of a sufficient size to contain medium plus sample without being more than three quarters full.

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

3.15 Sample Containers

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

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

3.16 Glassware and Plasticware

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

3.16.2 Graduated cylinders for measurement of sample volumes have a tolerance of 2.5% or less.

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

4. General Laboratory Practices

4.1 Sterilization Procedures

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

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

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

4.1.3 Membrane filter equipment is autoclaved at the start of the first filtration series of each day and after each filtration series. A filtration series ends when 30 minutes or longer elapse between individual sample filtration.

4.1.4 Membrane filter assemblies may be exposed to UV irradiation (germicidal lamp, 2537 angstroms) or submerged in boiling water for approximately two minutes if bacterial carry-over between individual sample filtration becomes a problem. (Filter assemblies submerged in boiling water are cooled to room temperature before filtering sample.)

4.2 Sample Containers

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

QC **4.2.2** Select at least one sample container at random from each batch of sterile sample bottles, or other EPA-approved containers, and confirm sterility by adding approximately a 25 mL volume of a sterile non-selective broth (e.g., tryptic soy, trypticase soy, or tryptone broth). Incubate at 35° ± 0.5°C for 24 hours and check for growth.

4.3 Reagent Water

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

QC **4.3.2** Test the quality of the reagent water or have it tested by a certified laboratory to assure it meets the criteria in the table below.

4.4 Dilution/Rinse Water

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

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

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

QC **4.4.4** Check each batch of dilution/rinse water for sterility by adding 50 mL of water to a 50 mL of a double strength non-selective broth (e.g., tryptic soy, trypticase soy or tryptose broth).

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

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

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

4.5 Glassware Washing

4.5.1 Use distilled or deionized water for final rinse.

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

4.6 Media—General Requirements

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

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

QC **4.6.3** For media prepared in the laboratory, record the date of preparation, type of medium, lot number, sterilization time and temperature, final pH, technician's initials.

QC **4.6.4** For liquid media prepared commercially, record date received, type of medium, lot number, and pH verification. Discard medium by manufacturer's expiration date.

4.7 Membrane Filter (MF) Media (needed only if laboratory conducts MF procedure)

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

4.7.2 Refrigerate MF broth no longer than 96 hours, poured MF agar plates no longer than 2 weeks, and ampouled m-Endo broth in accordance with manufacturer's expiration date.

4.8 Multiple Tube Fermentation Technique (MPN or MTF) Media

4.8.1 Double strength lauryl tryptose broth or lactose broth is used in the Presumptive Test and single strength brilliant green lactose bile (BGLB) broth in the Confirmed Test. Dispense broth medium volume of not less than 10 mL per tube and autoclave media at 121° C for 12-15 minutes. Final pH 6.8 ± 0.2 (7.2 ± 0.2 for BGLB broth).

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

4.8.3 Use m-Endo agar, m-Endo LES agar, or Levine Eosin Methylene Blue (EMB) agar for the Completed Test although the m-Endo LES agar is the medium of choice. Dissolve, using a sterile flask, in a boiling water bath (or direct heat if constantly attended) to bring medium just to the boiling point. Do not autoclave. Final pH 7.2 ± 0.2. Medium may be stored refrigerated for two weeks. If EMB agar is used for

Completed Test, either dissolve in a sterile flask using a boiling water bath (or direct heat if constantly attended) and bring medium to boiling point or autoclave medium at 121°C for 12-15 minutes. Final pH 7.1 ± 0.2. Use non-autoclaved medium on day of preparation; do not store. Refrigerate autoclaved medium and use within two weeks.

4.9 Heterotrophic Plate Count (HPC) Medium

Autoclave HPC agar at 121°C for 15 minutes, depending upon volume. Final pH 7.0 ± 0.2. Temper melted agar at 44°-46°C before pouring. Hold melted agar no longer than 8 hours. Do not melt sterile agar medium more than once.

5. Analytical Methodology

Note: on 12/31/90, significant changes will be made in this section to conform with the requirements of the revised total coliform rule.

5.1 EPA Approval

Approved analytical methodology is specified in the National Primary Drinking Water Regulations. Alternate methods must have EPA approval.

5.2 MF Procedure

5.2.1 Shake sample vigorously before analyzing. Sample volumes analyzed by the MF procedure must be 100 mL ± 2.5 mL.

5.2.2 Confluent growth is defined as bacterial growth with or without sheen covering the entire membrane filter. TNTC (too numerous to count) is defined as greater than 200 total bacterial colonies on the membrane filter.

5.2.3 Samples resulting in confluent growth or TNTC with less than five distinguishable sheen colonies are invalid. Record as "confluent growth" or "TNTC" with the number of discernable sheen colonies and request an additional sample from the same sampling site.

5.2.4 Samples resulting in confluent growth or TNTC with five or more distinguishable sheen colonies may be a MCL violation. Report as "confluent growth" or "TNTC" with the number of distinguishable sheen colonies.

5.2.5 Verify all sheen colonies for all unsatisfactory samples (>4 colonies/100 mL) regardless of the amount of sheen when the number of the sheen colonies is 5 or more up to 10/100 mL. When the number of sheen colonies exceeds 10/100 mL, randomly pick 10 colonies for verification.

5.2.6 Verify sheen colonies using either single strength lactose or LTB and then single strength BGLB media (same media used in MPN procedure), or EPA-approved cytochrome oxidase and β-galactosidase rapid test procedure.

5.2.7 Adjust initial counts based only upon verification data.

QC **5.2.8** Conduct MF sterility check at the beginning and the end of each filtration series. If controls indicate contamination, reject all data from affected samples and request immediate resampling.

QC **5.2.9** Laboratories which conduct the MF procedure and have two or more analysts should analyze one known coliform-positive sample monthly and each analyst should count the sheen colonies on the same membrane. The sheen colony counts should agree within 10%.

5.3 MPN Procedure

5.3.1 Conduct MPN Completed Test, quarterly, on not less than 10% of all unsatisfactory samples (> three positive confirmed tubes). Gram-staining is optional for potable water samples.

5.3.2 For unsatisfactory samples, adjust the number of positive confirmed tubes on the basis of the Completed Test.

5.3.3 If the MPN test is used on water supplies that have a history of confluent growth or TNTC by the MF procedure, all presumptive tubes with heavy growth without gas production are submitted to the Confirmed Test to check for coliform suppression.

QC **5.3.4** If no positive tubes result from potable water samples, perform the MPN procedure, quarterly, on a known coliform-positive sample. Confirm the positive presumptive tubes and perform the Completed Test on all positive confirmed tubes.

5.4 Minimal Medium ONPG-MUG (MMO-MUG) Test

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

QC **5.4.2** Check each lot of medium with a total coliform-positive control (e.g., *Klebsiella*

pneumonia) and a total coliform-negative control (e.g., *Pseudomonas aeruginosa*).

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

5.4.4 After incubation for 24 hours, if the sample color is indeterminate using a reference comparator, reincubate for another four hours (up to but not more than 28 hours). If the sample color remains indeterminate, the laboratory should consider the sample invalid and request another sample from the same site.

QC **5.4.5** Laboratories are strongly encouraged to perform parallel testing between the MMO-MUG Test and another EPA- approved procedure for enumerating total coliforms for at least several months and/or over several seasons to assess the effectiveness of the MMO-MUG Test for the wide variety of water types submitted for analysis.

5.5 HPC Procedure

5.5.1 Use the pour plate method to determine the HPC for potable water samples.

5.5.2 For most potable water samples, countable plates can be obtained by plating 1.0 mL or 0.1 mL volume of the undiluted sample.

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

5.5.4 Count colonies manually using a counting aid such as a Quebec colony counter. Consider only plates having 30 to 300 colonies in determining plate count, except for plates inoculated with 1.0 mL volume of undiluted sample. Counts less than 30 for such plates are acceptable. (Fully automatic colony counters are not suitable because of the size and small number of colonies observed when potable water is analyzed for HPC.)

5.5.5 Check each batch of HPC agar for sterility by pouring initial and final control plates. Reject data if controls are contaminated.

6. Sample Collection, Handling, and Preservation

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

6.1 Sample Collector

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

6.2 Sampling

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

6.3 Sample Icing

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

6.4 Sample Holding/Travel Time

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

6.5 Report Form

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

6.6 Chain-of-Custody

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

7. Quality Assurance

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

8. Records and Data Reporting

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

- Date, place, and time of sampling, name of persons who collected the sample.
- Identification of sample as to whether it is a routine distribution system sample, check sample, raw or process water sample, or other special purpose sample.
- Date and time of sample receipt and analysis.
- Laboratory and persons responsible for performing analysis.
- Analytical technique/method used
- Results of analysis. Base results of coliform analyses on data from Confirmed Test or Completed Test (for MPN Technique). Base MF results on initial counts or verified counts.

9. Action Response to Laboratory Results

9.1 Notification of Authorities

Promptly notify the proper authorities of unsatisfactory results on the basis of Confirmed Test (for MPN Technique) or unverified MF coliform data.

9.2 Adjustments in Coliform Counts

Although check sampling is to be initiated on the basis of MPN Confirmed Test and unverified MF coliform counts, data used to determine monthly compliance may be adjusted by using the MPN Completed Test and/or verified MF results.

9.3 High Concentrations of Non-Coliform Organisms

Alert proper authorities to the occurrence of high background levels of non-coliform organisms observed by the MF procedure, or turbid tubes lacking gas using the MPN procedure.

Laboratory _____

Street _____

City _____ State _____

Telephone Number _____

Survey by _____

Affiliation _____

Date _____

Codes for Marking On-Site Evaluation Forms

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

1. Personnel

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

2. Laboratory Facilities

Laboratory facilities clean, temperature and humidity controlled _____

Adequate lighting at bench top _____

Laboratory has provision for disposal of microbiological wastes _____

3. Laboratory Equipment, Supplies, and Materials

3.1 pH Meter

Manufacturer _____ Model _____

Accuracy ± 0.1 units _____

Scale graduation, 0.1 units _____

Maintains electrodes according to manufacturer's recommendations _____

pH buffer solution aliquots used only once _____

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

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

3.2 Balances (Top Loader or Pan)

Manufacturer _____ Model _____

Detects 100 mg at a 150 gram load _____

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

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

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

3.3 Temperature Monitoring Device

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

No separation in mercury column _____

QC Check calibration of glass/mercury thermometers annually and dial thermometers quarterly at the temperature used against a reference NBS thermometer or one meeting the requirements of NBS Monograph 150 _____

QC Recalibrate continuous recording devices used to monitor incubator temperature annually against a NBS thermometer or one meeting the requirements of NBS Monograph 150 _____

3.4 Incubator Unit

Manufacturer_____ Model_____

Maintains internal temperature of 35° ± 0.5°C _____

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

Immerse thermometer bulb in liquid _____

Culture dishes and tubes fit snugly in aluminum block incubator _____

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

3.5 Autoclave

Manufacturer_____ Model_____

Temperature gauge with sensor on exhaust _____

Operational safety valve _____

Maintains sterilization temperature during cycle _____

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

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

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

QC Establish service contract or internal maintenance protocol _____

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

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

3.6 Hot Air Oven

Manufacturer_____ Model_____

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

Thermometer graduated in no more than 10°C increments _____

Place thermometer bulb in sand _____

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

3.7 Colony Counter

Manufacturer_____ Model_____

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

3.8 Conductivity Meter

Manufacturer_____ Model_____

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

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

3.9 Refrigerator(s)

Manufacturer_____ Model_____

Maintains temperatures of 1° to 5°C _____

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

Thermometer bulb(s) immersed in liquid _____

QC Temperature recorded for days in use _____

3.10 Inoculating Equipment

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

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

3.11 Membrane Filtration Equipment, Membrane Filters and Pads

Manufacturer_____ Model_____

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

Units do not leak, not scratched or corroded _____

10 to 15X magnification device with fluorescent light source _____

Forcep tips without corrugations _____

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

Alternate pore size used _____

Membrane filters recommended by manufacturer for total coliform analysis _____

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

QC Record lot numbers of membrane filters and date received _____

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

3.12 Culture dishes

Use presterilized plastic or sterilized glass dishes _____

Incubate loose-lid dishes in a tight fitting container _____

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

Reseal open packs of disposable culture dishes between uses _____

3.13 Pipets

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

Reseal packs of disposable sterile pipets between major use periods _____

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

3.14 Culture Tubes and Closures

Tubes are borosilicate glass or other corrosion-resistant glass _____

Culture tubes are of sufficient size that medium plus sample does not exceed 3/4 full _____

Closures are stainless steel, plastic, aluminum, or screw caps with non-toxic liner _____

3.15 Sample Containers

Capacity at least 120 mL (4 oz) _____

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

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

3.16 Glassware and Plasticware

Glass made of borosilicate or other corrosion-resistant glass _____

Free of chips and cracks _____

Graduation marks are legible _____

Plastic items are clear and non-toxic _____

Graduated cylinders used to measure sample volume have a 2.5% tolerance or less _____

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

4. General Laboratory Practices

4.1 Autoclave Sterilization Procedures at 121°C

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

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

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

4.2 Sample Containers

Stock 10% sodium thiosulfate solution free of turbidity _____

Add sodium thiosulfate to sample containers prior to sterilization _____

Sterilized sampling bags contain sodium thiosulfate _____

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

4.3 Reagent Water

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

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

Parameter	Limits	Frequency
Conductivity	> 0.5 megohms or < 2 micromhos at 25°C	monthly

Metals—Pb , Cd, Cr, Cu, Ni, Zn	Not greater than 0.05 mg/L per contaminant. Collectively not greater than 0.1 mg/L	annually	_____
Total chlorine residual	None detected	monthly	_____
Heterotrophic Plate Count	< 500/mL	monthly	_____
Bacteriological quality of reagent water	Ratio 0.8-3.0	annually	_____

4.4 Dilution/Rinse Water

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

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

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

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

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

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

QC pH of peptone water is 6.8 ± 0.2 _____

QC Check dilution/rinse water for sterility _____

4.5 Glassware Washing

Use distilled or deionized water for final rinse _____

QC Perform inhibitory residue test on clean glassware _____

4.6 Media (General Requirements)

Commercially available dehydrated or prepared media used _____

Dehydrated media stored in cool, dry location _____

"Caked" or discolored dehydrated media discarded _____

Date dehydrated media when received and when initially opened _____

Discard dehydrated media that has passed the manufacturer's expiration date _____

Discard opened dehydrated media after 6 months; if stored in a desiccator, storage is extended to 12 months _____

QC Media Preparation Records include:

(a) Date of preparation _____

(b) Type of media _____

(c) Lot number _____

(d) Sterilization time and temperature _____

(e) Final pH _____

(f) Technician's initials _____

4.7 Membrane Filter Media

M-Endo or M-Endo LES broth or agar, final pH 7.2 ± 0.2 _____

Dissolution of m-Endo broth or agar and m-Endo agar LES:

(a) Boiling water bath _____

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

Prepare and store media in sterile flasks _____

Use only 95% ethanol, not denatured _____

Refrigerate membrane filter broth no longer than 96 hours _____

Refrigerate membrane filter poured agar plates no longer than 2 weeks _____

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

4.8 Multiple Tube Fermentation (MPN or MTF) Technique Media

Lauryl tryptose (lauryl sulfate) broth _____

Lactose broth _____

Dispense broth medium in volumes not less than 10 mL/tube _____

Use MPN media in tubes with loose-fitting closures within one week _____

Store MPN media in screw cap tubes no longer than three months; discard if evaporation exceeds 10% of original volume _____

Overnight incubation at 35°C of refrigerated sterilized MPN media _____

Lauryl tryptose (lauryl sulfate) broth:

Autoclave at 121°C for 12-15 minutes double strength; final pH 6.8 ± 0.2 _____

Lactose broth:

Autoclave at 121°C for 12-15 minutes, double strength; final pH 6.7 ± 0.2 _____

Brilliant green lactose bile broth:

Autoclave at 121°C for 12-15 minutes; final pH 7.2 ± 0.2 _____

Levine's Eosin Methylene Blue (EMB) agar (Completed Test):

Autoclave at 121°C for 12-15 minutes (store refrigerated two weeks) or use boiling water bath or direct heat for dissolution (use same day); final pH 7.1 ± 0.2 _____

m-Endo LES agar (Completed Test)

Prepare medium in a sterile flask using boiling water bath or direct heat to boiling point; final pH 7.2 ± 0.2 _____

4.9 Heterotrophic Plate Count (HPC) Medium

Temper melted agar (44° - 46°C) before pouring _____

Melted agar held no longer than 8 hours _____

Do not melt sterile medium more than once _____

Autoclave at 121°C for 15 minutes, time adjusted depending on volume _____

Final pH 7.0 ± 0.2 _____

5. Analytical Methodology

5.1 Approved methods used as referenced in 40 CFR 141 "National Primary Drinking Water Regulations." Alternate methods, if applicable, have EPA approval

5.2 Membrane Filter Technique

Filter funnels and receptacle sterile at start of series _____

Shake sample vigorously _____

Examine 100 mL ± 2.5 mL of sample _____

Rinse funnel by flushing several 20 to 30 - mL portions of sterile buffered water through membrane filter _____

Remove MF with a sterile forceps, grasping the area outside the effective filtering area _____

Roll MF onto medium pad or agar so air bubbles are not formed _____

Incubation Conditions:

Total incubation time 22 to 24 hours at 35° ± 0.5°C _____

Incubate in high humidity or in tight fitting culture dishes _____

Colony Counting:

Fluorescent light positioned for maximum reflection of colonies with sheen _____

Colonies uniformly dispersed over effective filtration area _____

Coliforms reported as coliform number per 100 mL

Confluent growth—membrane covered with bacterial growth; TNTC—greater than 200 total bacterial colonies _____

if reported as confluent growth or TNTC with less than 5 coliforms, request another sample from same sampling site _____

if reported as confluent growth or TNTC with 5 or more coliforms, request check samples _____

Verification procedure conducted on all unsatisfactory samples (>4 colonies/100 mL) _____

Use lactose broth or lauryl tryptose broth and confirm by BGLB media or EPA-approved rapid test _____

Adjust initial counts based on verification _____

QC Conduct MF sterility check at beginning and end of each filtration series _____

QC Analysts agree within 10% on the number of sheen colonies on same membrane filter _____

5.3 Total Coliform Multiple-Tube Fermentation Technique _____

Total Coliform Presumptive Phase

Five standard portions, either 10 or 100 mL _____

Sample shaken vigorously before test _____

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

Examined for gas (any size bubble) _____

24-hour gas-positive tube submitted to confirmed phase _____

Negative tubes returned to incubator _____

Examined for gas at 48 ± 3 hours; positive tubes submitted to confirmed phase _____

Total Coliform Confirmed Phase

Presumptive positive tubes shaken gently or mixed by rotating _____

One loopful or one dip of applicator transferred from presumptive positive tube to BGLB broth _____

Incubated at 35° ± 0.5°C; checked at 24 hours for gas production _____

Negative tubes reincubated for additional 24 hours; checked for gas production _____

Results recorded; MPN value calculated _____

Total Coliform Completed Test

Completed Test conducted quarterly on not less than 10% of all unsatisfactory samples (≥ three positive confirmed tubes) _____

Positive confirmed tubes streaked on m-Endo, m-Endo LES, or EMB agar plates for colony isolation _____

Incubated at 35° ± 0.5°C for 24 ± 2 hours _____

Growth from coliform colonies inoculated into lactose or LTB medium, incubated at 35° ± 0.5°C and observed for gas production within 48 hours _____

Adjust the number of positive confirmed tubes on the basis of the Completed Test _____

5.4 Minimal Medium ONPG-MUG (MMO-MUG) Test

When using bulk medium, each analysis or series of analyses includes a control consisting of test tube with MMO-MUG medium to which sterile water has been added _____

Each lot of medium checked with a total coliform-positive control and a total coliform-negative control _____

Tubes incubated at 35° ± 0.5°C for 24 hours and examined for production of yellow color _____

If test is indeterminate after 24 hours, the sample is reincubated for another 4 hours (up to but not more than 28 hours) _____

If sample color is indeterminate after 28 hours, sample is invalidated _____

Parallel testing between MMO-MUG Test and another EPA-approved procedure for enumerating total coliforms conducted for several months _____

5.5 Heterotrophic Plate Count (HPC) Procedure

Pour plate method used to determine HPC _____

Shake sample vigorously _____

Volume plated is between 0.1 mL and 1.0 mL _____

Add agar, tempered to 44°-46°C, and mix agar and sample _____

Incubate plates in inverted position at 35° ± 0.5°C for 48 ± 3 hours _____

	Do not stack plates more than four high	
	Count colonies using a Quebec colony counter	
	Count only plates in countable range, 30-300 colonies	
QC	Perform sterility check by pouring an initial and final control plate for each container and/or batch of HPC agar	

6. Sample Collection, Handling, and Preservation

6.1	Follow sample procedures described in *Standard Methods for the Examination of Water and Wastewater* or *Microbiological Methods for Monitoring the Environment*, U.S. EPA-600/8-78-017	
6.2	Sample collectors receive training	
6.3	Samples representative of distribution system	
6.4	Water taps free of any attachments and mixing type faucets	
6.5	Water run to waste for at least two minutes	
6.6	Sample volume is at least 100 mL with sufficient space for mixing sample	
6.7	Sample report form completed by collector	
6.8	Samples iced when carrying samples directly to laboratory	
6.9	Record date and time of sample arrival at laboratory and date and time analysis begins	
6.10	Transit time does not exceed 30 hours	
	If laboratory is required by State regulation to examine samples after 30 hours and up to 48 hours, data are indicated as possibly invalid	
	All samples arriving in laboratory after 48 hours are not analyzed for compliance use	
6.11	Compliance with State chain-of-custody regulations, if required	

7. Quality Assurance Program

7.1	Written QA Plan implemented and available for review	
7.2	Quality control records maintained for five years	
QC 7.3	PE sample is satisfactorily analyzed annually (if available)	

8. Data Reporting

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

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

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

Results of analyses

MPN data based on Confirmed or Completed Test and MF data based on initial or verified counts _____

9. Action Response by Laboratory

9.1 Notify the responsible authorities of unsatisfactory results _____

9.2 Notify responsible authorities of check sample results _____

9.3 Alert responsible authorities to high non-coliform levels in sample _____

Chapter VI
Radiochemistry

1. Personnel

1.1 Measurement of Gross Alpha and Gross Beta

Analyst or technician responsible only for the measurement of gross alpha and gross beta radioactivities.

1.1.1 Academic training: Minimum of a high school diploma or its equivalent, plus specialized training in standards and sample preparation, instrument calibration, calculations, and data handling.

1.1.2 Experience: Minimum of 6 months of on-the-job.

1.1.3 A technician may assist in routine sample preparation and radioanalytical procedures provided that such work is supervised and validated by an analyst with qualifications as described in section 1.2.

1.2 Measurement of Specific Radionuclides

Analyst responsible for the measurement of specific radionuclides described in the National Primary Drinking Water Regulations (NPDWR).

1.2.1 Academic training: Minimum of bachelor's degree in chemistry, radiochemistry, radioisotope technology, or equivalent.

1.2.2 Experience[1]: Minimum of 1 year of appropriate experience in radiation measurements and radiochemical procedures.

1.3 Laboratory Supervisor, Manager, or Director

1.3.1 Academic training: Minimum of bachelor's degree or its equivalent.

1.3.2 Experience[1]: Minimum of 5 years of experience.

2. Laboratory Facilities

2.1 General

The analysis of compliance monitoring samples should be conducted in a laboratory facility where security and integrity of the drinking water samples and analytical data are provided. In addition, a work-place for wet chemistry operations and for equipment that is critical to valid measurement of radioactive contaminants is necessary.

2.2 Location of Instruments

The counting instrument(s) necessary for measurement of those radionuclides described in the NPDWR must be located in a room other than the one in which samples and standards are being prepared and in which other types of wet chemical analyses are being performed. All instruments should be properly grounded, and a regulated power supply, either external or internal, should be available to each instrument.

2.3 Preparation of Standards

In areas where radioactive standards are being prepared, care must be taken to minimize contamination of surfaces, other samples and personnel. Either bench surfaces of an impervious material covered with adsorbent paper, or trays (stainless steel, plastic, or fiberglass) lined with adsorbent paper are acceptable.

2.4 Laboratory Fixtures

The following items are necessary in a laboratory performing even the most basic radiochemical measurements (gross alpha and gross beta radioactivities) for compliance monitoring of drinking water supplies.

2.4.1 Sink with tap water and connection to the sanitary sewer system.

[1] Each year of college-level training in related scientific fields of demonstrated equivalency shall be considered equal to 1 year of work experience. Such a substitution should not exceed one-half of the required experience.

2.4.2 Electrical outlets (120V AC grounded).

2.4.3 Source of distilled or deionized water.

2.4.4 Exhaust hood.

2.4.5 For laboratories that are performing wet chemistry separations that require filtration of a precipitated fraction of the sample, a vacuum source (pump or aspirator) should also be available.

3. Laboratory Equipment and Supplies

The following equipment and supplies are necessary for the analyses of regulated radionuclides. If a laboratory is not to be certified for a particular radionuclide parameter, instruments specified for analysis of that parameter are not necessary.

3.1 General Instrumentation and Equipment

3.1.1 Analytical balance: Precision, ± 0.1 mg. Minimum scale readability, 0.1 mg.

3.1.2 pH meter or specific ion meter:

3.1.2.1 pH meter: Accuracy, ± 0.5 units. Scale readability, ± 0.1 units.

3.1.2.2 Specific ion meter: Expanded scale millivolt capability. Readable and accurate to ± 0.1 mV.

3.1.3 Drying oven or lamp: Gravity convection type, or infrared drying lamp.

3.1.4 Desiccator: Glass or plastic models, depending on particular application.

3.1.5 Hot plate: Units with selectable temperature control for safe heating of laboratory reagents and samples.

3.1.6 Glassware: Borosilicate type glass. All volumetric glassware should be marked Class A, denoting that it meets Federal specifications and need not be calibrated before use.

3.1.7 Muffle furnace: Automatically controlled with a chamber capacity of at least 2,200 cc (10 x 9.5 x 23) and a maximum operating temperature of 1,000°C continuous and 1,100°C intermittent.

3.1.8 Centrifuge: General purpose table-top model with a maximum speed of at least 3,000 rpm and a loading option of 4 x 50 ml.

3.2 Radiation Counting Instruments

The types of radiation counting systems needed to comply with measurements described in the NPDWR are set forth below:

3.2.1 Liquid scintillation system: A liquid scintillation system is essential if the laboratory is to be certified for the measurement of tritium and/or radon in drinking water samples. The system needs to be such that the sensitivity will meet or exceed the requirements of section 141.25 of the NPDWR.

3.2.2 Gas-flow proportional counting system: A gas-flow proportional counting system may be used for the measurement of gross alpha and gross beta activities, radium-226, radium-228, strontium-89, strontium-90, cesium-134, and iodine-131 as described in the reference cited in section 141.25(a). The detector may be either a "windowless" (internal proportional counter) or a "thin window" type. A combination of shielding and a cosmic (guard) detector operated in anticoincidence with the main detector should be used to achieve low background beta counting capability. The alpha and beta background count of the system should be such that the sensitivity of the radioanalysis of water samples will meet or exceed the requirement of 40 CFR 141.25 with reasonable counting time (not more than 1,000 minutes).

3.2.3 Alpha scintillation counting system: For measurement of gross alpha activities and radium-226, a scintillation system designed for alpha counting may be substituted for the gas-flow proportional counter described. In such a system, a Mylar disc coated with a phosphor (silver-activated zinc sulfide) is either placed directly on the sample or on the face of a photo-multiplier tube, enclosed within a light-tight container, along with the appropriate electronics (high voltage supply, preamplifier, amplifier, timer and scaler).

3.2.4 Low background alpha and beta counting systems other than a gas-flow proportional counting system: Such a system should have a cosmic guard detector operated in anticoincidence with the signal from the sample detector, and shielding, such that the alpha background will not exceed 0.2 cpm and the beta background will not exceed 2.0 cpm for a 2 inch diameter counting planchet geometry.

3.2.5 Scintillation cell system: A scintillation system designed to accept scintillation flasks ("Lucas cells") should be used for the specific measurement of radium-226 by the radon

emanation method. The system consists of a light-tight enclosure capable of accepting the scintillation flasks, a detector (phototube), and the appropriate electronics (high voltage supply, amplifier, timers, and scalers). The flasks (cells) needed for this measurement may either be purchased from commercial suppliers or constructed by the laboratory.

3.2.6 Gamma spectrometer systems: Either a sodium iodide, NaI(Tl) crystal; a solid state lithium drifted germanium detector; or a gamma-X photon detector connected to a multichannel analyzer is needed if the laboratory is to be certified for analyses of manmade photon emitters.

3.2.6.1 If a sodium iodide detector is used, a cylindrical 7.5 cm x 7.5 cm NaI crystal is satisfactory. However, a 10 cm x 10 cm crystal is recommended. The detector should be shielded with a minimum of 10 cm of iron or equivalent. It is recommended that the distance from the center of the detector to any part of the shield should not be less than 30 cm. The multichannel analyzer, in addition to appropriate electronics, should contain a memory of not less than 200 channels and at least one readout device.

3.2.6.2 A system with a lithium drifted germanium, or a high purity germanium, or a gamma-X photon detector may be used for measurement of manmade photon emitters if the efficiency of the detector is such that the sensitivity of the system meets the minimum detectable activity requirements cited in 40 CFR 141.25. These detectors should be shielded with a minimum of 10 cm of iron or equivalent. The multichannel analyzer, in addition to appropriate electronics, should contain a memory of not less than 2,000 channels and at least one readout device.

4. General Laboratory Practices

(None specified)

5. Analytical Methodology

The approved methods indicated in the NPDWR or EPA-approved alternate methods, are to be used for drinking water compliance monitoring.

6. Sample Collection, Handling, and Preservation

Table VI-1 gives critical elements for sample handling including Preservation, and Applicable Counting Instrumentation.

7. Quality Assurance

7.1 Inspections

Quality control data and records are to be available for inspection.

7.2 Intercomparison Cross Check Studies

A laboratory needs to participate at least twice each year in those EPA laboratory intercomparison cross check studies that include each of the analyses for which the laboratory is, or wants to be, certified. Analytical results should be within control limits described in "Environmental Radioactivity Laboratory Intercomparison Studies Program -- FY 1981-1982" (EPA- 600/4-81-004), or in subsequent revisions.

7.3 Performance Evaluation Studies

A laboratory also needs to participate once each year in an appropriate water supply performance evaluation (blind sample) study administered by EPA. Analytical results must be within control limits established by EPA for each analysis for which the laboratory is, or wants to be, certified.

7.4 Operating Manuals

Operating manuals and calibration protocols for counting instruments should be available to analysts and technicians.

7.5 Maintenance of Records

Calibration data and maintenance records on all radiation instruments and analytical balances should be maintained in a permanently bound record.

7.6 Daily Quality Control

The following specifications are included in minimum daily quality control:

7.6.1 A minimum of 10-percent duplicate samples should be analyzed to verify internal laboratory precision for a specific analysis. The difference between duplicate measurements should be less than two times the standard deviation of the specific analysis as described in EPA-600/4-81-004, Table 3. If difference exceeds two standard deviations, prior measurements are suspect; calculations and procedures should be examined and samples should be reanalyzed when necessary.

7.6.2 When 20 or more specific analyses are performed each day, a counting standard and a background sample should be measured with each 20 samples. If less than 20 specific

Table VI-1: Sample Handling, Preservation, and Instrumentation

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

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

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

[3] A = Low background proportional system; B = Alpha scintillation system; C = Gamma spectrometer [NaI(Tl) or Ge(Li)]; D = Scintillation cell (radon) system; E = Liquid scintillation system (section C.2.a); F = Fluorometer (section C.1.i); G = Low background alpha and beta counting system other than gas-flow proportional.

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

analyses are performed in any 1 day, a counting standard and a background sample should be measured along with the samples.

7.7 Instrument Performance Charts/Records

Quality control performance charts, or performance records, should be maintained for each instrument.

7.8 QA Plan

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

8. Records and Data Reporting

8.1 Legal Defensibility

Compliance monitoring activities should be made legally defensible by the records kept of such activities.

8.2 Retention of Records

Records of radioanalyses for compliance monitoring of drinking water supplies are to be kept by the laboratory for not less than three years. This includes raw data, calculations, quality control data, and reports.

8.3 Information to be Recorded

Actual laboratory reports may be kept, or data may be transferred to tabular summaries provided that the following information is included:

8.3.1 Date, place, and time of sampling; name of person who collected the sample.

8.3.2 Identification of sample as to whether it is a routine distribution system sample, check sample, raw or process water sample, surface or ground water sample, or other special purpose sample.

8.3.3 Date of sample receipt and analysis.

8.3.4 Laboratory and persons responsible for performing analysis.

8.3.5 Analytical technique/method used.

8.3.6 Results of analysis.

8.4 Computer Programs

Computer programs should be verified initially by manual calculations and the calculations should be available for inspection.

9. Action Response to Laboratory Results

When action response is a designated laboratory responsibility, the proper authority must be notified promptly of noncompliance sample results, and a request must be made for resampling from the same sampling point.

Sample Forms for On-Site Evaluation of Laboratories Involved in Analysis of Public Water Supplies—Radiochemistry

Laboratory _____

Street _____

City _____ State _____

Survey By _____

Affiliation _____

Date _____ Telephone No. _____

Laboratory _____ Evaluator _____

Location _____ Date _____

Personnel

Position/title	Name	Academic training HS	BA/BS	MA/MS	Ph.D.	Present Specialty	Experience (years and area)
Analyst(s)/ technician(s)							
Supervisory analyst							
Laboratory supervisor/ director							
Support (e.g., electronic technician)							

Laboratory _____ Evaluator _____

Location _____ Date _____

Laboratory Facilities

Item	Available Yes	Available No	Comments
Laboratory Sink – with tap water and sanitary sewer connections			
Electrical outlets – 120V ac, grounded			
Distilled or deionized water			
Exhaust hood			
Vacuum source			
Counting Room Separate from wet chemistry, sample and standards preparation area			
Regulated power supply			
Adequate electrical ground			

Laboratory _____ Evaluator _____

Location _____ Date _____

General Laboratory Equipment and Instruments

Item	No. of Units	Manufacturer	Model	Age and Condition
Analytical balance				
pH meter				
Specific ion meter				
Conductivity meter				
Drying oven				
Infrared lamp				
Desiccator				
Hot plate				
Muffle furnace				
Centrifuge				
Fluorometer				

Laboratory _____ Date _____

Location _____ Evaluator _____

Thin Window Gas-Flow Proportional Counter

Instrument number	Manufacturer	Model	Year	Sample changing: Manual	Sample changing: Automatic	Capacity

	Counting gas	Window density (g/cm²)	Instrument background: Alpha Operating voltage	Alpha cpm	Beta Operating voltage	Beta cpm
Calibration Standard Type: Alpha Beta						

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

[1]Daily, weekly, monthly.
[2]Quarterly, semiannually, annually.
[3]Good operating but needs repair, not operating.

Are operating manuals readily available to the operator? Yes ☐ No ☐
Are calibration protocols available to the operator? Yes ☐ No ☐
Are calibrations kept in a permanent control chart record? Yes ☐ No ☐
Are permanent service maintenance records kept on these systems? Yes ☐ No ☐

Windowless Gas-Flow Proportional Counter

Instrument number	Manufacturer	Model	Year	Sample changing: Manual	Sample changing: Automatic	Capacity

	Counting gas	Sample dish diameter (in)	Instrument background: Alpha Operating voltage	Alpha cpm	Beta Operating voltage	Beta cpm
Calibration Standard Type: Alpha Beta						

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

[1]Daily, weekly, monthly.
[2]Quarterly, semiannually, annually.
[3]Good operating but needs repair, not operating.

Are operating manuals readily available to the operator? Yes ☐ No ☐
Are calibration protocols available to the operator? Yes ☐ No ☐
Are calibrations kept in a permanent control chart record? Yes ☐ No ☐
Are permanent service maintenance records kept on these systems? Yes ☐ No ☐

Laboratory _____ Date _____

Location _____ Evaluator _____

Low Background Alpha and Beta Counter (other than gas-flow proportional)

Instrument number	Manufacturer	Model	Year	Sample changing: Manual	Automatic	Capacity

	Sample dish diameter (in.)	Instrument background: Alpha Operating voltage	cpm	Beta Operating voltage	cpm	Gamma Operating voltage	cpm
Calibration Standard Type: Alpha Beta							

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

[1]Daily, weekly, monthly.
[2]Quarterly, semiannually, annually.
[3]Good operating but needs repair, not operating.

Are operating manuals readily available to the operator? Yes ☐ No ☐
Are calibration protocols available to the operator? Yes ☐ No ☐
Are calibrations kept in a permanent control chart record? Yes ☐ No ☐
Are permanent service maintenance records kept on these systems? Yes ☐ No ☐

Liquid Scintillation Counter

Instrument number	Manufacturer	Model	Year	Sample changing: Manual	Automatic	Capacity

	Discriminator channels: 1	2	3	Data readout: Visual	Channel printout: 1	2	3	External standard: Yes	No	Refrigeration: Yes	No
Calibration Standard Type:											

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

[1]Daily, weekly, monthly.
[2]Quarterly, semiannually, annually.
[3]Good operating but needs repair, not operating.

Are operating manuals readily available to the operator? Yes ☐ No ☐
Are calibration protocols available to the operator? Yes ☐ No ☐
Are calibrations kept in a permanent control chart record? Yes ☐ No ☐
Are permanent service maintenance records kept on these systems? Yes ☐ No ☐

Laboratory _____ Date _____

Location _____ Evaluator _____

Alpha Scintillation Counter

Instrument number	Manufacturer	Model	Year	Sample changing: Manual	Automatic	Capacity

	Alpha phosphor location: Photo tube	Samples	Instrument background: Operating voltage	cpm
Calibration Standard Type:				

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

[1]Daily, weekly, monthly.
[2]Quarterly, semiannually, annually.
[3]Good operating but needs repair, not operating.

Are operating manuals readily available to the operator? Yes ☐ No ☐
Are calibration protocols available to the operator? Yes ☐ No ☐
Are calibrations kept in a permanent control chart record? Yes ☐ No ☐
Are permanent service maintenance records kept on these systems? Yes ☐ No ☐

Radon Gas-Counting System

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

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

[1]Daily, weekly, monthly.
[2]Quarterly, semiannually, annually.
[3]Good operating but needs repair, not operating.

Are operating manuals readily available to the operator? Yes ☐ No ☐
Are calibration protocols available to the operator? Yes ☐ No ☐
Are calibrations kept in a permanent control chart record? Yes ☐ No ☐
Are permanent service maintenance records kept on these systems? Yes ☐ No ☐

Laboratory _____ Date _____

Location _____ Evaluator _____

Gamma Spectrometer Systems

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

[1]Daily, weekly, monthly.
[2]Quarterly, semiannually, annually.
[3]Good operating but needs repair, not operating.

Are operating manuals readily available to the operator? Yes ☐ No ☐
Are calibration protocols available to the operator? Yes ☐ No ☐
Are calibrations kept in a permanent control chart record? Yes ☐ No ☐
Are permanent service maintenance records kept on these systems? Yes ☐ No ☐

Laboratory _____ Date _____

Location _____ Evaluator _____

Sample Handling and Preservation

Parameter	Container Used	Preservative Used	Comments	Satisfactory Yes	Satisfactory No
Gross alpha activity					
Gross beta activity					
Strontium-89					
Strontium-90					
Radium-226					
Radium-228					
Cesium-134					
Iodine-131					
Tritium					
Uranium					
Photon emitters: a.					
b.					
c.					
d.					
e.					

Laboratory _____ Date _____

Location _____ Evaluator _____

Methodology

Parameter	Sample Load per Month	Method[1] used. Cite Edition, Year, and Page EPA	APHA	ASTM	HASL-300	[2]Approved Alternate	Other	Satisfactory Yes	No
Gross alpha activity									
Gross beta activity									
Strontium-89									
Strontium-90									
Radium-226									
Radium-228									
Cesium-134									
Iodine-131									
Tritium									
Uranium									
Photon emitters (identify): a.									
b.									
c.									
d.									
e.									

[1]Methods used, other than approved alternate methods, must be referenced in the Drinking Water Regulations (Federal Register)
[2]Cite approval date.

Laboratory _____ Date _____

Location _____ Evaluator _____

Quality Control

Item	Cross Check Studies (water)	A[1]	B[2]	Performance (Blind) Studies (Water)	A[1]	B[2]
Participation in intercomparison (cross check) studies and performance (blind) studies (studies conducted by EMSL-LV) Reporting Period: _____ To _____	Gross alpha			Gross alpha		
	Gross beta			Gross beta		
	Sr-89			Sr-89		
	Sr-90			Sr-90		
	Ra-226			Ra-226		
	Ra-228			Ra-228		
	Tritium			Uranium		
	Uranium			Cs-134		
	I-131			Cs-137		
	Cs-134			Co-60		
	Cs-137			Ru-106		
	Co-60			Written QA plan implemented and available for review		
	Ru-106					

	Yes	No	Frequency	Comments	Satisfactory Yes	Satisfactory No
Verification of sample results by duplicate sample analysis						
Use of quality control charts or records						
Calibration and maintenance records available						

[1]Scheduled frequency of participation by the Laboratory, times per year.
[2]Number of acceptable performances (results) in the past year, where an acceptable result is a normalized deviation from the known value of ≤ 3.0 sigma.

Laboratory _____ Date _____

Location _____ Evaluator _____

Data Reporting

Item	Comments: system(s) used, frequency, etc.
Records kept for 3 years	
Actual laboratory reports	
Tabular summary	
Information included:	
Date	
Place of sampling	
Time of sampling	
Person collecting sample	
Date of receipt of sample	
Date of analysis	
Type of analysis	
Laboratory and person responsible	
Method(s) used	
Results	

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 which can be used to trace the possession and handling of samples from the moment of collection through analysis. 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.

B. Sampling 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.); and
   h. Serial number on seals and transportation cases.
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. 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 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 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 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 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.

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 microbiological 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 must be established for audits of sample control information. Records should be examined to determine traceability, completeness, and accuracy.

Figure A-1 Sample Identification Tag Examples

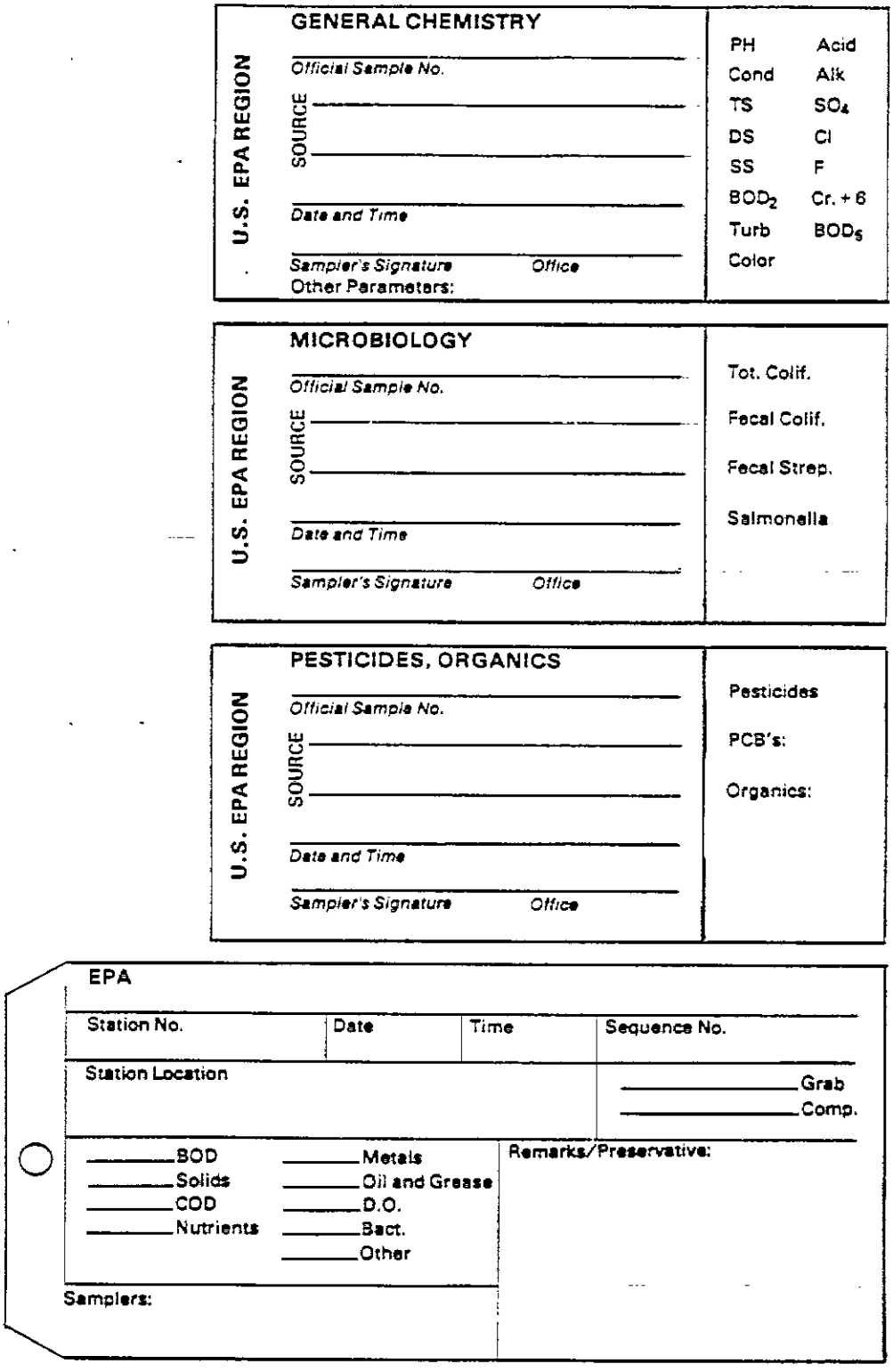

Figure A-2 Chain-of-Custody Record

Survey | **Samplers:** | *Signature*

Station Number	Station Location	Date	Time	Sample Type: Water (Comp.)	Sample Type: Water (Grab)	Sample Type: Air	Seq. No.	No. of Containers	Analysis Required

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

Method of Shipment:

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

Appendix B

Recommended Protocol for Regions Conducting On-Site Laboratory Evaluations

Before conducting the on-site evaluation, the Region shall:

- Hold a pre-evaluation conference with appropriate laboratory and field activity representatives to establish a schedule that would have a minimum impact on the laboratory activities.
- Request that a variety of tests be scheduled during the on-site evaluation.
- Arrange for the laboratory staff to be available during the on-site visit.

During the on-site visit, the team will:

- Evaluate the procedures and equipment used for those specific analyses for which the laboratory has requested certification, using the criteria in this manual.
- Review the records and written standard operating procedures for compliance with the required sampling frequency, sample collection, sample holding times, and if appropriate, resample notification.
- Insure that the laboratory has a QA plan in effect by:
  - Determining if the laboratory has written procedures (QA plan or equivalent) for conducting its quality assurance program.
  - Examining the quality assurance data to determine if the quality assurance program is being implemented.
- Complete the on-site checklists and other evaluation forms during the visit (see Chapters IV, V, and VI).
- Review the results of the evaluation with the director of the laboratory, the director of State water supply activities, and appropriate staff members. The review should:
  - Discuss any deviations in the observed procedures and records.
  - Recommend changes in equipment and supply needs, staffing requirements, and facility improvements, if necessary.
  - Discuss possible assistance the Region can provide the laboratory.

Evaluation Report for Principal State Laboratories and Laboratories in Non-Primacy States

After an on-site inspection, the evaluation team should prepare a narrative report and action memorandum. This report should contain all information pertinent to the evaluation and also recommend the certification status for all analyses evaluated. The report should then be forwarded for evaluation to the Regional Director of the Environmental Services Division and the Regional Director of the Water Division. After considering the report, they should transmit it to the Regional Administrator for action.

The Regional Administrator should decide the certification status of the laboratory within 30 days and notify the State. The State should be sent the complete report. If the report indicates that the laboratory not be given Certified status for an analysis, the Regional Administrator shall give the specific reasons.

The narrative report should be attached to each copy of the completed evaluation form. It should include the general headings and information listed below.

Title Page

The title page should contain the following:

Title:	Report of an on-site evaluation of the (name of laboratory)
At:	(city, State, and zip code)
On:	(date)
By:	(name, title, organization, and address of the certification team)

Certification Status

List either Certified, "Provisionally Certified," or Not Certified for each contaminant evaluated.

List of Deviations

List each deviation by item number used on the evaluation checklists. Describe the exact deviation and recommended changes.

Remarks

Recommend improvements which, while not affecting certification status, would improve laboratory operation. Other remarks might include reasons for failing the on-site evaluation, special recognition for outstanding performance, and description of unusual tests.

List of Personnel

List name and title of personnel along with the individual tests that each normally performs. Also identify the critical laboratory personnel.

Signature

Team members should sign the report.

Distribution

Copies of this report should be distributed to the State requesting the evaluation and EMSL-CI or EMSL-LV. For local laboratories in non-primacy States, reports should be distributed to appropriate Regional personnel.

Annually, each Region should submit to ODW a brief listing of laboratories in the Region having U.S. EPA or State certification status. The listing should include the names and location of each laboratory, and its certification status for all regulated contaminants. In addition, Regions should notify ODW of all changes in status soon after they occur so that ODW can maintain an updated list of certification status.

Appendix C
Abbreviations

CA—Certifying authority. Regional Administrator for principal State laboratories and laboratories in non-primacy States; EMSL-Cl and EMSL-LV Regional laboratories.

CFR—Code of Federal Regulations.

EMSL-Cl - Environmental Monitoring Systems Laboratory in Cincinnati, Ohio (ORD).

EMSL-LV—Environmental Monitoring Systems Laboratory in Las Vegas, Nevada (ORD).

DWLC—Drinking Water Laboratory Certification Work Group.

NPDWR—National Primary Drinking Water Regulations.

ODW—Office of Drinking Water.

ORD—Office of Research and Development.

PE—Performance evaluation.

RREL—Risk Reduction Engineering Laboratory (ORD)

QA—Quality assurance

QAMS—Quality Assurance Management Staff (ORD)

QC—Quality control

Appendix D

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 5 1989

MEMORANDUM

OFFICE OF WATER

SUBJECT: Third-Party Certification for Laboratories in Primacy States

FROM : Michael B. Cook, Director
Office of Drinking Water (WH-550A)

TO : Water Supply Representatives, Regions I-X
Environmental Services Division Directors, Regions I-X
Quality Assurance Officers, Regions I-X

This memorandum reissues and slightly modifies Water Supply Guidance VII-5, dealing with third-party certification for laboratories in primacy States. This memorandum should be retained as Water Supply Guidance VII-5A. No fundamental difference exists between the two versions; VII-5A merely clarifies the State's continuing responsibilities.

Under 40 CFR 142.10(b)(3), if a State does not perform all analytical measurements in its own laboratory, it must establish and maintain a program for the certification of laboratories as a condition of receiving and maintaining primary enforcement authority (primacy). This memorandum notifies States with primacy that they may contract with other organizations (third parties) to assist in certifying laboratories for drinking water analyses.

Several States have asked USEPA its position on third-party certification agents, i.e., private sector organizations which wish to operate the certification program for local laboratories in primacy States.

ODW endorses the third-party concept. This Office will not, however, pass judgment on any specific third-party program. It is the responsibility of each primacy State to assess the qualifications of the third-party and the adequacy of its program. The State must also retain the responsibility for overseeing the laboratory certification program. In assessing whether to choose a particular third-party, the State must

ier, as a minimum, the following features, some of which are
ied in the Manual for the Certification of Laboratories
ing Drinking Water:

technical criteria for chemistry, microbiology, and/or radiochemistry

use and quality of performance evaluation samples (unknown) and quality control samples (known)

frequency of on-site evaluations

evaluator capability

willingness to provide technical assistance

availability of records for review by State

adequacy of quality assurance program

criteria for downgrading/revoking certification

is essential that any third-party program be equivalent
revious program operated by the State and approved by
stated previously, States employing a third-party to
n certification must retain ultimate authority to decide
individual laboratories will be certified; this decision
be delegated to the third-party.

Regions should assist the State and third-party agent to
e program is sound. This could include reviewing the
helping the State obtain reference samples, and
technical assistance. In addition, Regions and States
sensitive to potential conflict-of-interest problems
third-party inspector and evaluated laboratories. For
inspectors employed by firms that provide analytical
in the drinking water area should not be put in the
of passing judgement on their competitors.

Appendix E

Required Analytical Capability for Principal State Laboratory Systems (As of June 1, 1979)

Volatile Organic Chemicals (40 CFR 141.24)

Benzene
Carbon tetrachloride
1,2-Dichloroethane
1,1-Dichloroethylene
p-Dichlorobenzene
1,1,1-Trichloroethane
Vinyl chloride
Trichloroethylene
Trihalomethanes
- Chloroform
- Bromodichloromethane
- Dibromochloromethane
- Bromoform

Organics other than VOCs (40 CFR 141.24)

Endrin
Lindane
Methoxychlor
Toxaphene
2,4-D
2,4,5-TP

Inorganics (40 CFR 141.23)

Arsenic
Barium
Cadmium
Chromium
Fluoride
Lead
Mercury
Nitrate-N
Selenium
Silver

Radionuclides (40 CFR 141.25)

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

Microorganisms (40 CFR 141.21)

Total coliforms

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

Appendix F

Additional Contaminants Scheduled for Future Rules (Rest of the 83 Not in Appendix E)

Volatile Organic Chemicals

o-Dichlorobenzene
cis-1,2-Dichloroethylene
trans-1,2-Dichloroethylene
1,2-Dichloropropane
Ethylbenzene
Methylene chloride
Monochlorobenzene
Tetrachloroethylene
Toluene
1,2,4-Trichlorobenzene
1,1,2-Trichloroethane
Xylenes (total)

Inorganics

Antimony
Asbestos
Beryllium
Copper
Cyanide
Nickel
Nitrite-N
Sulfate
Thallium

Radionuclides

Radon

Microorganisms (revised rules effective December 31, 1990)

Escherichia coli (not part of list of 83)
Fecal coliforms (not part of list of 83)
Giardia (no monitoring required under revised rules)
Heterotrophic bacteria (SPC or HPC)
Legionella (no monitoring required under revised rules)
Viruses (no monitoring required under revised rules)

Organics (other than VOCs)

Adipates (diethylhexyl)
Alachlor
Aldicarb
Aldicarb sulfoxide
Aldicarb sulfone
Aldrin
Atrazine
Acrylamide
Butachlor
Carbaryl
Carbofuran
Chlordane
Dalapon
1,2-Dibromo-3-chloropropane (DBCP)
2,4-DB
Dicamba
1,2-Dichoropropane
Dieldrin
Dinoseb
Diquat
Endothall
Ethylene dibromide (EDB)
Epichlorohydrin
Glyphosate
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Hexachlorocyclopentadiene
3-Hydroxycarbofuran
Methomyl
Metribuzin
Oxamyl (vydate)
PAHs (benzo(a)pyrene)
PCBs (decachlorobiphenyl)
Pentachlorophenol
Picloram
Phthalates (diethylhexyl)
Styrene
Simazine
2,4,5-T (silvex)
2,3,7,8-TCDD (dioxin)

Appendix G

§1445 Unregulated Chemicals to be Monitored (Final or Proposed)

40 CFR 141.40 (final rule published July 8, 1987)

Bromobenzene
Bromodichloromethane
Bromoform
Bromomethane

Chlorobenzene
Chlorodibromomethane
Chloroethane
Chloroform
Chloromethane
o-Chlorotoluene
p-Chlorotoluene

1,2-Dibro-3-chloropropane (DBCP)
Dibromomethane
m-Dichlorobenzene
o-Dichlorobenzene
1,1-Dichloroethane
cis-1,2-Dichloroethylene
trans-1,2-Dichloroethylene
Dichloromethane

1,2-Dichloropropane
1,3-Dichloropropane
2,2-Dichloropropane
1,1-Dichloropropene
1,3-Dichloropropene (cis and trans)

Ethylbenzene
Ethylene dibromide (EDB)

Styrene

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

m-Xylene
o-Xylene
p-Xylene

40 CFR 141.40 (proposed May 22, 1989)

Monitoring is required for the following contaminants if the State determines the system is vulnerable to contamination.

Synthetic Organics

Metribuzin
Hexachlorobenzene
Dalapon
Dinoseb
Picloram
Oxamyl (vydate)
Simazine
Glyphosate
Hexachlorocyclopentadiene
PAHs
Phthalates
2,3,7,8-TCDD (Dioxin)

Aldrin
Dieldrin
2,4-DB
Dicamba
2,4,5-T (silvex)
Carbaryl
3-Hydroxycarbofuran
Methomyl
Butachlor
Metolachlor
Propachlor

Inorganics

Antimony
Beryllium

Cyanide
Nickel

Sulfate
Thallium

Monitoring for the following contaminants is at the discretion of the State.

Ametryn
Aspon
Atraton
Azinphos methyl
Bolstar
Bromacil
Butylate
Carboxin
Chloropropham
Coumophos
Cycloate
Demeton-O
Demeton-S
Diazinon
Dichlofenthion
Dichlorvos
Diphenamid

Ethion
Ethoprop
Ethyl parathion
Famphur
Fenamiphos
Fenarimol
Fenitrothion
Fensulfothion
Fenthion
Fluridone
Fonofos
Hexazinone
Malathion
Merphos
Methyl paraoxon
Methyl parathion
Mevinphos

Disulfoton	MGK 264
Disulfoton sulfone	MGK 326
Disulfoton sulfoxide	Molinate
EPN	Napropamide
EPTC	Norflurazon
Pebulate	Chlorpyrifos
Phorate	DCPA
Phosmet	4,4'-DDD
Prometon	4,4'-DDE
Prometryn	4,4'-DDT
Pronamide	Dichloran
Propazine	Endosulfan I
Simetryn	Endosulfan II
Stirofos	Endosulfan sulfate
Tebuthiuron	Endrin aldehyde
Terbacil	Etridiazole
Terbufos	BCH-alpha
Terbutryn	BCH-beta
Trademefon	BCH-delta
Tricyclazole	BCH-gamma
Vernolate	cis-Permethrin
Chlorneb	trans-Permethrin
Chlorobenzilate	Trifluralin
Chloropropylate	Diquat
Chlorothalonil	Endothall

TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
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15. SUPPLEMENTARY NOTES

16. ABSTRACT

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

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

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Laboratory Certification Safe Drinking Water Act (SDWA)	Water Supply Drinking Water Quality Assurance Sanitary Microbiology Chemistry Radiochemistry	

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Manual for the Certification of Laboratories Analyzing Drinking Water

Criteria and Procedures Quality Assurance

Third Edition

Prepared by
The Laboratory Certification Program Revision Committee

Change 1 - October 1991

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

List of Effective Pages

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Page/Chapter No.	Change No.*
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ii (Effective Pages) (Added)	1
iii (Notice)	1
iv (Foreword) (Added)	1
v-vi (Preface)	1
vii-ix (Acknowledgments)	1
x (blank)	1
xi-xii (Contents)	1
1-36 (Chapters I-IV)	0
37-58.8 (Chapter V)	1
59-74 (Chapter VI)	0
75-86 (Appendices A-D)	0
87-91(Appendices E-G)	1
93-95 (Appendix H) (Added)	1

*Zero in this column indicates an original page.

Notice

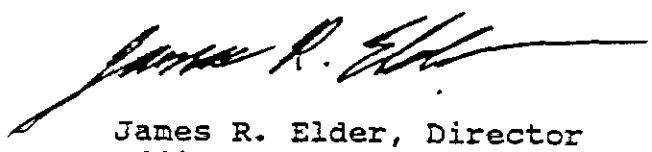
This manual has been reviewed by the Office of Drinking Water and the Office of Research and Development and approved for publication. The mention of commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.

Foreword

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

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

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



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

Preface

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

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

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

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

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

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

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

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

Acknowledgments

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

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Contents

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Chapter V
Microbiology

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

1. Personnel

1.1 Supervisor/Consultant

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

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

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

1.2 Analyst (or equivalent job title)

The analyst performs microbiological tests with minimal supervision.

1.2.1 Academic training: Minimum of high school education.

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

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

2. Laboratory Facilities

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

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

3. Laboratory Equipment and Supplies

3.1 pH Meter

3.1.1 Accuracy and scale graduations within ±0.1 units.

3.1.2 Use pH buffer aliquot only once.

3.1.3 Maintain electrodes according to manufacturer's recommendations.

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

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

3.2 Balance (top loader or pan)

3.2.1 Balance detects 100 mg at a 150 gram load.

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

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

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

3.3 Temperature Monitoring Device

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

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

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

3.4 Incubator Unit

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

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

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

3.5 Autoclave

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

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

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

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

QC 3.5.5 Check automatic timing mechanism with stopwatch quarterly.

3.6 Hot Air Oven

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

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

3.7 Colony Counter

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

3.8 Conductivity Meter

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

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

3.9 Refrigerator

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

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

3.10 *Inoculating Equipment*

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

3.11 Membrane Filtration Equipment (if MF procedure is used)

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

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

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

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

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

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

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

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

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

3.12 Culture Dishes (loose or tight lid)

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

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

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

3.13 Pipets

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

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

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

3.14 Culture Tubes, Containers, and Closures

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

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

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

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

3.15 Sample Containers

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

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

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

3.16 Glassware and Plasticware

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

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

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

3.17 Ultraviolet Lamp (if used)

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

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

4. General Laboratory Practices

4.1 Sterilization Procedures

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

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

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

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

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

4.2 Sample Containers

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

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

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

4.3 Reagent Water

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

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

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

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

4.4 Dilution/Rinse Water

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

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

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

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

4.5 Glassware Washing

4.5.1 Use distilled or deionized water for final rinse.

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

4.6 Analytical Media

4.6.1 General

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

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

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

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

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

or commercially available disks impregnated with the organism.

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

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

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

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

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

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

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

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

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

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

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

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

4.6.4.3 Final pH: 6.8 ± 0.2.

4.6.5 EC Medium (for fecal coliforms)

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

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

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

4.6.5.4 Final pH: 6.9 ± 0.2.

4.6.6 MMO-MUG Test Medium (for total coliforms)

4.6.6.1 Do not prepare this medium from basic ingredients.

4.6.6.2 Protect medium from light.

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

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

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

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

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

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

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

4.6.7.5 Final pH: 6.9 ± 0.2.

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

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

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

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

4.6.8.3 Final pH: 6.8 ± 0.2.

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

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

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

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

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

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

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

4.6.10.2 Final pH: 6.9 ± 0.1.

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

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

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

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

4.6.11.2 Final pH: 7.4.

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

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

5. Analytical Methodology

5.1 General

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

5.1.2 A laboratory should be certified for all analytical methods indicated below that it uses for compliance purposes. At a minimum, the laboratory must be certified for one total coliform method and one fecal coliform or *E. coli* method. In addition, for principal State laboratories and other laboratories that may enumerate heterotrophic bacteria for compliance purposes, the laboratory should be certified for the Pour Plate Method.

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

5.1.4 Shake water sample vigorously before analyzing.

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

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

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

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

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

5.2.4 Invalidate all samples resulting in confluent or TNTC (too numerous to count) growth. Record as "confluent growth" or "TNTC" and request an additional sample from the same sampling site. Confluent growth is defined as a continuous bacterial growth, without evidence of sheen colonies (total coliforms), covering the entire membrane filter. TNTC is defined as greater than 200 colonies on the membrane filter in the absence of detectable coliforms. Do not invalidate sample when the membrane filter contains at least one total coliform colony.

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

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

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

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

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

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

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

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

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

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

enumerating total coliforms for at least several months and/or over several seasons to assess the effectiveness of the MMO-MUG test for the wide variety of water types submitted for analysis.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.9.3 Count colonies manually using a counting aid such as a Quebec colony counter. Consider only plates having 30 to 300 colonies in determining plate count, except for plates inoculated with 1.0 mL volume of undiluted sample. Counts less than 30 for such plates are acceptable. (Fully automatic colony counters are not suitable because of the size and small number of colonies observed when potable water is analyzed for heterotrophic bacteria.)

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

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

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

5.10.2 Same as paragraph 5.9.3.

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

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

5.4.3 Confirm yellow cultures in BGLB broth.

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

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

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

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

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

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

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

5.5.6 Any amount of gas collected in the inverted vial of a tube showing growth is considered fecal coliform-positive.

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

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

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

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

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

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

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

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

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

5.6.9 Laboratories are encouraged to perform parallel testing between the MMO-MUG test and another EPA-approved procedure for

5.10.3 Same as paragraph 5.9.4.

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

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

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

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

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

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

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

5.12.3 Adjust initial counts based upon verification data.

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

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

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

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

5.13.3 Invalidate all samples which produce a turbid culture in the absence of gas production, in lauryl tryptose broth or lactose broth. Collect, or request the system collect, another sample. The laboratory may use another method to test the second sample. Alternatively, if a sample produces a turbid culture in the absence of gas production, perform a confirmed test. If the confirmed test is total coliform-positive, report the most probable number. If a confirmed test is total coliform-negative, invalidate the sample and request another one.

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

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

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

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

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

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

6. Sample Collection, Handling, and Preservation

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

6.1 Sample Collector

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

6.2 Sampling

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

6.3 Sample Icing

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

6.4 Sample Holding/Travel Time

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

6.5 Report Form

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

6.6 Chain-of-Custody

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

7. Quality Assurance

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

8. Records and Data Reporting

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

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

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

9. Action Response to Laboratory Results

9.1 Testing Total Coliform-Positive Cultures

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

9.2 Notification of Positive Results

(for Total Coliform Rule) Laboratory must notify proper authority promptly of a positive total coliform, fecal coliform, or *E. coli* result, so that appropriate follow-up actions (e.g., collection of repeat samples) can be conducted (see 40 CFR 141.21(b) and (e), 40 CFR 141.31, etc.). Total coliform-positive result is based on confirmed phase for the Multiple Tube Fermentation Technique and Presence-Absence (P-A) Coliform Test, or verified test for Membrane Filter Technique. No requirement exists for confirmation of positive MMO-MUG tests, fecal coliform test, or *E. coli* tests.

9.3 Invalidation of Total Coliform-Negative Sample

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

Laboratory _____

Street _____

City _____ State _____

Telephone Number _____

Survey by _____

Affiliation _____

Date _____

Codes for Marking On-Site Evaluation Forms

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

1. Personnel

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

2. Laboratory Facilities

Laboratory facilities clean, temperature and humidity controlled _____

Adequate lighting at bench top _____

Laboratory has provision for disposal of microbiological wastes _____

3. Laboratory Equipment, Supplies, and Materials

3.1 pH Meter

Manufacturer _____ Model _____

Accuracy ± 0.1 units _____

Scale graduation, 0.1 units _____

Maintains electrodes according to manufacturer's recommendations _____

pH buffer solution aliquots used only once _____

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

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

3.2 Balances (Top Loader or Pan)

Manufacturer _____ Model _____

Detects 100 mg at a 150 gram load _____

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

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

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

3.3 Temperature Monitoring Device

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

No separation in mercury column _____

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

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

3.4 Incubator Unit

Manufacturer_____ Model_____

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

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

Immerse thermometer bulb in liquid _____

Culture dishes and tubes fit snugly in aluminum block incubator _____

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

3.5 Autoclave

Manufacturer_____ Model_____

Temperature gauge with sensor on exhaust _____

Operational safety valve _____

Maintains sterilization temperature during cycle _____

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

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

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

QC Establish service contract or internal maintenance protocol _____

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

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

3.6 Hot Air Oven

Manufacturer_____ Model_____

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

Thermometer graduated in no more than 10°C increments _____

Place thermometer bulb in sand _____

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

3.7 Colony Counter

Manufacturer _____ Model _____

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

3.8 Conductivity Meter

Manufacturer _____ Model _____

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

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

3.9 Refrigerator(s)

Manufacturer _____ Model _____

Maintains temperatures of 1° to 5°C _____

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

Thermometer bulb(s) immersed in liquid _____

QC Temperature recorded for days in use _____

3.10 Inoculating Equipment

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

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

3.11 Membrane Filtration Equipment, Membrane Filters and Pads

Manufacturer _____ Model _____

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

Units do not leak, not scratched or corroded _____

10 to 15X magnification device with fluorescent light source _____

Forcep tips without corrugations _____

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

Alternate pore size used _____

Membrane filters recommended by manufacturer for total coliform analysis _____

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

QC Record lot numbers of membrane filters and date received _____

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

3.12 Culture dishes

Use presterilized plastic or sterilized glass dishes _____

Incubate loose-lid dishes in a tight fitting container _____

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

Reseal open packs of disposable culture dishes between uses _____

3.13 Pipets

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

Reseal packs of disposable sterile pipets between major use periods _____

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

3.14 Culture Tubes, Containers and Closures

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

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

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

3.15 Sample Containers

Capacity at least 120 mL (4 oz) _____

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

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

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

3.16 Glassware and Plasticware

Glass made of borosilicate or other corrosion-resistant glass _____

Free of chips and cracks _____

Graduation marks are legible _____

Plastic items are clear and non-toxic _____

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

Calibration of pre-calibrated containers spot checked _____

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

3.17 Ultraviolet lamp (if used)

Lamps cleaned monthly with a soft cloth moistened with ethanol _____

QC Lamp used for sanitization tested every quarter _____

4. General Laboratory Practices

4.1. Sterilization and Sanitation Procedures

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

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

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

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

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

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

4.2 Sample Containers

Stock 10% sodium thiosulfate solution free of turbidity _____

Add sodium thiosulfate to sample containers prior to sterilization _____

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

4.3 Reagent Water

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

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

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

4.4 Dilution/Rinse Water

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

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

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

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

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

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

QC pH of peptone water is 6.8 ± 0.2 _____

QC Check dilution/rinse water for sterility _____

4.5 Glassware Washing

Use distilled or deionized water for final rinse _____

QC Perform inhibitory residue test on clean glassware _____

4.6 Analytical Media

4.6.1 General

Commercially available dehydrated or prepared media used _____

Dehydrated media stored in cool, dry location _____

Caked or discolored media discarded _____

Dehydrated media dated when received and when initially opened _____

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

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

QC Media preparation records include:

(a) Date of preparation _____

(b) Type of medium _____

(c) Lot number _____

(d) Sterilization time and temperature _____

(e) Final pH _____

(f) Technician's initials _____

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

(a) Date received _____

(b) Type of medium _____

(c) Lot number _____

(d) pH verification _____

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

Prepared plates are refrigerated in sealed plastic bags or containers _____

4.6.2 Membrane Filter Media (for total coliforms)

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

Medium dissolved using:

(a) Boiling water bath _____

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

Media prepared in sterile flasks _____

Ethanol not denatured _____

MF broth refrigerated no longer than 96 hours _____

MF agar plates refrigerated no longer than 2 weeks _____

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

Uninoculated media discarded if growth or surface sheen observed _____

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

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

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

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

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

Brilliant green lactose bile broth used for confirmed phase _____

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

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

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

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

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

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

m-Endo LES agar used for the completed phase _____

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

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

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

Space allowed between bottles _____

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

4.6.5 EC Medium (for fecal coliforms)

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

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

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

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

4.6.6 MMO-MUG Test Medium (for total coliforms)

Commercial preparation used _____

Medium protected from light _____

Medium is not autoclaved _____

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

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

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

Inverted vial in test tube is not used _____

Final MUG concentration: 50 µg/ml _____

Final pH: 6.9 ± 0.2 _____

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

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

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

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

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

Final MUG concentration: 100 µg/ml

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

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

4.6.9 Heterotrophic Plate Count (HPC) Medium

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

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

Sterile agar medium melted not more than once

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

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

4.6.10 A-1 Medium (for fecal coliforms)

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

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

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

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

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

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

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

5. Analytical Methodology

5.1 General

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

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

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

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

Water sample shaken vigorously before analyzing _____

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

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

Sample volume analyzed is 100 mL ± 2.5 mL _____

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

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

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

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

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

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

Concentration of inoculated medium is correct _____

Sample volume analyzed is 100 mL ±2.5 mL _____

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

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

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

Cultures from gas-positive tubes incubated in BGLB broth _____

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

5.4 Presence-Absence (P-A) Coliform Test

Sample volume analyzed is 100 mL ± 2.5 mL _____

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

Yellow cultures confirmed in BGLB broth _____

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

5.5 Fecal Coliform Test

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

transfer technique

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

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

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

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

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

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

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

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

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

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

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

Inoculated test not exposed to prolonged direct sunlight

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.9 Pour Plate Method (for heterotrophic bacteria)

Appropriate volume of sample added to plate _____

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

Sample and melted agar mixed carefully _____

At least two replicate plates prepared for each sample _____

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

Plates stacked no more than four high _____

Sterile agar medium remelted only once _____

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

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

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

5.10 Spread Plate Method (for heterotrophic bacteria)

R2A agar medium used _____

Plates with solidified medium dried before use _____

Medium inoculated in accordance with *Standard Methods* _____

At least two replicate plates used for each sample _____

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

Plates stacked no more than four high _____

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

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

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

5.11 Membrane Filter Method (for heterotrophic bacteria)

Sample volume filtered yields filters with 20-200 colonies _____

Filter transferred to R2A agar medium _____

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

At least two replicate plates prepared for each sample _____

Stereoscopic microscope used to count colonies _____

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

5.12 MF Procedure (for total coliforms in source water)

Sample volume filtered yields 80 or fewer colonies/membrane _____

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

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

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

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

Initial counts adjusted, based upon verification data _____

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

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

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

Five or ten tubes/sample volume used _____

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

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

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

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

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

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

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

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

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

6. Sample Collection, Handling, and Preservation

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

6.2 Sample collectors receive training

6.3 Samples representative of distribution system

6.4 Water taps free of any attachments and mixing type faucets

6.5 Water run to waste for at least two minutes

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

6.7 Sample report form completed by collector

6.8 Samples iced when carrying samples directly to laboratory

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

6.10 Transit time does not exceed 30 hours

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

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

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

7. Quality Assurance Program

7.1 Written QA Plan implemented and available for review _____

7.2 Quality control records maintained for five years _____

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

8. Data Reporting

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

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

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

8.3 Results of analyses

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

9. Action Response by Laboratory

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

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

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

Appendix E

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

Volatile Organic Chemicals (40 CFR 141.24)

Benzene
Carbon tetrachloride
1,2-Dichloroethane
1,1-Dichloroethylene
p-Dichlorobenzene
1,1,1-Trichloroethane
Vinyl chloride
Trichloroethylene
trans-1,2-Dichloroethylene
Ethylbenzene
Styrene
Toluene
Total Trihalomethanes
  Chloroform
  Bromodichloromethane
  Dibromochloromethane
  Bromoform
o-Dichlorobenzene
cis-1,2-Dichloroethylene
1,2-Dichloropropane
Monochlorobenzene
Tetrachloroethylene
Xylenes (total)

Organics other than VOCs (40 CFR 141.24)

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

Inorganics (40 CFR 141.23, 141.89)

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

Radionuclides (40 CFR 141.25)

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

Microorganisms (40 CFR 141.21)

Total coliforms
Escherichia coli or fecal coliforms
Heterotrophic bacteria

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

Appendix F

Additional Contaminants Scheduled for Rules in 1992-1993

Volatile Organic Chemicals

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

Organics (other than VOCs)

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

Inorganics

Antimony
Beryllium
Cyanide
Nickel
Thallium
Sulfate

Radionuclides

Radon
Uranium

Appendix G

§1445 Unregulated Chemicals to be Monitored

40 CFR 141.40 (final rule July 8, 1987)

Bromobenzene
Bromodichloromethane
Bromoform
Bromomethane

Chlorodibromomethane
Chloroethane
Chloroform
Chloromethane
o-Chlorotoluene
p-Chlorotoluene

Dibromomethane
m-Dichlorobenzene
1,1-Dichloroethane
)ichloromethane

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

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

40 CFR 141.40 (final rule January 30, 1991)

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

Synthetic Organics

Aldrin
Hexachlorobenzene*
Dalapon*
Dinoseb*
Picloram*
Oxamyl (vydate)*
Simazine*
Glyphosate*
Hexachlorocyclopentadiene*
Benzo (a) pyrene*
Metolachlor
di(2-ethylhexyl) Phthalate*
2,3,7,8-TCDD (Dioxin)*
Diquat*
Dieldrin
Dicamba
Carbaryl
3-Hydroxycarbofuran
Methomyl
Butachlor
Endothall*
Metribuzin
Propachlor
di(2-ethylhexyl) Adipate*

Inorganics

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

* Being considered for regulation in 1992.

Appendix H

Analytical Methods for Microbiology

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Only the analytical method(s) specified in this paragraph, or otherwise approved by EPA, may be used to demonstrate compliance with the requirements of §§141.71, 141.72, and 141.73. Measurements for pH, temperature, turbidity, and residual disinfectant concentrations must be conducted by a party approved by the State. Measurements for total coliforms, fecal coliforms, and HPC must be conducted by a laboratory certified by the State or EPA to do such analysis. Until laboratory certification criteria are developed for the analysis of HPC and fecal coliforms, any laboratory certified for total coliform analysis by EPA is deemed certified for HPC and fecal coliform analysis. The following procedures shall be performed in accordance with the publications listed in the following section. This incorporation by reference was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR Part 51. Copies of the methods published in *Standard Methods for the Examination of Water and Wastewater* may be obtained from the American Public Health Association et al., 1015 Fifteenth Street, NW, Washington, DC 20005; copies of the Minimal Medium ONPG-MUG Method as set forth in the article "National Field Evaluation of a Defined Substrate Method for the Simultaneous Enumeration of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with the Standard Multiple Tube Fermentation Method" (Edberg et al.), Applied and Environmental Microbiology, Volume 54, pp. 1595-1601, June 1988 (as amended under Erratum, Applied and Environmental Microbiology, Volume 54, p. 3197, December, 1988), may be obtained from the American Water Works Association Research Foundation, 6666 West Quincy Avenue, Denver, Colorado 80235; and copies of the Indigo Method as set forth in the article "Determination of Ozone in Water by the Indigo Method" (Bader and Hoigne), may be obtained from Ozone Science & Engineering, Pergamon Press Ltd., Fairview Park, Elmsford, New York 10523. Copies may be inspected at the U.S. Environmental Protection Agency, Room EB15, 401 M Street, SW., Washington, DC 20460 or at the Office of the Federal Register, 1100 L Street NW, Room 8401, Washington, DC.

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

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

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

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

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