Appendices

Appendix A
Chain-of-Custody Evaluations

A. Introduction

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

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

1. It is in one's actual physical possession;

2. It is in one's view, after being in one's physical possession;

3. It is one's physical possession and then locked up so that no one can tamper with it;

4. It is kept in a secured area, restricted to authorized personnel only.

B. Sample Collection, Handling and Identification

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

   a. Unique sample or log number;

   b. Date and time;

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

   d. Preservative used;

   e. Analyses required;

   f. Name of collector(s);

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

   h. Serial number on seals and transportation cases;

   i. Comments.

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

3. The closed sample container should then be placed in a transportation case along with the chain-of-custody record form, pertinent field records, and analysis request form. The transportation case should then be sealed and labeled. All records should be filled out legibly in waterproof pen. The use of locked or sealed chests will eliminate the need for close control of individual sample containers. However, there will undoubtedly be occasions when the use of a chest will be inconvenient. On these occasions, the sampler should place a seal around the cap of the individual sample container which would indicate tampering if removed.

C. Transfer of Custody and Shipment

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

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

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

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

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

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

D. Laboratory Sample Control Procedures

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

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

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

3. A clean, dry, isolated room, building, and/or refrigerated space that can be securely locked from the outside must be designated as a "custody room."

4. The custodian must ensure that heat-sensitive samples, light-sensitive samples, radioactive samples, or other sample materials having unusual physical characteristics, or requiring special handling, are properly stored and maintained prior to analysis.

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

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

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

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

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

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

Figure A-1 Sample Identification Tag Examples

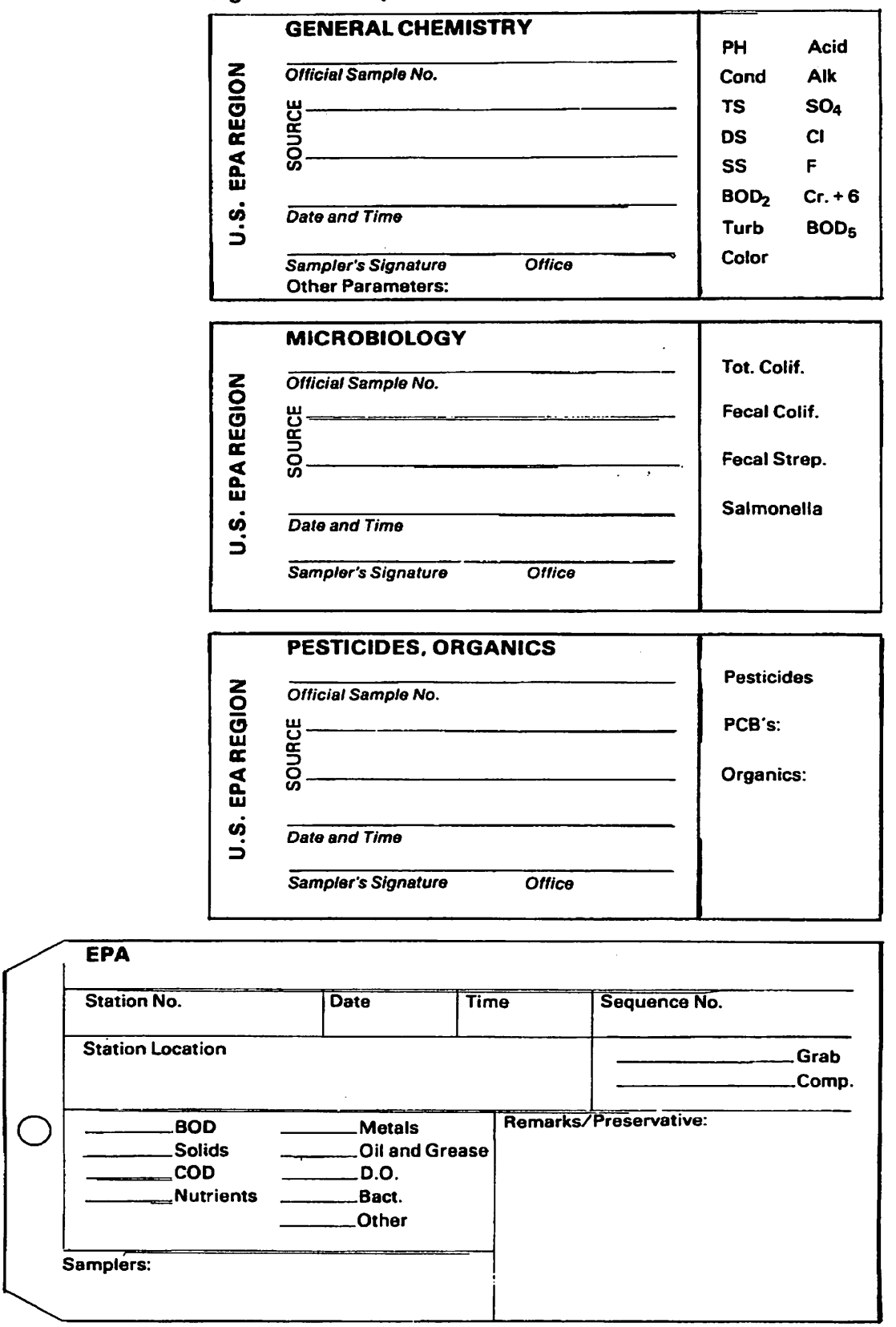

Figure A-2 Chain-of-Custody Record

Survey				**Samplers:** Signature					
Station Number	Station Location	Date	Time	Sample Type			Seq. No.	No. Of Containers	Analysis Required
				Water		Air			
				Comp	Grab.				

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

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

Method of Shipment:

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

Appendix B
Recommended Protocol for Regions Conducting On-Site Laboratory Evaluations

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

- Plan all the required activities to be completed during the assessment.

- 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 and review appropriate records.

- 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 shall:

- Conduct an opening conference or entrance interview.

- 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.

- Perform a data audit on at least one sample and one PE sample for at least one method but preferably for each method the laboratory performs.

- 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).

- Conduct a closing conference or exit interview in which the auditors 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.

    - Discuss a time frame for corrective actions and response.

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 Certification Program Manager for review. After reviewing and, if necessary, revising the report, it should be forwarded to the Certification Authority for signature.

The Certification Authority should decide the certification status of the laboratory within time constraints on page III-7 and notify the State. The State should be sent the complete report. If the report indicates that the laboratory should not be certified for an analysis, the Certification Authority should 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", "Administratively/Interim Certified", or "Not Certified" for each contaminant evaluated or if applicable (for VOCs, for instance) for each class of compounds 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. For local laboratories in non-primacy States, reports should be distributed to appropriate Regional personnel.

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

Appendix C
Definitions and Abbreviations

ASTM: American Society of Testing and Materials

AWWA: American Water Works Association

NERL-Ci: U.S. EPA National Exposure Research Laboratory in Cincinnati, Ohio (ORD).

NPDWR: National Primary Drinking Water Regulations.

OGWDW: U.S. EPA Office of Ground Water and Drinking Water.

ORD: U.S. EPA Office of Research and Development.

SDWA: The Safe Drinking Water Act as amended (42 U.S.C. 300f et seq.).

Accuracy: A measure of the closeness of an individual measurement or the average of a number of measurements to the true value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations. Refer to Standard Methods, Data Quality Section for a more detailed explanation.

Administrator: The administrator of the U.S. EPA or her/his authorized representative. See 40 CFR 142.2.

Agency: The U.S. EPA. See 40 CFR 142.2.

Auditor - A person who evaluates laboratories to determine if they meet the criteria to be certified. This person should be an experienced professional, who has effective communication skills, experience in quality assurance, the analytical techniques being evaluated, and familiarity with the drinking water regulations and this manual.

Bachelor Degree or Equivalent: A college degree with an equivalent 30 semester hours in a specific discipline. Equivalent is at least four years of experience in a specific scientific discipline.

Bias: The systematic or persistent distortion of a measurement process which causes errors in one direction.

Certification Authority: (CA) The person or designee who has the authority to certify laboratories conducting drinking water analyses and to certify the officials of the State responsible for the State's certification program in accordance with Section 1412 of the Safe Drinking Water Act. This authority is delegated to the Regional Administrator but may be redelegated.

Certification Program Manager: (CPM) The person responsible for managing the certification program which includes tracking the certification status of the State laboratories, ensuring that the regional and State certification officers are qualified and reviewing the certification evaluation reports.

Certification Officer: (CO) A State or Federal laboratory auditor who has passed the NERL certification officers training course (limited at this time to chemistry and microbiology). This person provides information to the CA or CPM for the purpose of making decisions on the certification status of a laboratory.

CFR: Code of Federal Regulations - A compilation of regulations is revised each time a regulation is promulgated. It is published every year in July.

Confirmation: Verification of the presence of a component through the use of an analytical technique based on a different scientific principle from the original method (e.g., second column, alternate wavelength or detector, etc.)

Conflict of Interest: a personal interest or relationship, as defined by law or regulation, that conflicts with the faithful performance of your official duty.

Data Audit: A qualitative and quantitative evaluation of the documentation and procedures associated with measurements to verify that the resulting data are acceptable.

Data Quality Objectives: qualitative and quantitative specifications used to design a study that will limit uncertainty to an acceptable level.

Data Reduction: The process of transforming the number of data items by arithmetic or statistical calculations, standard curves, concentration factors, etc. and collation into a more useful form. Data reduction is irreversible and generally results in the loss of detail.

Detection: Any concentration of an analyte which equals or exceeds the laboratory's detection limit. For VOCs, detection limit is defined as 0.0005 mg/L.

Drinking Water Laboratory: A laboratory that analyses samples as part of compliance monitoring for a public water supply.

Holding time: The allowed time from when a sample was taken (or extracted) until it must be analyzed.

IDC: Initial Demonstration of Capability - before analyzing compliance samples an analytical team must demonstrate acceptable precision, accuracy, sensitivity, and specificity for the method to be used.

LRB - Laboratory Reagent Blank: (Method blank) An aliquot of reagent water or other blank matrix that is treated exactly as a sample to determine if method analytes or other interferences are present.

LFB - Laboratory Fortified Blank: (Spike) An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample to determine whether the method is in control.

MCL: Maximum contaminant level means the maximum permissible level of a contaminant in water which is delivered to any user of a public water system. See 40 CFR Part 141.2.

MCLG: Maximum contaminant level goal means the maximum level of a contaminant in drinking water at which no known or anticipated adverse effect on the health of persons would occur, and which allows an adequate margin of safety. Maximum contaminant level goals are nonenforceable health goals. See 40 CFR 141.2.

Method Detection Limit: (MDL) the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The MDL is determined from analysis of a sample in a given matrix containing this analyte. See 40 CFR 136 App. B.

Method Reporting Limit: (MRL) the lowest concentration of standard used for calibration

Monitoring Trigger: The concentration of a regulated contaminant which triggers additional monitoring. (See Detection Limit 141.24(h)(iii)(18))

NELAC: National Environmental Laboratory Accreditation Conference - a voluntary organization of State, Federal and other groups to establish mutually acceptable standards for accrediting environmental laboratories.

Precision: The measure of mutual agreement among individual measurements.

Primacy: Primary responsibility for administration and enforcement of primary drinking water regulations and related requirements applicable to public water systems within a State.

Principal State Laboratory System: All facilities, whether part of the State laboratory or contracted by the State, producing data for the State and certified by the EPA, fulfilling the requirements for Primacy as listed in the 40 CFR 142.10(b)(4).

Profeciency Testing Samples (PTs): A sample provided to a laboratory for the purpose of demonstrating that the laboratory can successfully analyze the sample within specified acceptance limits specified in the regulations. The qualitative and/or quantitative composition of the reference material is unknown to the laboratory at the time of the analysis. See 40 CFR Part 141.2.

Public Water System: A system for the provision to the public of piped water for human consumption, if such system has at least fifteen service connections or regularly serves an average of at least twenty-five individuals daily at least 60 days out of the year. See 40 CFR Part 141.2.

Quality Assurance: An integrated system of management activities involving planning, quality control, quality assessment, reporting and quality improvement to ensure that a product or service meets defined standards of quality with a stated level of confidence.

Quality Control: The overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it meets the needs of the users; operational techniques and activities that are used to fulfill requirements for quality.

QA Plan: A comprehensive plan detailing the aspects of quality assurance needed to adequately fulfill the data needs of a program. This document is required before the laboratory is certified.

Regulatory Level: A concentration of a contaminant which is cited in the Federal Regulations (e.g., MCL, detect, etc.)

Shall: Denotes a mandatory requirement.

Should: Denotes a guideline or recommendation.

Standard Operating Procedure: A written document which details the method of an operation, analysis or action whose techniques and procedures are thoroughly prescribed and which is officially approved as the method for performing certain routine or repetitive tasks.

Third Party Auditor: Person or persons, not affiliated with a Region or State, who is designated by the Region or State to audit a laboratory. This person must pass the certification training course prior to auditing any laboratory unless he or she is a part of an audit team which includes a Regional/State certification officer. The third party auditor must also meet the educational/experience requirements specified in this manual. The certification decision remains with the Region.

Third Party Expert: Any person not designated as a certification officer or auditor, who is requested by the Region to assist in the audit of a laboratory because of his or her expertise in a particular area (e.g., asbestos). This person is not required to take the certification officers' course if he or she is part of an audit team which includes a certification officer.

Tribal Nation: Areas which for regulatory purposes are treated as independent States. On these lands, the Indian tribe has a Federally recognized governing body carrying out government duties and powers.

"Unregulated" Contaminants: Contaminants for which monitoring is required but which have no MCL.

Appendix D

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

January 16,1997

MEMORANDUM

SUBJECT: The Use of "Third-Parties" in the Drinking Water Laboratory Certification Program

FROM: Cynthia Dougherty, Director
Office of Ground Water and Drinking Water

TO: Water Supply Representatives, Regions I-X
Certification Authorities, Regions I-X
Quality Assurance Officers, Regions I-X
Regional Laboratories, Regions I-X

Purpose
This memorandum updates and clarifies the guidance memorandum from Michael Cook dated December 5, 1989 on "Third-Party Certification for Laboratories in Primacy States."

Action
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 for receiving and maintaining authority to administer the Safe Drinking Water Act in lieu of EPA (primacy). This memorandum notifies States with primacy that they may contract with other organizations (third parties) to assist the State in fulfilling this requirement. The authority for making certification decisions however, must remain with the State.

Discussion
Several States have asked USEPA its position on the use of third-parties, i.e., private sector organizations which assist the States with their certification program. OGWDW realizes that dwindling State resources may necessitate assistance from third-parties in the State certification programs. Consistent with the regulatory requirement at 40 CFR 142.10(b), providing for the "establishment and maintenance of a State program for the certification of laboratories," the State must retain ultimate authority to decide whether individual laboratories will be certified: this decision may not be abdicated to the third party.

This Office will not pass judgment on any specific third-party program. It is the responsibility of each primacy State to assess the qualifications of the third-party. In assessing whether to choose a particular third-party, the State should consider, as a minimum, the following items which are described in the

Manual for the Certification of Laboratories Analyzing Drinking Water:

- Ability to provide technical assistance and training
- Availability of records for review by the State
- Quality assurance program
- Freedom from conflicts of interest
- EPA policy, which provides that the auditor should pass an appropriate course on how to audit in the discipline for which he or she will be auditing.
- Experience of the auditor.
  The auditor should be an experienced professional with at least a bachelor's degree or equivalent education/experience in the discipline for which he or she audits.

  The auditor should have recent laboratory experience

Any State certification program using third party assistance should meet the requirements in the *Manual for the Certification of Laboratories Analyzing Drinking Water* just as it would if it were using State employees to perform these functions. The Regions should assist the State and third-party agent to assure that the certification program meets EPA guidelines.

Regions and States should be sensitive to potential conflict-of-interest problems between a third-parties and evaluated laboratories. For instance, inspectors employed by firms that provide analytical services in the drinking water area should not be put in the position of passing judgement on their competitors.

Further Information

If you have questions or need additional information or assistance, please contact the OGWDW Technical Support Center at 513-569-7904.

Appendix E
Required Analytical Capability for Principal State Laboratory Systems

INORGANICS
(40 CFR 141.23)
Asbestos
Cyanide
Fluoride
Nitrate
Nitrite
Antimony
Arsenic
Barium
Beryllium
Cadmium
Chromium
Mercury
Selenium
Thallium
(40 CFR 141.89)
Copper
Lead
Conductivity
Calcium
Alkalinity
Orthophosphate
Silica

VOLATILE ORGANICS
(40 CFR 141.24)
THMs
Benzene
Carbon tetrachloride
Chlorobenzene
o-Dichlorobenzene
p-Dichlorobenzene
1,2-Dichloroethane
1,1-Dichloroethylene
cis-1,2-Dichloroethylene
trans-1,2-Dichloroethylene
Dichloromethane
1,2-Dichloropropane
Ethylbenzene
Styrene
Tetrachloroethene
Toluene
1,2,4-Trichlorobenzene
1,1,1-Trichloroethane
1,1,2-Trichloroethane
Trichloroethene
Vinyl chloride
Xylenes

DBPs
HAA_5
Bromate
Chlorite

SOCs
(40 CFR 141.24)
Alachlor
Atrazine
Benzo(a)pyrene
Carbofuran
Chlordane
2,4-D
Di(2-ethylhexyl)adipate
Di(2-ethylhexyl)phthalate
Dibromochloropropane
Dalapon
Dinoseb
Dioxin (2,3,7,8-TCDD)
Diquat
Endothall
Endrin
Ethylenedibromide
Glyphosate
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Hexachlorocyclopentadiene
Lindane
Methoxychlor
Oxamyl
PCBs (as decachlorobiphenyl)
Pentachlorophenol
Picloram
Simazine
Toxaphene
2,4,5-TP

RADIONUCLIDES
(40 CFR 141.25)
Gross Alpha
Uranium
Gross Beta
Cesium-134
Strontium-89
Iodine-131
Strontium-90
Tritium
Other beta/photon emitters
Radium-226/228

MICROORGANISMS
(40 CFR 141.21)
Total coliforms
Escherichia coli or fecal coliforms
Heterotrophic bacteria

Appendix F

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460
Office of Ground Water and Drinking Water
Technical Support Center

OCT 1 2002

OFFICE OF WATER

MEMORANDUM

SUBJECT: Update on the Use of National Environmental Laboratory Accreditation Conference (NELAC) Standards for Certification of Laboratories Analyzing Drinking Water Samples

FROM: Cynthia C. Dougherty, Director
Office of Ground Water and Drinking Water

TO: Regional Drinking Water Representatives (Regions I-X)
Regional Laboratory Certification Officers (Regions I-X)

It has been almost five years since my memorandum of October 20, 1997 supporting the use of NELAC standards and I would like to report to you on recent developments.

Following NELAC program implementation, the Office of Ground Water and Drinking Water's (OGWDW's) Laboratory Certification Team conducted a review of 16 laboratory audits performed by eight of the NELAC Accrediting Authorities. The findings of this review were presented to the Accrediting Authorities during the "NELAC 8" meeting in Tampa, Florida on July 11, 2002. Significantly, the review verified equivalency to the criteria in OGWDW's "Manual for the Certification of Laboratories Analyzing Drinking Water," EPA 815-B-97-001, March 1997.

The NELAC standards, as amended through July 2002, may therefore be used as alternative guidance for use by States in the certification of laboratories under the Safe Drinking Water Act (SDWA). I continue to support the use of the NELAC standards in the certification of laboratories analyzing drinking water samples. Further, the use of NELAC standards fosters and increases the opportunity for national consistency. One of the Agency's primary goals in participating in NELAC is to encourage States to recognize certification of laboratories by other States, referred to as "reciprocity."

I would like to emphasize that NELAC is a voluntary program and that States may choose to continue to certify under the existing program based upon the criteria in OGWDW's aforementioned certification manual. Both options are acceptable in terms of maintaining primacy and producing data for compliance purposes.

I congratulate NELAC on their successful implementation of the standards and strongly encourage future reviews of the laboratory audits. Reviews conducted on a periodic basis, random in nature, and weighted toward larger programs, will allow continued assessment of equivalency and promote greater consistency within the program. I reiterate that the drinking water program will benefit nationwide through State participation in the accreditation program.

If you have questions concerning the drinking water laboratory certification program or its relationship to NELAC, please contact Caroline Madding at 513-569-7402.

cc: Nanci Gelb (4601M)
Ephraim King (4607M)
Gregory Carroll (Ci-TSC-140)
Ed Glick (Ci-TSC-140)
Patricia Hurr (Ci-TSC-140)
Caroline Madding (Ci-TSC-140)

Appendix G

Analytical Methods for Microbiology

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

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

(f) *Analytical methodology.*

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

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

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

Organisms	Methodology[12]	Citation[1]
Total Coliforms[2]	Total Coliform Fermentation Technique[3,4,5]	9221 A, B
	Total Coliform Membrane Filter Technique[6]	9222 A, B, C
	Presence-Absence (P-A) Coliform Test[5,7]	9221 D
	ONPG-MUG Test[8]	9223
	Colisure Test[9]	
	E*Colite® Test[10]	
	m-ColiBlue24® Test[11]	
	Readycult® Coliforms 100[13]	
	Membrane Filter Technique using Chromocult® Coliform Agar[14]	
	Colitag Test[15]	

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

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

[2]The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10 deg. C during transit.

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

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

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

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

[7] Six-times formulation strength may be used if the medium is filter-sterilized rather than autoclaved.
[8] The ONPG-MUG Test is also known as the Autoanalysis Colilert System.
[9] A description of the Colisure Test, Feb 28, 1994, may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092. The Colisure Test may be read after an incubation time of 24 hours.
[10] A description of the E*Colite® Test, "Presence/Absence for Coliforms and *E. Coli* in Water," Dec 21, 1997, is available from Charm Sciences, Inc., 36 Franklin Street, Malden, MA 02148-4120.
[11] A description of the m-ColiBlue24® Test, Aug 17, 1999, is available from the Hach Company, 100 Dayton Avenue, Ames, IA 50010.
[12] EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the method(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for β-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent confirmation tests. False-positive and false- negative information is often available in published studies and/or from the manufacturer(s).
[13] The Readycult® Coliforms 100 Presence/Absence Test is described in the document, "Readycult® Coliforms 100 Presence/Absence Test for Detection and Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters," (November 2000, Version 1.0) and is available from EM Science [now EMD Chemicals, Inc.], an affiliate of Merck KGgA, Darmstadt Germany), 480 S. Democrat Road, Gibbstown, NJ 08027-1297. Telephone number is (800) 222-0342, E-Mail address is: adellenbusch@emscience.com. [E-Mail address is now adellenbusch@emdchemicals.com. Website is www.emdchemicals.com]
[14] Membrane Filter Technique using Chromocult® Coliform Agar is described in the document, "Chromocult® Coliform Agar Presence/Absence Membrane Filter Test Method for Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters," November 2000, Version 1.0, available from EM Science [now EMD Chemicals, Inc.] (an affiliate of Merck KGgA, Darmstadt Germany), 480 S. Democrat Road, Gibbstown, NJ 08027-1297. Telephone number is (800) 222-0342, E-Mail address is: adellenbusch@emscience.com. [E-Mail address is now adellenbusch@emdchemicals.com. Website is www.emdchemicals.com]
[15] Colitag® product for the determination of the presence/absence of total coliforms and *E. coli* is described in "Colitag® Product as a Test for Detection and Identification of Coliforms and *E. coli* Bacteria in Drinking Water and Source Water as Required in National Primary Drinking Water Regulations," August 2001, available from CPI International, Inc., 5580 Skylane Blvd., Santa Rosa, CA, 95403, telephone (800) 878-7654, Fax (707) 545-7901, Internet address http://www.cpiinternational.com.

(4) [Reserved]

(5) Public water systems must conduct fecal coliform analysis in accordance with the following procedure. When the MTF Technique or Presence-Absence (PA) Coliform Test is used to test for total coliforms, shake the lactose-positive presumptive tube or P-A vigorously and transfer the growth with a sterile 3-mm loop or sterile applicator stick into brilliant green lactose bile broth and EC medium to determine the presence of total and fecal coliforms, respectively. For EPA-approved analytical methods which use a membrane filter, transfer the total coliform-positive culture by one of the following methods: remove the membrane containing the total coliform colonies from the substrate with a sterile forceps and carefully curl and insert the membrane into a tube of EC medium (the laboratory may first remove a small portion of selected colonies for verification), swab the entire membrane filter surface with a sterile cotton swab and transfer the inoculum to EC medium (do not leave the cotton swab in the EC medium), or inoculate individual total coliform-positive colonies into EC Medium. Gently shake the inoculated tubes of EC medium to insure adequate mixing and incubate in a waterbath at 44.5± 0.2° C for 24 ± 2 hours. Gas production of any amount in the inner fermentation tube of the EC medium indicates a positive fecal coliform test. The preparation of EC medium is described in Method 9221 E (paragraph 1a) in *Standard Methods for the Examination of Water and Wastewater*, 18th edition (1992), 19th edition (1995), and 20th edition (1998); the cited method in any one of these three editions may be used. Public water systems need only determine the presence or absence of fecal coliforms; a determination of fecal coliform density is not required.

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

methods:

(i) EC medium supplemented with 50 µg/mL of 4-methylumbelliferyl-beta-D-glucuronide (MUG) (final concentration), as described in Method 9222G in Standard Methods for the Examination of Water and Wastewater,19th edition (1995) and 20th edition (1998). Either edition may be used. Alternatively, the 18th edition (1992) may be used if at least 10 mL of EC medium, as described in paragraph (f)(5) of this section, is supplemented with 50 µg/mL of MUG before autoclaving. The inner inverted fermentation tube may be omitted. If the 18th edition is used, apply the procedure in paragraph (f)(5) of this section for transferring a total coliform-positive culture to EC medium supplemented with MUG, incubate the tube at 44.5 ± 0.2°C for 24 ± 2 hours, and then observe fluorescence with an ultraviolet light (366 nm) in the dark. If fluorescence is visible, *E. coli* are present.

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

(iii) Minimal Medium ONPG-MUG (MMO-MUG) Test, as set forth in the article ``National Field Evaluation of a Defined Substrate Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with Presence-Absence Techniques" (Edberg et al.), Applied and Environmental Microbiology, Volume 55, pp. 1003-1008, April 1989. (Note: The Autoanalysis Colilert System is an MMO-MUG test).
If the MMO-MUG test is total coliform-positive after a 24-hour incubation, test the medium for fluorescence with a 366-nm ultraviolet light (preferably with a 6-watt lamp) in the dark. If fluorescence is observed, the sample is *E. coli*-positive. If fluorescence is questionable (cannot be definitively read) after 24 hours incubation, incubate the culture for an additional four hours (but not to exceed 28 hours total), and again test the medium for fluorescence. The MMO-MUG
Test with hepes buffer in lieu of phosphate buffer is the only approved formulation for the detection of *E. coli*.

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

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

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

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

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

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

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

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

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

(a) *Analytical requirements.* Only the analytical method(s) specified in this paragraph, or otherwise approved by EPA, may be used to demonstrate compliance with §§141.71, 141.72 and 141.73. Measurements for pH, turbidity, temperature and residual disinfectant
concentrations must be conducted by a person approved by the State. Measurement for total coliforms, fecal coliforms and HPC must be conducted by a laboratory certified by the State or EPA to do such analysis. Until laboratory certification criteria are developed for the
analysis of fecal coliforms and HPC, any laboratory certified for total coliforms analysis by the State or EPA is deemed certified for fecal coliforms and HPC analysis. The following procedures shall be conducted in accordance with the publications listed in the following section.
This incorporation by reference was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies of the methods published in *Standard Methods for the Examination of Water and Wastewater* may be obtained from the American Public Health Association et al., 1015 Fifteenth Street, NW., Washington, DC 20005; copies of the Minimal Medium ONPG-MUG Method as set forth in the article ``National Field Evaluation of a Defined Substrate Method for the Simultaneous Enumeration of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with the Standard Multiple Tube Fermentation Method" (Edberg et al.), Applied and Environmental Microbiology, Volume
54, pp. 1595-1601, June 1988 (as amended under Erratum, Applied and Environmental Microbiology, Volume 54, p. 3197, December, 1988), may be obtained from the American Water Works Association Research Foundation, 6666 West Quincy Avenue, Denver, Colorado, 80235; and copies of the Indigo Method as set forth in the article ``Determination of Ozone in Water by the Indigo Method" (Bader and Hoigne), may be obtained from Ozone Science & Engineering, Pergamon Press Ltd., Fairview Park, Elmsford, New York 10523. Copies may be inspected at the U.S. Environmental Protection Agency, Room EB15, 401 M St., SW., Washington, DC 20460 [Note: current location of EPA's Drinking Water Docket is 1301 Constitution Avenue, NW., EPA West, Room B102, Washington, DC 20460;] or at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

(1) Public water systems must conduct analysis of pH and temperature in accordance with one of the methods listed at §141.23(k)(1). Public water systems must conduct analysis of total coliforms, fecal coliforms, heterotrophic bacteria, and turbidity in accordance with one of the following analytical methods and by using analytical test procedures contained in *Technical Notes on Drinking Water Methods*, EPA-600/R-94-173, October 1994, which is available at NTIS PB95-104766.

Organism	Methodology	Citation[1]
Total Coliform[2].....	Total Coliform Fermentation Technique[3,4,5]	9221 A. B. C
	Total Coliform Membrane Filter Technique[6]	9222 A, B, C
	ONPG-MUG Test [7]	9223
Fecal Coliforms[2].....	Fecal Coliform Procedure[8]	9221 E
	Fecal Coliform Filter Procedure	9222 D
Heterotrophic bacteria[2]..	Pour Plate Method	9215 B
	SimPlate[11]	
Turbidity	Nephelometric Method	2130 B
	Nephelometric Method	180.1[9]
	Great Lakes Instruments	Method 2[10]
	Hach FilterTrak	10133[12]

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

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

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

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

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

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

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

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

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

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

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

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

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

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