DEPARTMENT OF PUBLIC HEALTH

NOTICE OF PROPOSED AMENDMENTS

1) Heading of the Part: Certification and Operation of Environmental Laboratories

2) Code Citation: 77 Ill. Adm. Code 465

3)

Section Numbers:	Proposed Action:
465.120	Amend
465.125	Amend
465.200	Amend
465.310	Amend
465.330	Amend
465.340	Amend
465.350	Amend
465.360	Amend
465.370	Amend
465.390	Amend
465.400	Amend
465.430	Amend
465.Appendix A	Repeal

4) Statutory Authority: Implementing Section 1401(1)(D) of the Safe Drinking Water Act (42 U.S.C. 300f(1)(D)), Subpart C of the National Primary Drinking Water Regulations (40 CFR 141.21 through 141.30 (1991)), the Illinois Environmental Protection Act [415 ILCS 5] and the Civil Administrative Code of Illinois [20 ILCS 5], and authorized by Sections 4(o) and (p) of the Illinois Environmental Protection Act [415 ILCS 5/4(o) and (p)] and Sections 2310-575, 2310-580, and 2310-30 of the Civil Administrative Code of Illinois [20 ILCS 2310].

5) A Complete Description of the Subjects and Issues Involved:

To establish compliance with 40 CFR Parts 141 and 142, National Primary Drinking Water Regulations: Revisions to the Total Coliform Rule as published in the Federal Register, Vol. 78, No. 30, February 13, 2013 by:

- Updating reference 40 CFR 141, 142, National Primary Drinking Water Regulations: Revisions to the Total Coliform Rule (Including E. coli), April 15, 2013)
- Updating referenced versions of Standard Methods for the Examination of Water and Wastewater eliminating the 18th and 19th editions and adding the 21st and online editions.

- Repealing Appendix A – Colisure is referenced in added versions of Standard Methods
- Replacing Colitag method with Modified Colitag method
- Eliminating Fluorocult LMX, Coliscan, and R2A

Adding certification for Cryptosporidium by:

- Adding reference to the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule) (40CFR 9, 141, 142)
- Adding reference to "Supplement 2 to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water", November 2012, known as EPA 815-F-08-006
-

Updating Section 465.360 to contain all methods referenced in federal or state rules.

Adding reference to "Supplement 1 to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water", June 2008, known as EPA 815-F-08-006.

The economic effect of this proposed rulemaking is unknown. Therefore, the Department requests any information that would assist in calculating this effect.

The Department anticipates adoption of this rulemaking approximately six to nine months after publication of the Notice in the *Illinois Register*.

6) Published studies or reports, and sources of underlying data used to compose this rulemaking: None

7) Will this rulemaking replace any emergency rulemaking currently in effect? No

8) Does this rulemaking contain an automatic repeal date? No

9) Does this rulemaking contain incorporations by reference? Yes

10) Are there any other proposed rulemakings pending on this Part? No

11) Statement of Statewide Policy Objectives: This rulemaking will impose a State Mandate on units of local government.

DEPARTMENT OF PUBLIC HEALTH

NOTICE OF PROPOSED AMENDMENTS

12) Time, Place and Manner in which interested persons may comment on this proposed rulemaking:

Interested persons may present their comments concerning this rulemaking within 45 days after the publication of this issue of the *Illinois Register* to:

Susan Meister
Division of Legal Services
Illinois Department of Public Health
535 W. Jefferson St., 5th floor
Springfield, Illinois 62761

217/782-2043
e-mail: dph.rules@illinois.gov

13) Initial Regulatory Flexibility Analysis:

A) Types of small businesses, small municipalities and not for profit corporations affected: Laboratories certified for water microbiology

B) Reporting, bookkeeping or other procedures required for compliance: Laboratory quality control and reports of sample analysis

C) Types of professional skills necessary for compliance: *Cryptosporidium* supervisors and analysts must have bench experience with *Cryptosporidium* and FA microscopy and with using EPA method 1623 or 1623.1.

14) Regulatory Agenda on which this rulemaking was summarized: July 2013

The full text of the Proposed Amendments begins on the next page:

DEPARTMENT OF PUBLIC HEALTH

NOTICE OF PROPOSED AMENDMENTS

TITLE 77: PUBLIC HEALTH
CHAPTER I: DEPARTMENT OF PUBLIC HEALTH
SUBCHAPTER d: LABORATORIES AND BLOOD BANKS

PART 465
CERTIFICATION AND OPERATION OF ENVIRONMENTAL LABORATORIES

SUBPART A: GENERAL PROVISIONS

Section

SUBPART B: MICROBIOLOGICAL ANALYSES
OF PUBLIC WATER SUPPLY SAMPLES

Section

DEPARTMENT OF PUBLIC HEALTH

NOTICE OF PROPOSED AMENDMENTS

AUTHORITY: Implementing Section 1401(1)(D) of the Safe Drinking Water Act (42 U.S.C. 300f(1)(D)), Subpart C of the National Primary Drinking Water Regulations (40 CFR 141.21 through 141.30 (1991)), the Illinois Environmental Protection Act [415 ILCS 5] and the Civil Administrative Code of Illinois [20 ILCS 5], and authorized by Sections 4(o) and (p) of the Illinois Environmental Protection Act [415 ILCS 5/4(o) and (p)] and Sections 2310-575, 2310-580, and 2310-30 of the Civil Administrative Code of Illinois [20 ILCS 2310].

SOURCE: Adopted at 22 Ill. Reg. 14294, effective July 15, 1998; amended at 35 Ill. Reg. 14494, effective August 12, 2011; amended at 38 Ill. Reg. _____, effective _____.

SUBPART A: GENERAL PROVISIONS

Section 465.120 Definitions

For purposes of this Part unless otherwise specifically defined or the context clearly requires a different meaning:

"Act" means Sections 4(o) and (p) of the Environmental Protection Act [415 ILCS 5/4(o) and (p)].

"American Association for Laboratory Accreditation (A2LA)" means an association that offers accreditation of proficiency testing providers, located at 5301 Buckeystown Pike, Suite 350, Frederick, MD 21704, 301-644-3248

"Analyst" means any person who performs analyses for certain or all parameters on samples submitted to the environmental laboratory and who meets the qualifications set forth in Section 465.310(b).

"ASTM International (ASTM)" means ~~"ASTM" means the American Society for Testing and Materials West Conshohocken PA,~~ a not-for-profit, voluntary

standards development system, located at 100 Barr Harbor Drive, P.O. Box C700, West Conshocken, PA.

"Certification" means a status of approval granted to an environmental laboratory that meets the criteria established by this Part or in accordance with a reciprocity agreement entered into pursuant to Section 465.240. Certification is not a guarantee of the validity of the data generated.

"Certification Officer" means any person who is designated by the Department to inspect and evaluate environmental laboratories for compliance in meeting the criteria set forth in this Part. Certification officers shall meet the educational and experience qualifications for laboratory supervisors as set forth in Section 465.310(a).

"Department" means the Illinois Department of Public Health.

"Deficiency" means a failure of an environmental laboratory to meet any requirement of this Part.

"Environmental Laboratory" means any facility that performs analyses on environmental samples ~~in order~~ to determine the quality of food, milk, public water supplies, surface water, ground water, recreational waters, wastewater, air, or land.

"General Education Development (GED) tests" means a group of five subject tests that, when passed, certify that the test taker has American or Canadian high school-level skills.

"Laboratory Pure Water" means water meeting the standards set forth in Section 465.380.

"Laboratory Supervisor" means a person who supervises the performance of the analytical procedures within an environmental laboratory and who meets the qualifications set forth in Section 465.310(a).

"Major Remodeling" means any remodeling of the laboratory facility that requires the acquisition of a local building permit.

~~"MUG" means 4-methyl-umbelliferyl-beta-d-glucuronide.~~

~~"NIST" means the "~~United States Department of Commerce, Technology ~~Administration, National Institute of Standards and Technology~~ (NIST), ~~(formerly National Bureau of Standards) .~~

"P-A Coliform Test" means "Presence-Absence Coliform Test".

"Proficiency Testing Samples ~~or "~~(PTs)" means samples provided to a laboratory for the purpose of demonstrating that the laboratory can successfully analyze the sample within acceptance limits specified in 40 CFR 141.2 . The ~~qualitative and/or quantitative~~ composition of the reference material is unknown to the laboratory at the time of the analysis.

"Provisional Certification" means a certification status granted to an environmental laboratory ~~in order~~ to allow time for the correction of a deficiency. Failure to correct a deficiency during the provisional certification period allows the Department to revoke certification as specified in Section 465.180. While on provisional certification, an environmental laboratory remains approved for the analyses covered by its certification.

"Public Water Supply" means all mains, pipes and structures through which water is obtained and distributed to the public, including wells and well structures, intakes and cribs, pumping stations, treatment plants, reservoirs, storage tanks and appurtenances, collectively or severally, actually used or intended for use for the purpose of furnishing water for drinking or general domestic use and that serve at least 15 service connections or that regularly serve at least 25 persons at least 60 days per year.

"Quality Assurance" means 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 Assurance Plan" means 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.

"Quality Control" means 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.

"Readily Accessible" means that the referenced item is located upon the premises.

~~"Standard Methods" means "Standard Methods for the Examination of Water and Wastewater," 21st Edition, 2005, American Public Health Association, 1015 Fifteenth Street, NW, Washington DC 20001, 202-628-8303.~~

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

~~"State" means the Illinois Environmental Protection Agency for Community Public Water Supply samples and Illinois Department of Public Health for Non-Community Public Water Supply samples.~~

"Too numerous to count" (TNTC) means ~~"too numerous to count" or~~ greater than 200 colonies on the membrane filter in the absence of detectable coliforms when analyzing drinking water for total coliforms.

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.125 Incorporated and Referenced Materials

~~a)~~ ~~Abbreviations and short-name listing of references. The following names and abbreviated names, presented in alphabetical order, are used in this Part to refer to materials incorporated by reference:~~

~~1)~~ ~~"Colitag® Test" means "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," 2004, available from CPI International, 5580 Skylane Boulevard, Santa Rosa CA 95403, 707-525-5788.~~

~~2)~~ ~~"Membrane Filter Technique using Chromocult Coliform Agar" means Chromocult Coliform Agar Presence/Absence Membrane Filter Test Method for Detection and Identification of Coliform Bacteria and Escherichia coli in Finished Waters, November 2000. Version 1.0, available from EMD Chemicals Incorporated, 480 South Democrat Road, Gibbstown NJ 08027, 800-222-0342.~~

~~3)~~ ~~"ONPG-Mug Test" (meaning "minimal medium ortho-nitrophenyl-beta-d-galactopyranoside-4-methyl-umbelliferyl-beta-d-glucuronide test"), also called the "Autoanalysis Colilert System," is Method 9223, available in "Standard Methods for the Examination of Water and Wastewater," 21st Edition, 2005, from American Public Health Association, 1015 Fifteenth Street, NW, Washington DC 20001, 202-628-8303.~~

~~4)~~ ~~"New medium for the simultaneous detection of total coliform and Escherichia Coli in water" by Brenner, K.P., et al., 1993, Applied and Environmental Microbiology 59:3534-3544. EPA Method 1604, which can be found online at www.epa.gov/microbes, is identical.~~

~~5)~~ ~~"Readycult Coliforms 100 Presence/Absence Test" and "Fluorocult LMX" means "Readycult Coliforms 100 Presence/Absence Test for Detection and Identification of Coliform Bacteria and Escherichia coli in Finished Waters," Version 1.1, 2007, available from EMD Chemicals Incorporated, 480 South Democrat Road, Gibbstown NJ 08027, 800-222-0342.~~

~~6)~~ ~~"SimPlate Method" means "IDEXX SimPlate™ HPC Test Method for Heterotrophs in Water," approved under USEPA 40 CFR 141.74, Vol. 97, No. 209, Oct. 29, 2002, and as included in Standard Methods for Water and Wastewater, On-Line Edition, Section 9215E, available from IDEXX Laboratories, Incorporated, One IDEXX Drive, Westbrook ME 04092, 800-321-0207 www.idexx.com.~~

~~7)~~ ~~"Standard Methods" means "Standard Methods for the Examination of Water and Wastewater," (referred to as "Standard Methods"). American Public Health Association, 1015 Fifteenth Street, NW, Washington DC 20001, 202-628-8303.~~

a~~b~~) The following publications and federal regulations are incorporated by reference:

DEPARTMENT OF PUBLIC HEALTH

NOTICE OF PROPOSED AMENDMENTS

1) "Chromocult Coliform Agar Presence/Absence Membrane Filter Test Method for Detection and Identification of Coliform Bacteria and Escherichia coli in Finished Waters," November 2000, Version 1.0~~.~~; available from EMD Chemicals Inc. (an affiliate of Merck KGaA, Darmstadt, Germany), 480 S. Democrat Road, Gibbstown NJ 08027-1297~~,~~ ~~Telephone:~~ 800-222-0342. www.emdchemicals.com .

2) "Readycult Coliforms 100 Presence/Absence Test for Detection and Identification of Coliform Bacteria and Escherichia coli in Finished Waters," Version 1.1 2007~~,~~; available from EMD Chemicals Inc., 480 S. Democrat Road, Gibbstown NJ 08027-1297~~,~~ ~~Telephone:~~ 800-222-0342. www.emdchemicals.com .

3) "IDEXX SimPlate™ HPC Test Method for Heterotrophs in Water"(SimPlate Method), November 2000. IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092~~,~~ ~~Telephone:~~ 800-321-0207.

~~4) United States Environmental Protection Agency - Manual for the Certification of Laboratories Analyzing Drinking Water, 5th edition, January 2005, U.S. Environmental Protection Agency, Ariel Rios Building, 1200 Pennsylvania Avenue, NW, Washington DC 20460, 202-272-0167.~~

4) "Membrane Filtration Method m-ColiBlue24® Broth" (m-ColiBlue24®), Revision 2, August 17th, 1999; available from Hach Company, P.O. Box 389, Loveland, CO 80539, 800-604-3493.

5) Method 1604: Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium), September 2002, known as EPA 821-R-02-024~~,~~; available from the U.S. Environmental Protection Agency, Ariel Rios Building, 1200 Pennsylvania Avenue, NW, Washington DC, 20460, 202-272-0167.

6) Method 1623 Cryptosporidium and Giardia in Water by Filtration/IMS/FA, December 2005, known as EPA 815-R-05-002; available from the U.S. Environmental Protection Agency, Ariel Rios Building, 1200 Pennsylvania Avenue, NW, Washington DC, 20460, 202-272-0167.

7) Method 1623.1 Cryptosporidium and Giardia in Water by Filtration/IMS/FA, January 2012, known as EPA 816-R-12-001; available from the U.S Environmental Protection Agency, Ariel Rios Building, 1200 Pennsylvania Avenue, NW, Washington DC, 20460, 202-272-0167.

8) "Charm E*Colite™ Presence/Absence Test for Detection and Identification of Coliform Bacteria and Escherichia coli in Drinking Water" (E*Colite*®), January 9th, 1998; available from Charm Sciences, Inc., 659 Andover Street, Lawrence, MA 01843-1032, 800-343-2170.

9) "Modified Colitag™ Product as a Test for Detection and Identification of Coliforms and E. coli Bacteria in Drinking Water and Source Water as Required in the National Primary Drinking Water Regulations" (Modified Colitag®); available from CPI International, 5580 Skylane Boulevard, Santa Rosa, CA 95403, 707-525-5788.

10~~6~~) Manual for the Certification of Laboratories Analyzing Drinking Water," USEPA 570/9-90/008A, 5th Edition (January 2005). A copy of this manual can be obtained by contacting the U.S. Environmental Protection Agency, Ariel Rios Building, 1200 Pennsylvania Avenue, NW, Washington DC ~~20465~~, 20460, 202-272-0167.

11) Supplement 1 to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water, June 2008, known as EPA 815-F-08-006; available from the U.S Environmental Protection Agency, Ariel Rios Building, 1200 Pennsylvania Avenue, NW, Washington DC, 20460. Telephone: 202-272-0167.

12 Supplement 2 to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water, November 2012, known as EPA 815-F-12-006; available from the U.S Environmental Protection Agency, Ariel Rios Building, 1200 Pennsylvania Avenue, NW, Washington DC, 20460. Telephone: 202-272-0167.

13~~7~~) United States Environmental Protection Agency National Primary Drinking Water Regulations (40 CFR 141), July 2006~~,~~; available from the U.S. Environmental Protection Agency, Ariel Rios Building, 1200 Pennsylvania Avenue, NW, Washington DC 20460, 202-272-0167.

148) Occupational Safety and Health Standards (29 CFR 1910), July 2007~~,~~; available from the U.S. Department of Labor, Occupational Safety & Health Administration, 200 Constitution Avenue, NW, Washington DC 20210.

~~9) 40 CFR 141, 142, National Primary Drinking Water Regulations; Total Coliforms (Including Fecal Coliforms and E. coli) (June 29,1989).~~

~~10) 40 CFR 9, 141, 142, National Primary Drinking Water Regulations: Interim Enhanced Surface Water Treatment (December 16, 1998).~~

15) 40 CFR 141,142, National Primary Drinking Water Regulations; Revisions to the Total Coliform Rule (February 13, 2012).

1611) 40 CFR 9, 141, 142 National Primary Drinking Water Regulations: Ground Water Rule (November 8, 2006).

17) 40 CFR 9, 141,142, National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule (January 5, 2006).

1812) Good Automated Laboratory Practices, known as EPA 2185, Office of Information Management, Research Triangle Park NC 27711, August 10, 1995.

19) Standard Methods for the Examination of Water and Wastewater, either the 20th Edition, 1998; 21st Edition, 2005; or 22nd Edition, 2012; and online version as cited per method in 40 CFR 141 and 142, February 13, 2013; available from the American Public Health Association, 1015 Fifteenth Street, NW, Washington DC 20001.

20) ASTM E617-13, Standard Specification for Laboratory Weights and Precision Mass Standards; available from ASTM International (ASTM); 100 Barr Harbor Drive, P.O. Box C700, West Conshohocken, PA, 610-832-9500, www.astm.org.

21) NIST Handbook 150-2G, National Voluntary Laboratory Accreditation Program, Calibration Laboratories, Technical Guide for Mechanical Measurements, March 2004; available from National Voluntary Laboratory Accreditation Program, National Institute of Standards and

Technology, 100 Bureau Drive, Stop 2140, Gaithersburg, MD, 20899-2140, 301-975-4016.

be) These incorporations by reference refer to the edition of the document on the date specified and do not include any subsequent amendments or editions.

cd) The following laws and rules are referenced in this Part:

1) Safe Drinking Water Act (42 USC 300f(1)(D))

2) Civil Administrative Code of Illinois [20 ILCS 5]

3) Illinois Environmental Protection Act [415 ILCS 5]

4) Illinois Plumbing Code, Illinois Department of Public Health (77 Ill. Adm. Code 890)

5) Primary Drinking Water Standards, Pollution Control Board (35 Ill. Adm. Code 611)

6) Electronic Commerce Security Act [5 ILCS 175]

7) Local Records Act [50 ILCS 205]

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.200 Proficiency Testing Samples (PTs)

a) An environmental laboratory is required to participate in proficiency testing samples (PTs) analyses for each analytical parameter or method for which it seeks or wishes to maintain certification in accordance with the certification procedures of Section 465.130(c), the certification renewal procedures of Section 465.140(a), and the quality assurance requirements contained in Subpart B ~~of this Part~~.

b) Heterotrophic plate count and coliform Microbiological Water Supply (WS) PT samples shall be analyzed annually (every 12 months). Cryptosporidium PT samples shall be analyzed every four to six months. PT samples shall be analyzed in the same manner as routine samples. The laboratory shall document ~~be able to~~

~~provide documentation~~ that the analyst analyzing any PT sample is a laboratory employee who routinely analyzes drinking water compliance samples.

c) Laboratories shall acquire the PT sample from a provider accredited under A2LA ~~a supplier acceptable to the Department~~.

d) For methods used to test the presence or absence of an organism in a sample, each set shall contain 10 ~~ten~~ samples, all shipped at the same time in either a lyophilized, dehydrated, or aqueous state. The set shall include samples, in various combinations, that contain total coliforms, fecal coliforms, E. coli, non-coliforms, and at least one blank. Each set shall be used only with a single analytical method. For a PT result to be acceptable, the laboratory shall have no false negative results and no more than one false positive result for each set. ~~To be acceptable, a laboratory shall correctly analyze a minimum of nine of ten samples, with no false negative result (i.e., a single false positive result may be acceptable). For quantitative methods, one PT sample may be analyzed.~~

e) For quantitative methods, each set shall contain one sample. For a PT to be acceptable, the laboratory result shall be statistically acceptable as determined by the PT provider.

f~~e~~) Unless otherwise specified in Subpart B ~~of this Part~~, within 60 days after receipt of a PT sample, the environmental laboratory shall analyze the sample and report the test results to the PT provider ~~Department~~. The PT provider shall submit the laboratory's results and acceptable ranges to the Department. No fee shall be charged to the Department ~~for the~~ ~~analyses~~.

(Source: Amended at 38 Ill. Reg. _____, effective _____)

SUBPART B: MICROBIOLOGICAL ANALYSES
OF PUBLIC WATER SUPPLY SAMPLES

Section 465.310 Personnel Requirements

a) The microbiology laboratory supervisor shall have ~~be a person holding~~ a minimum of a bachelor's degree in microbiology, biology, chemistry, or related natural or physical science field, shall have completed a training course conducted or approved by the Department, and shall have received Department approval to

serve as laboratory supervisor. In addition, the laboratory supervisor shall have had a minimum of 80 hours of on-the-job training in water microbiology at a certified laboratory. The supervisor shall demonstrate the ability to properly perform representative test procedures under his or her supervision while under observation by the certification officer. A laboratory supervisor shall be a full-time employee who is on-site at the certified laboratory. If the laboratory supervisor position becomes vacant, then a replacement supervisor shall be in place within 60 days.

b) The parasitology principal analyst/supervisor shall have a minimum of a bachelor's degree in microbiology or a closely related field, shall have a minimum of one year of bench experience with Cryptosporidium and immunofluorescence assay (FA) microscopy, a minimum of six months experience using Method 1623 or 1623.1, and have analyzed a minimum of 100 samples using Method 1623 or 1623.1. The principal analyst/supervisor shall participate in a monthly analyst verification, supervise and verify the processing and microscopy in the laboratory and may perform the same duties as an analyst. The prinicipal analyst/supervisor shall ensure that all laboratory personnel are able to perform the analyses to which they are assigned and that all data reported by the laboratory meet the required quality assurance and regulatory criteria.

c~~b~~) A microbiology analyst ~~is a person who~~ performs microbiological analyses on water, shall have ~~has~~ a minimum of a high school diploma ~~in academic or laboratory oriented vocational courses~~, and shall have ~~has had a minimum of three months~~ of ~~bench experience in a microbiological analytical laboratory. The analyst shall have~~ a minimum of 30 days of on-the-job training in drinking water microbiology under an experienced analyst. In addition, an analyst shall be able to perform representative test procedures with which he or she is involved while under the observation of the certification officer. Analysts shall be under the direct supervision of the laboratory supervisor. Before analyzing compliance samples, the analyst shall demonstrate acceptable results on samples spiked with known culture controls.

d) A parasitology analyst establishes Kohler illumination for the microscope, may perform the same duties as a technician and is able to examine samples using the microscope. An analyst shall have a minimum of two years of college with courses in microbiology or a closely related field, a minimum of six months of bench experience with Cryptosporidium and FA microscopy, and a minimum of

three months of experience using Method 1623 or 1623.1. The analyst shall participate in a monthly analyst verification.

e) A parasitology technician filters samples, performs centrifugation, elution, concentration, and purification using immunomagnetic separation (IMS), and prepares purified samples on slides for microscopic examination; but does not perform microscopic protozoan identification. A technician shall have a minimum of three months of experience in filter extraction and processing of protozoa samples by Method 1623 or 1623.1 and have analyzed a minimum of 50 samples using Method 1623 or 1623.1 for the specific procedures that he or she will be using.

~~e~~f) The Department may waive the need for the academic training required by this Section, on a case-by-case basis, for highly experienced analysts who have passed the GED tests.

g) The Department may ~~also~~ waive the need for the ~~above-specified~~ college education and training required by this Section, on a case-by-case basis, for supervisors of microbiology laboratories that analyze only samples from drinking water systems with which the laboratory is associated. The supervisor shall have a minimum of 10 years experience in water microbiology and shall have demonstrated a working knowledge of Quality Assurance activities as justification for the waiver.

h) The Department may waive college education in lieu of experience for a parasitology supervisor or analyst who has greater than 10 years experience of protozoan identification duties.

i) If a waiver ~~for supervisor~~ is granted, the Department will prepare a written and signed justification for the waiver.

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.330 Laboratory Equipment

Only those instruments that are needed to analyze for the parameters for which the laboratory is being certified are required, but those instruments shall meet the following minimum specifications. A laboratory performing all of the analyses described in Section 465.360 shall

have, or have access to, within the same building, all of the equipment listed in this Section with the minimum specifications cited.

a) A top loading or trip pan balance shall be clean~~,~~ and not corroded~~, and provided with standardized Class S or S-1, or equivalent ASTM 1, 2, or 3, weights, certified by the manufacturer as meeting the requirements established by NIST. The certificate of accuracy shall accompany the weights~~.

1) A torsion or trip pan balance used for weighing materials of 2 grams or more shall detect 100 mg of weight accurately at a 150 gram load.

2) An analytical balance used for weighing quantities of less than 2 grams shall be sensitive to 1 mg at a10 gram load.

b) A magnetic stirrer shall be capable of achieving variable speeds and shall be used with a Teflon-coated stirring bar. The magnetic stirrer may be equipped with a heating element.

c) A pH meter shall have an accuracy of at least ± 0.1 units and a scale readability of at least ± 0.1 units. The pH meter may be either line/bench or battery/portable operated.

d) A conductivity meter and cell combination, suitable for checking laboratory pure water quality, shall be readable in ohms or mhos, and have a range capable of determining the conductivity or resistivity of laboratory pure water as described in Section 465.380(a). The conductivity meter may be either line/bench or battery/portable operated.

e) An autoclave shall be horizontal ~~—~~chambered and shall meet all of the following specifications:

1) When observed during the operational cycle or when time-temperature charts are read, the autoclave shall be in good operating condition;

2) An operating safety valve shall be included;

3) Separate temperature and pressure gauges shall be located on the exhaust side;

4) The autoclave shall reach and maintain a temperature of 121º ± 1º C during the sterilization cycle, and no more than 45 minutes shall be required for a complete cycle of carbohydrate media;

5) Depressurization shall not produce gas bubbles in fermentation media; and

6) Pressure cookers shall not be used.

f) A hot-air sterilization oven shall operate at a minimum of 175º C, shall be equipped with a thermometer inserted through the top porthole or be equipped with a temperature-recording device, and shall be equipped with a thermostatic control that will not allow the temperature to deviate by more than ± 5º C from the temperature setting.

g) An incubation unit shall maintain an internal temperature of 35º ± 0.5º C or 36° ± 1° C or 44.5º ± 0.2º C and shall be of the following type: air or water jacketed incubator, incubator room, water bath, or aluminum block incubator. Incubation units of the aluminum block type shall have culture dishes and tubes that are snug fitting in the block. Water baths shall be circulating with covers. Laboratories that use the enzyme substrate tests with air-type incubators shall note the product incubation details indicated in Section 465.360(h)(7).

h) An ultraviolet (UV) sterilizer shall be free from radiation leaks and shall be UV efficiency tested quarterly as described in "Standard Methods for the Examination of Water and Wastewater." Proper eye protection shall be available for users of the ultraviolet sterilizer. The ultraviolet sterilizer shall not be used as a substitute for an autoclave. The unit shall be disconnected monthly and the lamps cleaned by wiping with a soft cloth moistened with ethanol.

i) A refrigerator shall maintain a temperature of between 1º and 5° C and shall be equipped with a thermometer located on the top shelf. The thermometer shall be graduated in not greater than 1º C increments, and the thermometer bulb shall be immersed in liquid.

j) An agar tempering water bath shall be of appropriate size for holding melted medium and shall be thermostatically controlled at 45º ± 1º C.

k) The following standards shall apply to temperature-monitoring devices:

1) Glass or electronic thermometers shall be graduated in not greater than 0.5º C units for use in 35º or 36° C incubators.

2) Glass or electronic thermometers shall be graduated in not greater than 0.2º C units for use in 44.5º C water baths or aluminum block type incubators.

3) Glass or electronic thermometers shall be graduated in not greater than 1.0º C units for use in 55º to 65º C incubators.

4) Electronic thermometers with thermocouplings and continuous temperature-recording devices shall be sensitive to not greater than 0.5º C when used in 35° or 36° C incubators, shall be sensitive to not greater than 0.2º C when used for 44.5º C water baths or aluminum block type incubators, and shall be sensitive to not greater than 1º C when used on 55º to 65º C incubators.

5) An NIST certified thermometer, or one of equivalent accuracy graduated in 0.2º C or less, shall be available for calibration use and shall be accompanied by its certification papers and procedures for use. All thermometers and temperature-recording devices shall be calibrated annually at temperature of use against the certified thermometer to within ± 1.0º C. NIST thermometers shall be calibrated at least every five years at each temperature of use.

6) Each laboratory shall have a maximum registering thermometer in the range of 80º to 200º C graduated in increments no greater than 1º C.

7) Each laboratory shall use separate thermometers for determining the temperatures of water baths, ovens, autoclaves, samples, refrigerators, storage areas, etc.

8) The liquid column of glass thermometers shall have no separations.

9) Dial thermometers are not permitted.

l) Optical counting equipment shall include a low-power magnification device of the dissecting or stereomicroscope type with a magnification power of 10 to 15 diameters, and an external daylight fluorescent light source for sheen discernment at an angle of 60º to 80º above the colonies.

m) A mechanical hand tally shall be available for counting colonies on membrane filters or agar pour plates.

n) Where metal inoculation loops are used, loops shall be of 22 to 24 gauge chrome, or platinum-iridium wire, with loop diameters of at least 3 mm. Hot-air sterilized wooden applicator sticks, pre-sterilized cotton swabs or pre-sterilized plastic loops may be used.

o) Membrane filter equipment shall be non-leaking, uncorroded, and made of stainless steel, glass, or autoclavable plastic. Disposable single-use equipment made of plastic is also acceptable. Metal plating on membrane filter equipment shall not be worn so as to expose base metal. ~~Calibration shall be checked before first use with Class A graduated cylinders, and a record shall be maintained. Tolerance shall be ± 2.5%.~~

p) Membrane filters shall be white, grid marked, 47 mm diameter, with 0.45 micron pore size, and made from cellulose ester materials. Another pore size may be used if the manufacturer gives performance data equal to or better than the 0.45 micron membrane filter. Membrane filters shall be autoclavable or presterilized.

q) Absorbent pads shall be of uniform thickness to permit 1.8 to 2.2 mL media absorption and shall be autoclavable or presterilized. Filter paper shall be free from growth-inhibiting substances.

r) Forceps used to handle membrane filters and absorbent pads shall have a round tip without corrugations.

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.340 Laboratory Glassware, Plastic Ware and Metal Utensils

a) Except for disposable plastic ware, items shall be resistant to effects of corrosion, high temperature, and vigorous cleaning operations. Metal utensils made of

stainless steel are preferred. Plastic items shall be of inert, non-toxic material and shall retain accurate graduations or calibration marks after repeated autoclaving. Glassware that is used for purposes that may subject it to damage from heat or chemicals shall be of borosilicate glass. All glassware shall be free of chips, cracks, or excessive etching. All volumetric glassware shall be Class A, denoting that it meets federal specifications and is certified by the manufacturer as meeting the standards established by the ~~American Society for Testing and Materials (~~ASTM~~)~~.

b) Graduated cylinders for measurement of sample volumes shall have a tolerance of 2.5% or less. Precalibrated sample containers shall have clearly marked volumes of 2.5% tolerance. ~~The calibration of each precalibrated sample container shall be checked before first use by measuring the volume of 10 calibrated containers per lot.~~

c) Media-preparation utensils shall be of borosilicate glass or stainless steel, and shall be clean and free from foreign residues or dried medium.

d) Micropipettors ~~Micropipetters~~ (also referred to as Mechanical Pipettors or Pipettors ~~Pipetters or Pipetters~~) shall meet the specifications set forth in "Standard Methods for the Examination of Water and Wastewater." Pipets delivering volumes of 10 mL or less shall be accurate to within a 2.5% tolerance. Micropipettors ~~Micropipetters~~ shall be fixed volume and calibrated. Micropepettors ~~Micropipetters~~ shall be used with tips that are sterile. ~~Micropipetters shall be calibrated annually and replaced if the precision or accuracy is greater than 2.5% tolerance. Micropipetters shall be calibrated with 10 consecutive weighings annually (using a separate tip for each weighing), and the average of all 10 weighings shall be ± 2.5% of specified delivery volume. For volumes e 1.0 mL, check volume by using a Class A graduated cylinder.~~ Containers for glass pipets shall be of either stainless steel or aluminum. Opened packages of sterile disposable pipets shall be securely resealed between uses. A pipet aid shall be used when using pipets; mouth pipetting is prohibited. The pipet shall be clean and dry. Pipet aids used to pipet outside of the certified water microbiology testing laboratory shall not be used.

e) Culture dishes shall be sterile and shall be of the tight-lid or loose-lid plastic or loose-lid glass type. In addition, culture dishes shall be of 100 mm x 15 mm (for Plate Count), 50mm x 12 mm, 60 mm x 15 mm, or other appropriate size (for

membrane filter methods), and shall be clear, flat bottomed, and free from bubbles and scratches. Containers for culture dishes shall be of aluminum or stainless steel, or culture dishes shall be wrapped in heavy aluminum foil or char-resistant paper. Open packages of sterile disposable culture dishes shall be securely resealed between uses. Loose-lid dishes shall be incubated in a tight-fitting container, e.g., a plastic vegetable crisper containing a moistened paper towel, to prevent dehydration of membrane filter and medium.

f) Culture tubes shall be of borosilicate glass or other corrosion-resistant glass, and shall be of sufficient size to contain culture medium, as well as the sample portions employed, without being more than three-fourths full. Culture tube closures shall be loose-fitting stainless steel, or plastic caps, or aluminum caps, or plastic screw caps with non-toxic liners. Cotton plugs and foam plugs shall not be used.

g) Dilution bottles shall be of borosilicate glass or other corrosion-resistant glass or autoclavable plastic and shall be free of chips and cracks at the lip. A graduation level shall be distinctly marked on the side of dilution bottles at 99 mL. Dilution bottle closures shall be plastic screw caps with leak-proof liners and shall not produce toxic substances during the sterilization process. ~~The accuracy of dilution blank volumes shall be verified by checking one bottle for every 25 prepared or purchased. The tolerance shall be ±2 mL for a 99-mL volume.~~

h) Sample bottles shall be sterile, of plastic or hard glass, and wide mouthed, and shall have a capacity of at least 120 mL (4 oz.) to allow at least a 1-inch head space. Reusable sample bottle closures shall be glass stoppers or screw caps (metal or plastic), capable of withstanding repeated sterilization, with leak-proof liners, and shall not produce toxic substances during the sterilization process. Glass-stoppered bottle closures shall be covered with aluminum foil or char-resistant paper for sterilization. Metal caps with exposed bare metal on the inside shall not be used. Presterilized containers including bags, with or without a dechlorinating reagent, may be used.

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.350 General Laboratory Practices

a) The following requirements shall apply to sterilization procedures:

1) Autoclaving of the following items shall be carried out at 121º ± 1º C for the durations specified below:

Item	Minimum duration of autoclaving at 121° ± 1° C
Membrane filters and pads	10 minutes
Carbohydrate-containing media (lauryl tryptose, brilliant green lactose bile broth, etc.)	12-15 minutes
Contaminated materials and discarded tests	30 minutes
Membrane filter assemblies (wrapped), sample collection bottles (empty), and individual glassware items	15 minutes
Rinse water volumes of 500 mL to 1000 mL	45 minutes
Rinse water volumes in excess of 1000 mL	Time adjusted for volume; check for sterility
Dilution water blanks	15 minutes

2) Membrane filters and pads and all media shall be removed from the autoclave immediately after completion of the sterilization cycle.

3) The maximum elapsed time for exposure of carbohydrate-containing media to any heat (from the time of closing the loaded autoclave to unloading) shall be 45 minutes.

4) Membrane filter assemblies shall be autoclaved between each sample filtration series. A UV sterilizer or boiling water may be used on

membrane filter assemblies for at least two minutes to prevent bacterial carryover between sample filtrations, but shall not be used as a substitute for autoclaving between sample filtration series.

5) Dried glassware to be sterilized in a hot-air sterilizing oven shall be kept at 175º ± 5º C for at least 2 hours.

6) Empty sample containers shall be moistened with several drops of distilled water before autoclaving to prevent an "airlock" sterilization failure.

b) Laboratory pure water, which may be distilled or deionized, or other processed water shall meet the standards set forth in Section 465.380. Only water determined to be laboratory pure water shall be used for performing bacteriological analyses.

c) Rinse and dilution water shall be prepared in the following manner:

1) A stock phosphate buffer solution of potassium dihydrogen phosphate (KH_2PO_4) and a magnesium chloride solution shall be prepared as specified in "Standard Methods for the Examination of Water and Wastewater." The pH of stock phosphate buffer solution is 7.2 ± 0.5.

2) The phosphate buffer solution and magnesium chloride solution shall be autoclaved or filter sterilized, labeled, dated, and stored at 1º to 5º º C.

3) The stored stock phosphate buffer solution and magnesium chloride solution shall be free of turbidity.

4) Rinse and dilution water shall be prepared by adding 1.25 mL of stock phosphate buffer solution and 5.0 mL of magnesium chloride solution per liter of laboratory pure water.

5) Alternatively, commercially prepared phosphate buffer and magnesium chloride solution may be used when preparing rinse and dilution water. The date received, expiration date, proof of sterility, and pH of phosphate buffer shall be recorded.

d) The following minimum requirements shall be met for storing and preparing

media:

1) Laboratories shall use commercial dehydrated media or commercially manufactured prepared media for routine bacteriological procedures.

2) All media shall be prepared according to the media specifications of "Standard Methods for the Examination of Water and Wastewater."

3) Dehydrated media containers shall be kept tightly closed and stored in a cool, dry location. Discolored or caked dehydrated media shall not be used.

4) All water used shall be laboratory pure water.

5) Dissolution of the media shall be completed before dispensing to culture tubes or bottles.

6) Multiple Tube Fermentation (MTF) media, when prepared in tubes with loose-fitting caps, shall be used within one week after preparation. If MTF media are refrigerated after sterilization, they shall be incubated overnight at 35º C to confirm usability. Tubes of MTF media showing growth or gas bubbles shall be discarded. Refrigerated M Endo agar ~~LES~~ shall be used within two weeks after refrigeration ~~or discarded~~.

7) MTF media in screw cap containers may be held up to three months, provided that the media are stored in the dark and evaporation does not exceed 1.0 mL per 10 mL total volume.

~~8)~~ ~~The laboratory using commercially manufactured prepared media shall record the date received, type of medium, lot number, sample performance when checked against cultures known to give positive and negative results, and pH verification. Media shall be discarded by the manufacturer's expiration date.~~

~~9)~~ ~~Each new lot of prepared commercial medium and each batch of laboratory prepared medium shall be checked before use with positive and negative culture controls. Additionally, each batch of prepared media (whether commercially prepared or laboratory prepared) shall be checked for sterility. Control organisms (total coliform, fecal coliform, and/or E.~~

~~coli, as appropriate) shall be either known stock cultures (periodically checked for purity) or commercially available cultures impregnated with the organism. Results shall be recorded. The following table identifies a few positive and negative culture controls that laboratories might consider.~~

~~**Group**~~	~~**Positive Culture Control**~~	~~**Negative Culture Control**~~
~~Total Coliforms~~	~~Escherichia coli~~ ~~Enterobacter aerogenes~~	~~Staphylococcus aureus~~ ~~Proteus vulgaris~~ ~~Pseudomonas aeruginosa~~
~~Fecal Coliforms~~	~~Escherichia coli~~ ~~Klebsiella pneumoniae (thermotolerant)~~	~~Enterobacter aerogenes~~
~~E. coli~~	~~Escherichia coli (MUG-positive strain)~~	~~Enterobacter aerogenes~~ ~~Klebsiella pneumoniae (thermotolerant)~~
~~Enterococci~~	~~Enterococcus faecalis~~ ~~Enterococcus faecium~~	~~Staphylococcus aureus~~ ~~E. coli~~ ~~Serratia marcesens~~

~~10)~~ ~~Examples of appropriate American Type Culture Collection strains include the following:~~

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

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.360 Methodology

A laboratory shall be certified for all analytical methods listed below that it uses for compliance purposes. At a minimum, the laboratory shall be certified for one total coliform method and one

fecal coliform or E. coli method. In addition, for laboratories that may enumerate heterotrophic bacteria (as measured by the Heterotrophic Plate Count) for compliance with the Surface Water Treatment Rule (SWTR), the laboratory shall be certified for either the Pour Plate Method or the SimPlate method for heterotrophic bacteria.

a) The following methodology, as specified in the listed references, shall be followed for individual parameters:

Method References

Methodology Category	Method	Method Citations				
		RTCR[6,7] (Detect)	SWTR[6] (Count)	LT2 ESWTR[6] (Count)	New Main Construction[2,9] (Detect)	GWR[2,9] (Detect)
Total Coliforms						
Lactose fermentation methods	Standard Total Coliform Fermentation Technique (LTB' BGLB Broth)	9221B.1,B.2[1,2] 9221B.1.B.2-99[4]	9221A,B,C[1,2] 9221A,B,C -99[4]		9221A,B.1[1,2] 9221A,B.1-99[4]	
	Presence-Absence (P-A) Coliform Test (P-A Broth ' BGLB Broth)	9221D.1, D.2[1,2] 9221D.1,D.2-99[4]				
Enzyme substrate methods	Colilert®, Colilert-18®	9223B[1,2] 9223B-97[4]	9223B[3] 9223B-97[4]			
	Colisure®	9223B[1,2] 9223B-97[4]				
	Readycult®	[9]				
	E*Colite®	[9]				
	Modified Colitag™	[9]				

Methodology Category	Method	Method Citations				
		RTCR[6,7] (Detect)	SWTR[6] (Count)	LT2 ESWTR[6] (Count)	New Main Construction[2,9] (Detect)	GWR[2,9] (Detect)
Membrane filtration methods	Standard Total Coliform Membrane Filter Procedure (M-Endo or LES-Endo LTB, BGLB Broth)	9222B[1,2] 9222B-97[4]	9222A,B,[1,2] 9222A,B-97[4]			
	Standard Total Coliform Membrane Filter Procedure (M-Endo)				9222B,.1 9222B.2.a,b,c,d.e[1,2]	
	MI Medium	Method 1604	Method 1604			
	m-ColiBlue24® [9]					
Fecal Coliforms						
Fermentation broth methods	A-1 broth (from mFC LTB A-1 broth)		9221E,[3] 9221E--99[4]			
	EC broth (from mFC LTB EC broth)		9221E,[3] 9221E-99[4]			
Membrane filtration methods	mFC		9222D[3] 9222D-97[4]			
Escherichia coli						

Methodology Category	Method	Method Citations				
		RTCR[6,7] (Detect)	SWTR[6] (Count)	LT2 ESWTR[6] (Count)	New Main Construction[2,9] (Detect)	GWR[2,9] (Detect)
Enzyme substrate methods	Colilert® or Colilert-18®	9223B[1,2] 9223B-97[4]		9223B[1]		9223B[1,2,3]
	Colisure®	9223B[1,2] 9223B-97[4]				9223B[1,2,3]
	E*Colite®	[9]				[9]
	Readycult®	[9]				[9]
	Modified Colitag®	[9]				[9]
Escherichia coli procedure following lactose fermentation methods	EC-MUG medium	9221F.1[1,2]				9221F[3]
Escherichia coli partition method	EC broth with MUG (EC-MUG)	9222G.1c(2)[1,2]		9222G.1c(2)[1]		
	NA-MUG medium	9222G.1c(1)[1,2]		9222G.1c(1)[1]		9222G.1c(1)[1]
Membrane filtration methods	MI Medium	Method 1604		Method 1604		Method 1604
	m-ColiBlue24®	[9]		[9]		[9]
	Chromocult®	[9]				
Heterotrophic Bacteria						
Heterotrophic Plate Count	Pour plate method		9215B[3]			
Multiple enzyme substrate method	SimPlate®		[9]			

Methodology Category	Method	Method Citations				
		RTCR[6,7] (Detect)	SWTR[6] (Count)	LT2 ESWTR[6] (Count)	New Main Construction[2,9] (Detect)	GWR[2,9] (Detect)
Cryptosporidium	Filtration/ IMS/FA			Method 1623[8], Method 1623.1[8]		

Approved Methods	Media	Method[1] Citation	TCR[2] (Detect)	SWTR[2] (Count)	New Main Construction[2] (Detect)
Fermentation broth method	LTB' BGLB Broth	SM9221	X	X	X
	P-A Broth ' BGLB Broth	SM9221	X		
Enzyme substrate methods method	Colilert®; Colilert-18®	SM9223	X	X	
	Colisure®	SM9223	X		
	Readycult® or Fluorocult LMX®		X		
	E*Colite®		X		
	Modified Colitag™®		X		
Membrane filtration methods filter method	Standard Total Coliform Membrane Filter Procedure	SM9222B,C	X	X	X
	M-Endo or LES-Endo ' LTB, BGLB Broth				
	MI Medium	EPA Method 1604	X	X	
	m-ColiBlue24®		X		
	Chromocult®		X		
	Coliscan®		X	X	

Approved Methods	Media	Method[1] Citation	TCR[2,] (Detect)	SWTR[2] (Count)	New Main Construction[2] (Detect)
Fecal Coliforms					
Lactose fermentation methods Fermentation broth method	LTB or P/A broth ' EC broth	(SM 9221B,D) SM9221E	X	X	
	A-1 broth	SM9221E		X	
Membrane filtration methods filter method	M-Endo medium ' EC broth	(SM[1] 9222B) SM[1] 9221E[1,2,3] 9221E-99[4]	X	X	
	mFC	SM[1] 9222D[3] 922D-97[4]		X	
Enzyme substrate methods method	Colilert® or Colilert-18®	SM9223B[1,2,3] 9223B-97[4]	X		
	Colisure®	SM[1,2,3,4] 9223B	X		
	E*Colite®		X		
	Readycult® or Fluorocult LMX®		X		
	LTB, P/A broth, M-Endo ' EC-MUG	(SM[1] 9221B,D; SM[1] 9222B) SM[1,2,3] 9221F	X		
	Modified Colitag®		X		
Membrane filtration methods filter method	MI Medium	EPA Method 1604	X		
	m-ColiBlue24®		X		
	Chromocult®		X		

~~Approved Methods~~	~~Media~~	~~Method[1] Citation~~	~~TCR[2,] (Detect)~~	~~SWTR[2] (Count)~~	~~New Main Construction[2] (Detect)~~
	~~Coliscan®~~		~~X~~		
	~~M-Endo or LES-Endo – NA-MUG~~	~~(SM[1] 9222B) SM[1,2] 9222G~~	~~X~~		
~~Pour plate method~~	~~Plate count agar~~	~~SM[1,2,3,4] 9215B~~		~~X~~	
~~Multiple enzyme substrate method~~	~~SimPlate®~~	~~9~~		~~X~~	
~~Pour plate, spread plate, or membrane filter methods~~	~~R2A~~		~~X[3]~~		

1 ~~SM =~~ Standard Methods for the Examination of Water and Wastewater, ~~18th, 19th or~~ 20th, edition.

2 Standard Methods for the Examination of Water and Wastewater, 21st edition.

3 Standard Methods for the Examination of Water and Wastewater, 22nd edition.

4 Standard Methods for the Examination of Water and Wastewater, online version; the year in which each method was approved by the Standard Methods Committee is designated by the last two digits following the hyphen in the method number. The methods listed are the only online versions that may be used.

5 ~~MC =~~ "Manual for the Certification of Laboratories Analyzing Drinking Water," ~~USEPA 570/9-90/008A, 5th Edition (January 2005). A copy of this manual can be obtained by contacting the U.S. Environmental Protection Agency, Washington DC 20465. This manual as published and dated is exclusive of subsequent amendments or editions.~~

6 RTCR = Revised Total Coliform Rule (40 CFR 141.852) ~~(40 CFR 141.21(f))~~, SWTR=Surface Water Treatment Rule (40 CFR 141.74(a)), New Main Construction (see 35 Ill. Adm. Code 652.203(b)). GWR = Ground Water Rule (40 CFR 141.402), LT2ESWTR = Long Term 2 Enhanced Surface Water Treatment Rule (40 CFR 141.704 and 40 CFR 141,705).

~~3 For possible use if system operates under a variance to the TCR.~~

[7] The laboratory shall use the same technique for E. coli analysis that the laboratory is certified to use for drinking water under 40 CFR 141.74 (e.g., membrane filtration, multiple-well, multiple-tube).

[8] Supplement 2 to the Fifth edition of Manual for the Certification of Laboratories Analyzing Drinking Water, November 2012

[9] See Section 465.125.

b) Laboratories shall perform parallel testing between a newly approved test and another EPA-approved procedure for enumerating total coliforms. The laboratory shall conduct at least 25 parallel tests between methods using waters normally tested. Results between methods shall vary by less than 10%.

c) Water samples shall be shaken vigorously at least 25 times in a complete up and down or back and forth movement.

d) Sample volume analyzed for total coliforms in drinking water shall be 100 mL.

e) Aseptic practices shall be used for all microbiological procedures.

f) All samples shall be handled as though they are positive and have the potential to contaminate other samples if handled improperly. All spills shall be promptly disinfected.

g~~e~~) Fermentation broth methods. The water level of the water bath shall be above the upper level of the medium in the culture tubes.

h~~f~~) Multiple tube fermentation technique (for detecting total coliforms in drinking water and enumerating total coliforms in source water):

1) For drinking water samples: Various testing configurations can be used (Standard Methods 9221B), as long as a total sample volume of 100 mL is examined for each test.

2) For source water samples: Laboratories shall use at least three series of five tubes each with appropriate sample dilutions of source water (e.g., 0.1 mL, 0.01 mL, 0.001 mL).

i~~g~~) Media

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

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

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

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

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

6) For drinking water samples: Each total coliform positive sample shall be tested for the presence of either fecal coliforms or E. coli.

j~~h~~) Invalidation of total coliform-negative samples

1) For drinking water samples: All samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, shall be invalidated. The laboratory shall collect, or request that the system collect, another sample from the same location as the original invalidated sample within 24 hours. ~~(~~Before invalidation, the laboratory may perform

a confirmed test and/or a fecal coliform/E. coli test on the total coliform-negative culture to check for coliform suppression. If the confirmed test is coliform positive or fecal coliforms/E. coli are detected, the sample shall be reported as such. A fecal coliform/E. coli-positive result is considered a total coliform positive, fecal coliform/E. coli-positive sample, even if the presumptive or confirmed total coliform test is negative. If the follow-up test or tests are negative, the sample shall be invalidated because high levels of non-coliform bacteria in the presumptive tubes may have injured, killed, or suppressed the growth of any coliforms in the sample.~~)~~

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

k~~i~~) Enzyme (chromogenic/fluorogenic) substrate tests

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

2) Media shall not be prepared from basic ingredients, but rather from a commercially available source.

3) Media shall be protected from light.

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

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

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

7) Incubators, especially small low-wattage air-type incubators, may not bring a cold 100 mL water sample or samples to the specified incubation temperature for several hours. The problem may cause false negative results with the enzyme substrate tests and possibly other tests as well. Laboratories with air-type incubators shall observe the following instructions for chromogenic/fluorogenic substrate test:

Test	**Pre-incubation sample instructions** [1,2]
Colilert (Presence/Absence)	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35° ± 0.5° C [1]
Colilert Quanti-Tray	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35° ± 0.5° C
Colilert-18 (Presence/Absence)	Prewarm sample in 35° ± 0.5° C water bath for 20 minutes or 44.5° C for 7-10 minutes

Colilert-18 Quanti-Tray	Allow sample to equilibrate to room temperature (20-30° C) before beginning 18-hour incubation time
Colisure	Allow sample to equilibrate to room temperature (20-30° C) before beginning 24-hour incubation time
Readycult Coliforms~~/ Fluorocult LMX~~	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35° ± 0.5° C or 36° ± 1° C
Modified Colitag™	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35° ± 0.5° C

[1] If the laboratory plans to put a large load into a small incubator, samples shall be brought to room temperature before incubation.

[2] Information based on manufacturer's instructions.

8) If a water bath is used, the water level shall be above the upper level of the medium.

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

10) The enzyme substrate tests shall not be used to confirm a presumptive total coliform-positive result that was obtained in fermentation broth (e.g., LTB, LB) or on a membrane filter.

11) Any sample that produces an atypical color change (e.g., greenish black or black) in the absence of a yellow color shall be invalidated.

12) Any reference comparator provided by the manufacturer shall be discarded by the manufacturer's expiration date.

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

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

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

15) If the Quanti-Tray or Quanti-Tray 2000 test is used, the sealer shall be checked monthly by adding a dye (e.g., bromcresol purple) to the water. If dye is observed outside the wells, maintenance shall be performed or another sealer shall be used.

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

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

18) For the Readycult Coliforms 100 Presence/Absence test, the contents of a snap pack shall be added to a 100-mL water sample, followed by incubation at 35° ± 0.5° C or 36° ± 1° C for 24 ± 1 hours. If coliforms are present, the medium changes color from a slightly yellow color to blue-green. In addition, if E. coli is present, the medium will emit a bright light-blue fluorescence when subjected to a long wave (365-366 nm) UV light. If confirmation of E. coli is desired, Kovac's indole reagent shall be added to the broth; the immediate formation of a red ring confirms the presence of E. coli.

~~19)~~ ~~Fluorocult LMX broth is identical to Readycult, except that it is a dehydrated culture medium in granulated form packed primarily in a 500 g plastic bottle. For testing a 100-mL water sample, 34 g of Fluorocult LMX shall be suspended in 1 L purified water and boiled to dissolve completely. Transfer 100-mL aliquots to 250-mL bottles and autoclave for 15 minutes at 121° C. Cool to room temperature, add the 100-mL water sample, and incubate. Do not add E. coli/Coliform Supplement to the medium.~~

19~~20~~) For the Modified Colitag™ test, samples shall be incubated at 35° ± 0.5° C for 24 ± 2 hours. During incubation, trimethylamine-N-oxide in the Modified Colitag™ medium causes the pH of the medium to increase from 6.2 to 6.8-7.2. A yellow color in the medium indicates the presence of total coliforms. A coliform-positive sample that fluoresces under a UV light indicates the presence of E. coli.

l~~j~~) Membrane filter (MF) methods

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

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

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

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

5) Membrane filters shall be handled with sterile forceps that are sterilized before each use by dipping in 95% ethyl or absolute methyl alcohol and flaming. The membrane filters shall be grasped outside the effective filtration area.

mk) Media used for ~~total coliforms, fecal coliforms, and E. coli by MF method for~~ detecting total coliforms and E. coli in drinking water, enumerating total coliforms or fecal coliforms in source water, and detecting E. coli in ground water.

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

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

3) Using MI medium (with or without agar) for detecting total coliforms and E. coli in drinking water or enumerating total coliforms in source water: Commercially made ~~Do not autoclave commercially made~~ pre-sterilized bottled MI agar or broth shall not be autoclaved. Bottled ~~Melt bottled~~ agar shall be melted in a boiling water bath ~~(~~or by other processes recommended by the manufacturer~~)~~. As soon as complete melting has occurred, the medium shall be cooled ~~cool~~ slightly and ~~pour~~ immediately poured into sterile plates. Care shall be taken to prevent overheating the agar, as excessive heat destroys the effectiveness of the antibiotic cefsulodin. If dehydrated culture medium is used, it shall be prepared and autoclaved according to the manufacturer's instructions. The ~~Cool the~~ agar shall be cooled, ~~add~~ freshly prepared filter-sterilized cefsulodin shall be added, and the mixture shall be ~~pour~~ immediately poured into sterile plates. The final pH of MI agar shall be 6.95 ± 0.2; the final pH of MI broth shall be 7.05 ± 0.2. The preparation and use of MI agar and MI broth are referenced in Section 465.125(a)(4). EPA Method 1604, which can be found online at www.epa.gov/microbes, is identical.

4) ~~Using~~ Chromocult® Coliform agar for detecting total coliforms and E. coli in drinking water shall not be autoclaved or overheated~~: Do not autoclave or overheat~~. The final pH shall be 6.8 ± 0.2. If a heavy background of heterotrophic bacteria is expected (especially Pseudomonas and Aeromonas species), ~~add~~ cefsulodin solution shall be added to the cooled (45° to 50° C) medium (dissolve 10 mg cefsulodin in 2 mL deionized or distilled water, and ~~add~~ solution added to 1 L of medium).

~~5) Using Coliscan® for detecting total coliforms and E. coli in drinking water or enumerating total coliforms in source water: Coliscan is available as a dry powder agar mix or as a presterilized bottled agar. For reconstitution and antibiotic addition, follow the protocol of the manufacturer (Micrology Laboratories, LLC). Do not overheat the antibiotic cefsulodin. The final pH of Coliscan agar shall be 7.00 ± 0.2.~~

5~~6~~) ~~Using~~ m-FC broth (with or without agar) for enumerating fecal coliforms in source water shall not be autoclaved~~: Do not autoclave.~~ The ~~Bring~~ medium shall be brought just to the boiling point. The final pH shall be 7.4 ± 0.2.

6~~7~~) When stored, prepared medium shall be refrigerated. Petri dishes containing medium shall be stored in a plastic bag or tightly closed container, and used within two weeks. Before use, refrigerated sterilized medium shall be brought to room temperature. Plates with laboratory-prepared broth medium shall be discarded after 96 hours, poured MF agar plates discarded after two weeks, and ampuled M-Endo broth and other prepared media discarded in accordance with the manufacturer's expiration date. Broth, plates, or ampules shall be discarded earlier if growth or (for M-Endo agar) surface sheen is observed. The ~~Record~~ date and time prepared shall be recorded.

7~~8~~) Incubation conditions and colony color of inoculated medium

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

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

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

n~~l~~) Invalidation of a total coliform-negative drinking water sample: All samples resulting in confluent or TNTC (too numerous to count) growth shall be invalidated unless total coliforms are detected. If no total coliforms are detected, the sample shall be recorded ~~record~~ as "confluent growth" or "TNTC" and ~~request~~ an additional sample shall be requested from the same sampling site. Confluent growth is defined as a continuous bacterial growth covering the entire membrane filter without evidence of total coliform type colonies. TNTC is defined as greater than 200 colonies on the membrane filter in the absence of detectable coliforms. Laboratories shall not invalidate samples when the membrane filter contains at least one coliform type colony (i.e., sheen colony for M-Endo medium, red or blue colony for m-ColiBlue24 agar, fluorescent or blue colony for MI agar, salmon to red or dark blue to violet colonies for Chromocult Coliform agar~~, pink-magenta or blue-purple colony for Coliscan)~~. ~~(~~Before invalidation, the laboratory shall ~~may~~ perform a verification test on the total coliform negative culture, i.e., on confluent or TNTC growth, and an ~~a fecal coliform/~~E. coli test. If the verification test is total coliform positive, the sample shall be reported as total coliform positive. If the test is total coliform negative, the sample shall be invalidated. An ~~A fecal coliform/~~E. coli positive result is considered a total coliform-positive, ~~fecal coliform/~~E. coli positive sample, even if the sample tests negative for total coliform ~~initial and/or verification total coliform test is negative~~.~~)~~

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

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

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

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

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

sr) Nutrient Agar- ~~+~~ MUG Test (for detection of E. coli in drinking water or ground water)

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

2) Positive and negative controls shall be tested as stated in Section 465.350(d)(9). Control cultures shall be filtered or spot-inoculated onto a membrane filter on M-Endo agar LES or M-Endo broth or agar, and shall be incubated ~~incubate~~ at 35° ± 0.5° C for 24 hours. The ~~Then transfer the~~ filter shall then be transferred to Nutrient Agar- ~~+~~ MUG and incubated ~~incubate~~ at 35° C for another four ~~4~~ hours. The results shall be read and recorded.

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

4) Inoculated medium shall be incubated at 35° ± 0.5 C° for 4 hours.

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

~~s)~~ ~~MF method for detecting enterococci/fecal streptococci in ground water~~

~~1)~~ ~~For mE agar (SM 9230C) for the detection of enterococci: Basal Prepare basal mE agar . Then autoclave and cool in a 44° 46° C water bath. *Dissolve 0.48 g* nalidixic acid and 0.4 mL 10 N NaOH shall be dissolved into 10 mL of reagent grade distilled water and mixed mix. The Filter sterilize the solution , and add 5.2 mL per liter of basal mE agar added. For triphenyl tetrazolium chloride (TTC), add 0.25 g of TTC to 25 mL of reagent grade water , and warm to dissolve. Filter sterilize the solution and add 15 mL per liter of basal mE agar added. Final pH shall be 7.1 ± 0.2.~~

~~2)~~ ~~For m Enterococcus agar (SM 9230C) for the detection of fecal streptococci (not enterococci): Heat to dissolve ingredients, but do not autoclave. Dispense into sterile petri plates (9 X 50 mm) (about 4 mL) and allow to solidify. Final pH shall be 7.2 ± 0.2.~~

~~3)~~ ~~For mEI agar (EPA Method 1600) for the detection of enterococci: Add 0.75 g indoxyl b D glucoside to 1L of basal mE agar, and proceed according to subsection (s)(1), except that the preparation of TTC is as~~

~~follows: Add 0.1 g of TTC to 10 mL of reagent-grade distilled water, and warm to dissolve.~~ The ~~Filter-sterilize the solution, and add 2 mL per liter of medium . Final pH shall be 7.1 ± 0.2.~~

~~4)~~ ~~After filtering a 100-mL sample,~~ the ~~place membrane in a petri dish on one of the agar media listed in subsection (s)(1), (s)(2) or (s)(3). Serial dilutions should not normally be necessary for detecting enterococci in ground water.~~

~~5)~~ ~~If m-Enterococcus agar is used, incubate inverted plate at 35° ± 0.5° C for 48 hours and, using magnification and a fluorescent lamp, count all light and dark red colonies as fecal streptococci.~~

~~6)~~ ~~If mE agar is used, incubate inverted plate for 48 hours at 41° ± 0.5° C, and then transfer filter to EIA medium. Incubate at 41° ± 0.5° C for 20-30 minutes and, using magnification and a fluorescent lamp, examine the colonies. Pink to red colonies on mE agar with a black or reddish brown precipitate on the underside of filter on EIA indicates the presence of enterococci.~~

~~7)~~ ~~If mEI agar is used,~~ the ~~incubate inverted plate for 24 hours at 41° ± 0.5° C. Using magnification and a small fluorescent lamp, examine both the top and bottom of the plate for colonies with a blue halo. A colony with a blue halo, regardless of colony color, indicates the presence of enterococci.~~

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

1) The Pour Plate Method (Standard Methods 9215B) or the SimPlate Method shall be used for determining compliance with 40 CFR 141.74(a)(l) and shall also be used for testing reagent grade water. ~~For systems that have been granted a variance from the Total Coliform Rule's maximum contaminant level (see variance criteria in 40 CFR 141.4), any method in Standard Methods, Section 9215, Heterotrophic Plate Count, may be used with R2A medium for enumerating heterotrophic bacteria in drinking water.~~

2) Media

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

3) (For Pour Plate Method) Melted agar shall be tempered at 44°-46° C in a water bath before pouring. Agar temperature control accompanies media from tempering through use. Melted agar shall be held no longer than three hours. Sterile agar medium shall not be melted more than once. The center of media in containers shall be no greater than 2.5 cm from some surface.

~~4)~~ ~~(For Spread Plate Method) 15 mL of R2A agar medium (or other medium) shall be poured into a petri dish (100 x 15 mm or 90 x 15 mm) and allowed to solidify.~~

4~~5~~) Refrigerated medium may be stored in bottles or in screw-capped tubes for up to three months, or in petri dishes for up to two weeks. ~~Prepared petri dishes with R2A medium may be stored for up to one week.~~

5~~6~~) For most potable water samples, countable plates can be obtained by plating 1.0 mL and/or 0.1 mL volumes of the undiluted sample (dilutions may not be necessary for SimPlate, which has a counting range up to 738/mL). At least duplicate plates per dilution shall be used.

6~~7~~) (For Pour Plate Method) The sample shall be aseptically pipetted onto the bottom of a sterile petri dish. Then at least 10-12 mL of tempered melted (44°-46° C) agar shall be added to each petri dish. The sample and melted agar shall be mixed carefully to avoid spillage. After agar plates have solidified on a level surface, the plates shall be inverted and incubated at 35° ± 0.5° C for 48 ± 3 hours. Plates shall be stacked no more than four high and shall be arranged in the incubator to allow proper air circulation and to maintain uniform incubation temperature. Excessive ~~Avoid~~

~~excessive~~ humidity in the incubator shall be avoided to reduce the possibility of spreader formation on the agar medium. Excessive ~~Also avoid excessive~~ drying of the agar medium shall also be avoided; agar medium in plates shall ~~should~~ not lose more than 15% by weight during 48 hours of incubation. Agar weight loss shall be determined quarterly.

~~8)~~ ~~(For Spread Plate Method) 0.1 or 0.5 mL of the sample (or dilution) shall be pipetted onto the surface of the pre-dried agar in the plate, and then spread over the entire surface of the agar using a sterile bent-glass rod. The inoculum shall be absorbed completely by the agar before the plate is inverted and incubated. The plate shall be incubated at 20°-28° C for 5-7 days.~~

~~9)~~ ~~(For Membrane Filter Technique) The volume to be filtered shall yield between 20-200 colonies. The filter is transferred to a petri dish containing 5 mL of solidified R2A medium, and incubated at 20°-28° C for 5-7 days. If plates with loose-fitting lids are used, plates shall be placed in a plastic box with a close fitting lid containing moistened paper towels. Paper towels shall be rewetted as necessary to maintain moisture. Colonies shall be counted using a stereoscopic microscope at 10-15X magnification.~~

7~~10~~) (For SimPlate Method) Unit Dose (for a single sample): A 10-mL volume of test sample shall be ~~is~~ added to a test tube containing dehydrated SimPlate medium. Then the dissolved medium shall be poured onto the center of a plate containing 84 small wells (provided by the manufacturer, IDEXX Laboratories, Inc.). Alternatively, 9 mL of sterile diluent (D.I. water, distilled water, or buffered water (Standard Methods, 9050C, 1 a)) can be added to the tube, followed by a 1-mL sample. Then ~~follow~~ the procedure ~~as~~ indicated ~~above~~ for the 10-mL sample shall be followed. The mixture shall be distributed evenly to the 84 wells on the plate, and the excess liquid shall be drained into an absorbent pad on the plate. The plate shall then be inverted (the fluid in each well is held in place by surface tension), and incubated for 45-72 hours at 35° ± 0.5° C. Bacterial density is determined by counting the number of wells that fluoresce under a 365-366 nm UV light, and converting this value to a Most Probable Number using the Unit Dose MPN table provided by the manufacturer. If a 10-mL sample is used, ~~read~~ the Unit Dose MPN/mL shall be read directly. If a 1-

mL sample is used, then ~~correct~~ the MPN/mL value shall be corrected by multiplying it by 10.

8~~11~~) (For SimPlate Method) Multiple Dose (for 10 samples of 1 mL each): A 100-mL sterile diluent shall be added to the dehydrated SimPlate medium to reconstitute, and shaken to dissolve. Then a 1.0-mL test sample shall be pipetted to the center of a plate containing 84 small wells, followed by 9 mL of the reconstituted medium. The plate shall be gently swirled ~~Gently swirl plate~~ to mix the sample and medium, and ~~distribute~~ the mixture shall be distributed evenly to the 84 wells on the plate. Then ~~continue with~~ the procedure indicated in subsection (t)(10) shall be followed, except that the Multi-Dose table supplied by the manufacturer shall be used to determine the MPN/mL. If a dilution is made during sample preparation, then ~~multiply~~ the MPN/mL value shall be multiplied by the dilution factor.

9~~12~~) (For Pour Plate Methods ~~and Spread Plate Techniques~~) Colonies shall be counted manually using a dark-field colony counter. In determining sample count, laboratories shall count only plates having 30 to 300 colonies, except for plates inoculated with 1.0 mL of undiluted sample. Counts less than 30 ~~for such plates~~ are acceptable. ~~(~~Fully automatic colony counters are not suitable because of the size and small number of colonies observed when potable water is analyzed for heterotrophic bacteria.~~)~~

10~~13~~) Each batch or flask of agar shall be checked for sterility by pouring a final control plate. Data shall be rejected if control is contaminated.

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.370 Sample Collection, Handling and Preservation

When the laboratory has been delegated responsibility for sample collection, handling, and preservation, there shall be strict adherence to correct sampling procedures, complete identification of the sample, and prompt transfer of the sample to the laboratory as specified in "Standard Methods for the Examination of Water and Wastewater." In addition, the following standards for sample collection, handling, and preservation of potable water samples shall be met:

a) For ~~In order for~~ the sample to be representative of the potable water system, the

sampling program shall include examination of the finished water at selected sites that systematically cover the distribution network.

b) Minimum sampling frequency shall be as specified in 35 Ill. Adm. Code 611, Subpart L (Microbiological Monitoring and Analytical Requirements).

c) Water shall be sampled from cold water taps that are free of aerators, strainers, hose attachments, and water purification devices. Prior to sampling, a steady flow of water shall be maintained from the tap for two to three minutes to clear the service line.

d) The sample bottle shall be filled allowing at least 1 inch of air space from the top to provide space for mixing. A minimum sample volume of 100 mL shall be collected. If a sample bottle is filled too full to allow for proper mixing, rather than pouring off and discarding ~~do not pour off and discard~~ a portion of the sample, ~~.Rather, pour~~ the entire sample shall be poured into a larger sterile container, mixed ~~mix~~ properly, and ~~proceed with~~ the analysis shall proceed.

e) The sample report form shall be completed in indelible ink immediately after collecting the sample and shall contain the following information: name of system (public water system site identification number, if available); sample identification (if any); date and time of collection; sample site location; sample collector's name and organization (if not the water system); persons transporting the samples from the system to the laboratory (if not the sampler); transportation condition (e.g., <10º C, protection from sunlight); sample type (e.g., routine, repeat); and total chlorine residual (if applicable).

f) When sample containers are prepared within the laboratory, the dechlorinating agent, 0.1 mL of a 3% solution of sodium thiosulfate, shall be added to a 120 mL bottle to neutralize up to 5 mG/L. Volume ~~Adjust volume~~ added to larger bottles shall be adjusted to provide the same level of neutralization. Stock sodium thiosulfate solution shall be free of turbidity.

g) When the sample is delivered to the laboratory:

1) The following information shall be added to the sample report form:

A) Date and time of sample arrival;

B) Name of carrier; and

C) Name of the person receiving the sample for the laboratory; and

2) Each sample shall be assigned a laboratory number. If the sample is ~~In the event of~~ a repeat or replacement sample, the number assigned to the original sample shall also be recorded.

h) Records necessary to establish chain-of-custody of the samples shall be maintained.

i) For the analysis of total coliform in drinking water, the time between sample collection and the placement of the sample in the incubator shall not exceed 30 hours. All samples received in the laboratory shall be analyzed on the day of receipt, unless the laboratory receives the sample late in the day, ~~(~~in which case, the sample shall be refrigerated overnight~~)~~, as long as analysis begins within 30 hours after sample collection.

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

k) Samples of potable water for heterotrophic plate count analysis shall be refrigerated and delivered to the laboratory within six hours after collection, and analyzed within two hours after receipt in the laboratory.

l) Source water samples shall be held at <10º C and the time of initiation of analyses shall not exceed eight hours from time of collection.

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.390 General Quality Control Procedures

a) A written description of the current laboratory quality control and quality assurance program shall be maintained and made available to analysts in an area of the laboratory where analytical work takes place. The quality assurance plan shall address all of the items listed in the Manual for the Certification of Laboratories Analyzing Drinking Water. The quality assurance plan shall be reviewed annually and updated as necessary. A record of analytical quality control tests and quality control checks on media, materials, and equipment shall be prepared and retained for five years.

b) ~~A laboratory manual containing complete written instructions~~ Standard operating procedures for each parameter for which the laboratory is certified and for all required quality control procedures shall be maintained and made available to analysts in an area of the laboratory where analytical work takes place.

c) The following minimum requirements shall apply to analytical quality control tests for general laboratory practices and methodology:

1) Each laboratory shall successfully analyze at least one set of proficiency testing (PT) samples once every 12 months, for each method for which it is certified. When PT sample results indicate technical error, the Department will provide appropriate technical assistance to determine the cause and make suggestions for correction of the problem.

~~2)~~ ~~Each analyst approved for the total coliform presence/absence procedure by the membrane filter technique shall verify quarterly total coliform analyses by swabbing three plates from a known positive sample and inoculating lauryl tryptose broth and brilliant green lactose bile broth from each plate. The lauryl tryptose broth and brilliant green lactose bile broth shall be incubated at 35.0° ± 0.5° C for 24 to 48 hours. Turbid growth with gas production indicates a positive result.~~

2~~3~~) Each analyst approved for the total coliform count procedure by the membrane filter technique for source water samples (SWTR) shall verify monthly ~~quarterly~~ 10 colonies, including each type of atypical colony observed. Counts shall be adjusted based on percent verification.

~~4)~~ ~~Each analyst approved for EC verification shall inoculate quarterly three tubes of EC medium with the same swabs used to perform the quarterly total coliform verification. EC medium shall be incubated at 44.5º ± 0.2º C for 24 hours.~~

3~~5~~) Each analyst approved for the fecal coliform procedure by the membrane filter technique for source water samples (SWTR) shall verify a positive water sample monthly. At ~~quarterly fecal coliform analyses by picking at~~ least 10 isolated colonies shall be chosen from membranes containing typical blue colonies and, if present, atypical colonies of different morphological types and shall be transferred ~~transferring~~ to lauryl tryptose broth. Positive tubes shall be transferred ~~and~~ to EC medium. The lauryl tryptose broth shall be incubated at 35.0º ± 0.5º C for 24 to 48 hours. The EC medium shall be incubated at 44.5º ± 0.2º C for 24 hours. Turbid growth with gas production indicates a positive result. Counts shall be adjusted based on percent verification.

4~~6~~) If there is more than one analyst in the laboratory, at least once each month each analyst shall count the same heterotrophic plate count plate, total coliform membrane, and fecal coliform membrane (per certified method used to test source water samples under the SWTR). Colony counts between analysts shall agree within 10 percent.

5~~7~~) The standards for laboratory pure water specified in Section 465.380 shall be met.

d) The following quality control tests for heterotrophic plate count shall be used ~~utilized~~:

1) Sterility controls shall be poured for each bottle of sterile melted, tempered medium used. These controls shall be the last plate poured from each bottle used.

2) Pipets shall be checked for sterility during each series of samples plated. All affected samples shall be marked “laboratory accident,” and results shall not reported when the sterility check indicates that the pipets used within the series were not sterile. ~~Sterility of pipets and petri dishes shall be determined.~~

3) Microbial density of the air during plating procedures shall be determined for each series of samples plated. The air control plate shall be the first plate set up and shall be located so that it is within the area of the plating activity. The agar shall be exposed to the air for 15 minutes as determined by the laboratory timer. The inside of the plate lid shall not be exposed. When 15 or more colonies appear on an exposed plate after a 15-minute exposure period and 48 hours of incubation at 35º C, corrective action shall be taken.

4) The sterility of dilution water, if used, shall be determined. All affected samples shall be marked "laboratory accident," and results shall not be reported when the sterility check indicates that the dilution water used within the series was not sterile.

5) Records of all sterility test results shall be maintained.

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.400 Quality Controls for Media, Equipment and Supplies

The following minimum requirements shall apply to quality control checks of laboratory media, equipment, and supplies:

a) The pH meter or meters shall be standardized before each use period with pH 7.0 and either pH 4.0 or pH 10.0 standard buffers, whichever range covers the desired pH of the media or reagent. A record of the standardization, including the percent slope, shall be maintained. Percent slope shall be 95 to 105%. If the pH meter does not have a feature to automatically calculate the slope, but can provide the pH in millivolts, the following formula shall be used: Slope (as %) = mV at pH 7 – mV at pH 4 or pH 10 X 1000/77. Each buffer aliquot shall be used only once. Commercial buffer solutions shall be dated and shall not be used~~. Do not use~~ past the expiration date. Electrodes shall be maintained ~~Maintain electrodes~~ according to manufacturer's recommendations.

b) Balances shall be calibrated monthly using NIST standardized Echelon I or II ~~Class "S" or "S-1"~~, or equivalent ASTM 1, 2, or 3 weights. A minimum of three weights that bracket the weighing requirements of the laboratory shall be used, and these weights shall be recertified every five years. A certificate shall

accompany the weights. The certificate shall state either that the weights are compliant with the requirements of ASTM E1617-13 class 1, 2 or 3 tolerances or that they are compliant with the NIST Handbook 150-2G. The certificate shall list corrective data. ~~listing correction data shall accompany the weights.~~ Electronic balances shall be calibrated annually by a qualified service representative who is not affiliated with the laboratory. A certificate of calibration from the service representative shall be available for inspection.

c) Glass and electronic thermometers and temperature-recording devices, including data loggers, shall be calibrated annually at temperature of use against an NIST certified thermometer to within ± 1.0° C. Mercury NIST-certified thermometers shall be checked at the ice point annually and recalibrated at least every five years at each temperature of use. Digital NIST-certified thermometers shall be checked at the ice point annually and recalibrated at least every five years to demonstrate linearity. Digital thermometer probe and meter shall be calibrated as a unit. The calibration factor, date calibrated, temperature of calibration, and analyst's initials shall be tagged on each thermometer. In addition, the laboratory shall record the following information in a Quality Control (QC) record book:

1) Serial number or unique identifier of laboratory thermometer;

2) Serial number of NIST-traceable thermometer;

3) Temperature of laboratory thermometer;

4) Temperature of NIST-traceable thermometer;

5) Correction (or calibration) factor;
6) Date of calibration; and

7) Analyst's initials.

d) Temperature in incubation equipment shall be recorded continuously by a temperature-recording device or recorded twice daily (at times separated by at least four hours) from in-place thermometers immersed in liquid and placed on the top and bottom shelves of the use area. Documentation shall include the date and time of reading, temperature (as determined using the correction factor of the thermometer in use), and analyst's initials. Temperature readings from walk-in

incubators with a continuous temperature reading device shall be supplemented by readings from in-place thermometers placed on various shelves other than where the recorder probe is located.

e) Date, contents, sterilization time and temperature, total time in autoclave, and analyst's initials shall be recorded each time the autoclave is used. Charts, if used, are to accompany written records.

f) Hot air ovens shall be equipped with a thermometer registering up to at least 180º C, or with a temperature-recording device. The oven thermometer shall be graduated in 10º C increments or less, with the bulb placed in sand during use. Date, contents, sterilization time and temperature, total time in oven, and analyst's initials shall be recorded each time the hot air oven is used.

g) Only membrane filters recommended for water analysis by the manufacturer shall be used ~~utilized~~. Manufacturer data sheets containing information as to lot number, ink toxicity, recovery, retention, and absence of growth-promoting substances for membrane filters shall be entered into the laboratory's record system. ~~Membrane filters with new lot numbers shall be compared with membrane filters previously found acceptable using student's t test as specified in Standard Methods. Unacceptable membranes shall be returned to the vendor.~~ The lot numbers of membrane filters and date received shall be recorded. Membrane filters shall not be brittle or distorted, and the manufacturer's specification/certification sheet shall be available. Positive control shall be run on each new lot of membrane filters. Any gridline inhibition shall be recorded as unacceptable. Unacceptable membranes shall be returned to the vendor.

h) Washing processes shall provide clean glassware with no stains or spotting. ~~Use~~ Distilled ~~distilled~~ or deionized water shall be used for final rinse. Laboratory glassware shall be washed with a detergent designed for laboratory use. A glassware inhibitory residue test (Standard Methods, Section 9020B, under Laboratory Supplies) shall be performed, and acceptable results obtained, before the initial use of a detergent and whenever a different formulation, lot number, container or washing procedure is used. Results shall be recorded.

i) A representative piece of each type of glassware or plastic ware from each batch of clean, dried glassware or plastic ware shall be tested for residual alkaline or

acid residue using bromothymol blue indicator. If the result of the indicator test is not green, corrective action shall be taken by re-rinsing, then air drying and retesting.

j) At least one bottle per lot or batch of sterilized sample bottles shall be checked before first use for sterility by adding approximately 25 ml of sterile non-selective broth media to each bottle. The bottle shall be capped and rotated so that the broth comes in contact with all surfaces and shall be incubated at 35º ± 0.5º C and checked after 24 and 48 hours for growth. Sample ~~Prepared sample~~ bottles ~~from each batch~~ shall not be used unless satisfactory results are obtained ~~from the tested bottle~~.

k) At least one bottle per lot or batch of sterilized sample bottles prepared with sodium thiosulfate shall be checked for a sufficient amount of the dechlorinating reagent by collecting a potable sample at the laboratory tap, then checking for residual chlorine. Corrective action shall be taken if there is any residual chlorine, and bottles ~~from the batch~~ checked shall not be used until corrective action has been completed.

l) At least one bottle per lot of precalibrated sample containers shall be checked before first use by measuring the volume with a Class A graduated cylinder. Tolerance shall be ± 2.5%.

m~~l~~) Current service contracts or in-house protocols shall be maintained on balances, autoclaves, hot-air sterilization ovens, water stills, deionizers, reverse osmosis apparatus, water baths, incubators, etc. Service records on the ~~such~~ equipment shall include the date, name of the servicing person, and a description of the service provided.

n~~m~~) Records shall be available for inspection on all batches of sterilized media, showing the type of medium, lot numbers, date, sterilization time and temperatures, final pH, and the names ~~name~~ of the persons responsible for all or any part of the recorded data. The final pH of the medium at 25° C shall be:

Media	pH
M-Endo broth	7.2 ± 0.2
M-Endo agar	7.2 ± 0.2
M-Endo LES agar	7.2 ± 0.2

brilliant green lactose bile broth	7.2 ± 0.2
P-A coliform test medium	6.8 ± 0.2
EC Medium	6.9 ± 0.2
plate count agar	7.0 ± 0.2
M-FC broth/agar	7.4 ± 0.2
lauryl tryptose broth	
single strength	6.8 ± 0.2
double strength	6.7 ± 0.2

o) The laboratory using commercially manufactured prepared media shall record the date received, type of medium, lot number, sample performance when checked against cultures known to give positive and negative results, and pH verification per subsection (m). Media shall be used or discarded by the manufacturer's expiration date.

p) Each new lot of prepared commercial medium and each batch of laboratory prepared medium shall be checked before use with positive and negative culture controls. Additionally, each batch of prepared media (whether commercially prepared or laboratory prepared) shall be checked for sterility. Control organisms (e.g. total coliform, fecal coliform, and E. coli) shall be either known stock cultures (periodically checked for purity) or commercially available cultures impregnated with the organism. Results shall be recorded. The following table identifies a few positive and negative culture controls that laboratories might consider.

Group	**Positive Culture Control**	**Negative Culture Control**
Total Coliforms	Escherichia coli Enterobacter aerogenes	Staphylococcus aureus Proteus vulgaris Pseudomonas aeruginosa
Fecal Coliforms	Escherichia coli Klebsiella pneumoniae (thermotolerant)	Enterobacter aerogenes
E. coli	Escherichia coli (MUG-positive strain)	Enterobacter aerogenes Klebsiella pneumoniae (thermotolerant)
Enterococci	Enterococcus faecalis	Staphylococcus aureus

	Enterococcus faecium	Escherichia coli Serratia marcesens

q) Examples of appropriate American Type Culture Collection (ATCC) strains include the following:

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

r ~~n~~) Lactose broth may be used in lieu of LTB if the laboratory conducts at least 25 parallel tests between this medium and LTB using water normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliforms, using lactose broth, is less than 10%.

s ~~o~~) A maximum registering thermometer shall be used during each autoclave and hot air oven cycle to verify sterilization temperatures. The oven maximum registering thermometer shall be placed in sand. The autoclave maximum registering temperature shall be placed in a container of water. Spore strips or ampules shall be used monthly to confirm sterilization of the autoclave. ~~Use spore strips or ampules on a monthly basis, including a positive control.~~ Spore strips shall be used monthly to confirm sterilization for the hot air oven. ~~Do not use ampules~~ Ampules shall not be used in the hot air oven because they may explode or melt. Strips or ampules that have not been placed in the autoclave or hot air oven shall be used as positive controls each time the sterilization is checked. A record of these results shall be maintained to include the date, material sterilized, and the initials of the analyst involved. Automatic ~~Check automatic~~ timing mechanisms on autoclaves shall be checked quarterly with a stopwatch. For a 15-minute sterilization period, the autoclave time shall be within 60 seconds of the stopwatch ~~clock~~ time.

t ~~p~~) When a media-dispensing apparatus is used, the media preparer shall check and

maintain a record of the accuracy of the dispenser with a graduated cylinder at the start of each volume change and periodically throughout extended runs.

u) Micropipettors shall be calibrated annually and replaced if the precision or accuracy is greater than 2.5% tolerance. Micropipettors shall be calibrated with 10 consecutive weighings annually (using a separate tip for each weighing), and the average of all 10 weighings shall be ± 2.5% of specified delivery volume. For volumes e 1.0 mL, volume shall be checked by using a Class A graduated cylinder.

v~~q~~) The refrigerator temperature shall be determined daily by an accurate thermometer immersed in liquid and placed on the top shelf. The refrigerator unit shall be visibly clean. Outdated materials in the refrigerator and freezer compartments shall be discarded.

w~~r~~) Ultraviolet sterilization lamps shall be tested quarterly by exposing agar spread plates containing 200 to 250 microorganisms to the light for two minutes. If the ~~such~~ irradiation does not reduce the count of control plates by 99 percent, the lamps shall be replaced. Alternatively, ~~replace~~ lamps shall be replaced if they emit less than 70% of the initial output. Ultraviolet ~~Cleaning of ultraviolet~~ sterilization lamps shall be cleaned ~~done~~ at least monthly by disconnecting the unit and cleaning the lamps with a soft cloth moistened with ethanol. Protective ~~Use protective~~ eye wear shall be used when checking the operation of a 254 nm lamp.

x~~-s~~) Water baths shall be cleaned at least monthly. The use of distilled or deionized water for water baths is recommended.

y~~t~~) Media shall be used on a first in, first out basis. Records shall be kept of the kind, amount, date received, and date opened for bottles of media. The date opened and the date received shall be written on the bottles. Bottles of dehydrated media shall be used within six months after opening, except that media stored in a desiccator ~~may~~ shall be used ~~up to one year after opening~~ by the manufacturer's expiration date. All media shall be discarded if visible deterioration is observed (e.g. clumping, color change). It is recommended that media be ordered in quantities to last no longer than one year, and that media be ordered in quarter pound multiples rather than one pound bottles ~~in order~~ to keep the supply sealed and protected as long as possible. Any media that have passed the manufacturer's expiration date shall be discarded.

zu) The ~~Calibrate the~~ conductivity meter shall be calibrated at least monthly, following the manufacturer's recommendations, using a certified and traceable low level standard of 20 micromhos or less. The meter reading shall be within 2% of the value of the standard. If an in-line unit cannot be calibrated, it shall not be used to check reagent-grade water.

aav) A spectrophotometer or colorimeter (if used) shall have wavelengths in the visible range. A calibration standard and method specific blank shall be analyzed every day that the instrument is used prior to sample analysis. The calibration standard shall give a reading in the desired absorbance range and shall be obtained from an outside source.

bbw) Each ~~Check each~~ batch of prepared or each lot of commercial dilution/rinse water shall be checked for sterility by adding 50 mL of water to 50 mL of double-strength, nonselective broth. The batch shall be incubated ~~Incubate~~ at 35° ± 0.5° C, and checked ~~check~~ for growth after 24 and 48 hours. The ~~Discard~~ batch shall be discarded if growth is detected.

ccx) Each ~~Check each~~ batch of prepared or each lot of commercial dilution water blanks shall be checked for pH; pH shall be 7.2 ± 0.2.

ddy) ~~Check one of 25 dilution water blanks per batch of prepared lot of commercial dilution water blanks for volume~~ The accuracy of dilution blank volumes shall be verified by checking one bottle for every 25 prepared or purchased using a Class A graduated cylinder or a MacCaffrey flask. Volume shall be 99 mL ± 2 mL. Purchased dilution blanks shall be used by manufacturer's expiration date.

ee) Each lot of purchased single use membrane filtration equipment shall be checked before use with a Class A graduated cylinder, and a record shall be maintained. Tolerance shall be ± 2.5%. Sterility check shall be performed before and after analysis per Section 465.360(j)(2).

ff) Membrane filter equipment calibration shall be checked before first use with Class A graduated cylinders, and a record shall be maintained. Tolerance shall be ± 2.5%.

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.430 Action Response to Laboratory Results

a) For laboratory results concerning samples from public water supplies and their sources, presumptive positive microbiological test results are to be reported to the ~~Illinois Environmental Protection Agency~~ state regulatory agency that has jurisdiction over the public water supply and the ~~community~~ public water supply ~~system~~ as preliminary without waiting for membrane filter verification or multiple tube fermentation (MTF) completion. After membrane filter verification or MFT completion or both, the adjusted results shall be reported. ~~The Illinois Environmental Protection Agency~~ The state regulatory agency and the public water supply shall be notified when results indicate that non-coliforms may have interfered with the total coliform analysis, as described in 40 CFR 141.21(c)(2).

b) If any sample is ~~fecal coliform or~~ E. coli-positive, the system shall notify the state regulatory agency and the public water supply ~~State~~ by the end of the day. ~~when the public water supply system is notified of the test result, unless the public water supply system is notified of the result after the State office is closed, in which case the system shall notify the State before the end of the next business day (see 40 CFR 141.21(e)(1)).~~

c) A total coliform-positive result is based on the confirmed phase if the Multiple Tube Fermentation Technique or Presence/Absence (P/A) Coliform Test is used, or the verified test for the Membrane Filter Technique if M-Endo medium or LES Endo agar is used. No requirement exists to confirm a total coliform-positive result using Colilert, Colisure, MI agar, E*Colite, m-ColiBlue24, Chromocult, Readycult/Fluorocult, Coliscan, or Colitag test. ~~Also, no requirement exists to confirm and/or verify as such, but if~~ If a sample is found to be ~~fecal coliform or~~ E. coli-positive, the sample is ~~considered~~ to be reported as total coliform-positive even when the total coliform confirmation is negative. ~~and fecal coliform/E. coli-positive.~~

(Source: Amended at 38 Ill. Reg. _____, effective _____)

Section 465.APPENDIX A Colisure P/A and Colisure Multiple Tube P/A (Repealed)

~~I. Storage~~

~~Store at 2.0º - 4.0º C. Discard on or before expiration date on product package.~~

~~II. Quality Control Testing~~

~~1A. Colisure P/A~~

~~Add 100 ml of sterile laboratory pure water to the medium in one 125 ml bottle. Shake to dissolve. Transfer a 20 ml aliquot to each of 4 25 ml sterile glass tubes.~~

~~2A. Colisure Multiple Tube P/A~~

~~Rehydrate 3 vials with 20 ml of sterile laboratory pure water. Shake to dissolve.~~

~~B. Test by inoculating with E. coli, a total coliform other than E. coli, and a non-coliform. If Pseudomonas is used as the non-coliform, use a nonfluorescent species. Use the uninoculated tube as an additional control.~~

~~C. Incubate at 35º ± 0.5º C for 28 hours and observe for the following results:~~

~~Organism~~	~~Color~~	~~Fluorescence~~
~~E. coli~~	~~Red or magenta~~	~~Positive~~
~~Coliform (non-E. coli)~~	~~Red or magenta~~	~~Negative~~
~~Non-coliform~~	~~Yellow~~	~~Negative~~
~~None~~	~~Yellow~~	~~Negative~~

~~III. Test Procedure~~

~~A. Allow medium and samples to reach room temperature.~~

~~B. Add a 100 ml water sample to the bottle containing the dehydrated~~

~~Colisure medium for a single bottle test. Then recap the bottle.~~

~~OR~~

~~Add a 20 ml water sample to each of the 5 tubes containing the dehydrated Colisure medium for a multiple tube test. Then recap each tube.~~

~~C.~~ ~~Shake and invert the bottle or tube(s) to thoroughly mix the contents. The solution will appear yellow with a fine precipitate.~~

~~D.~~ ~~Incubate the bottle or tube(s) at 35º ± 0.5º C for 28 hours.~~

~~IV.~~ ~~Interpretation~~

~~A.~~ ~~Check each tube or bottle visually for color.~~

~~Color~~	~~Interpretation~~
~~Red or magenta~~	~~Coliform Positive~~
~~Pale red/orange~~	~~Re-incubate up to 48 hours~~
~~No change (yellow)~~	~~Coliform Negative~~

~~B.~~ ~~Examine each positive result for fluorescence using a long wave (366 nm) ultraviolet light. If the solution glows a uniform, bright, light blue throughout the bottle or tube, fluorescence is present. This indicates the presence of E. coli.~~

(Source: Repealed at 38 Ill. Reg. _____, effective _____)