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	Amendment
465.125	Amendment
465.200	Amendment
465.310	Amendment
465.360	Amendment

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: This rulemaking seeks to allow laboratories that test drinking water to use additional vendors as proficiency test providers for microbiology drinking water to include those test providers recognized by The NELAC Institute (TNI) in addition to those accredited by the American Association for Laboratory Accreditation. Laboratories will not be required to use the vendors, but rather may use the vendor the laboratory determines will best benefit the laboratory operation. The addition of additional methods of testing and vendors will increase competition with the potential for reduction of costs to the laboratory. Currently, there is only one acceptable testing method and only venders accredited by the American Association for Laboratory Accreditation may be used. The rulemaking adds certification for "TECTA EC/TC Automated Microbiology System" as an alternate test procedure for detecting coliform and E. coli in water samples. The rulemaking also changes references to the General Education Development (GED) test to high school equivalency certificate.

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

DEPARTMENT OF PUBLIC HEALTH

NOTICE OF PROPOSED AMENDMENTS

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.

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:

Elizabeth Paton
Assistant General Counsel
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

14) Regulatory Agenda on which this rulemaking was summarized: This rulemaking was not included on either of the two most recent Regulatory Agendas as the need for this rulemaking was not apparent at the time those Agendas were prepared.

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 USC 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 Section 4(o) and (p) of the Illinois Environmental Protection Act 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. 16240, effective July 15, 2014; amended at 39 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 Section~~Sections~~ 4(o) and (p) of the Environmental Protection Act ~~[415 ILCS 5/4(o) and (p)]~~.

~~"American Association for Laboratory Accreditation" or "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" or "ASTM" means a not-for-profit, voluntary standards development system~~,~~ located at 100 Barr Harbor Drive, P.O. Box C700, West Conshohocken 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 to determine the quality of food, milk, public water supplies, surface water, ground water, recreational waters, wastewater, air~~,~~ or land.

~~"General Education Development Tests" or "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.

"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 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 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 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.

"The NELAC Institute" or "TNI" is an organization that recognizes associations that offer accreditation of microbiology drinking water proficiency testing providers, located at P.O. Box 2439, Weatherford TX 76086, 817-598-1624.

"Too Numerous to Count" or "TNTC" means greater than 200 colonies on the membrane filter in the absence of detectable coliforms when analyzing drinking water for total coliforms.

(Source: Amended at 39 Ill. Reg. _____, effective _____)

Section 465.125 Incorporated and Referenced Materials

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

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, 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, 800-222-0342, www.emdchemicals.com.

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

4) "Membrane Filtration Method m-ColiBlue24® Broth" (m-ColiBlue24®), Revision 2, August 17, 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 9, 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) 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 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) 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.

14) 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.

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

16) 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).

18) 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.

22) TECTA EC/TC Method, May 22, 2014; available from Veolia Water Solutions and Technologies, Suite 4697, Biosciences Complex, 116 Barrie Street, Kingston, Ontario K7L 3N6 Canada, 866-362-0993.

23) 40 CFR 141, National Primary Drinking Water Regulations: Expedited Approval of Alternative Test Procedures for the Analysis of Contaminants Under the Safe Drinking Water Act; Analysis and Sampling Procedures (January 19, 2014).

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

c) 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 39 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 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 acceptable to TNI~~accredited under A2LA~~.

d) For methods used to test the presence or absence of an organism in a sample, each set shall contain 10 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.

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) Unless otherwise specified in Subpart B, 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. 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 39 Ill. Reg. _____, effective _____)

SUBPART B: MICROBIOLOGICAL ANALYSES
OF PUBLIC WATER SUPPLY SAMPLES

Section 465.310 Personnel Requirements

a) The microbiology laboratory supervisor shall have 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, have 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, shall 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) A microbiology analyst performs microbiological analyses on water, shall have a minimum of a high school diploma and 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.

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 hold a high school equivalency certificate~~have passed the GED tests~~.

g) The Department may waive the need for the 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 is granted, the Department will prepare a written and signed justification for the waiver.

(Source: Amended at 39 Ill. Reg. _____, effective _____)

Section 465.360 Methodology

A laboratory shall be certified for all analytical methods listed in subsection (a)~~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® or 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]				
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]				
	TECTA EC/TC	[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						
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]			
Cryptosporidium	Filtration/ IMS/FA			Method 1623[8], Method 1623.1[8]		

[1] Standard Methods for the Examination of Water and Wastewater, 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] "Manual for the Certification of Laboratories Analyzing Drinking Water".

[6] RTCR = Revised Total Coliform Rule (40 CFR 141.852), 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).

[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 5th edition of the 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) Fermentation broth methods. The water level of the water bath shall be above the upper level of the medium in the culture tubes.

h) 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) 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 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 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) 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 shall be reported. If the test is total coliform negative, the sample shall be invalidated.

k) 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 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, 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 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	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, 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) 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) 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.

m) Media used 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 used in the rehydration procedure shall 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 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 pre-sterilized bottled MI agar or broth shall not be autoclaved. 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 slightly and 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 agar shall be cooled, freshly prepared filter-sterilized cefsulodin shall be added, and the mixture shall be 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) Chromocult® Coliform agar for detecting total coliforms and E. coli in drinking water shall not be autoclaved or overheated. The final pH shall be 6.8 ± 0.2. If a heavy background of heterotrophic bacteria is expected (especially Pseudomonas and Aeromonas species), 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 solution added to 1 L of medium).

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

6) 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 date and time prepared shall be recorded.

7) 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]
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) 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 as "confluent growth" or "TNTC" and 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. Before invalidation, the laboratory shall perform a verification test on the total coliform negative culture, i.e., on confluent or TNTC growth, and an 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 E. coli positive result is considered a total coliform-positive, E. coli positive sample, even if the sample tests negative for total coliform.

o) 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) 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, up to five red questionable sheen colonies and up to five red non-sheen colonies representing different morphological types shall be verified. Alternatively, 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.

q) For drinking water samples: Total coliform-positive colonies shall be tested for E. coli. 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. When coliforms or EC Medium-MUG 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 three different media, EC-MUG Medium, LTB, and BGLBB, in that order. If Nutrient Agar-MUG is used, the Nutrient Agar-MUG section shall be followed.

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

s) 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 at 35° ± 0.5° C for 24 hours. The filter shall then be transferred to Nutrient Agar-MUG and incubated at 35° C for another four 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 it is 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 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.

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.

2) Media

Method	**Medium**	**Final pH**
Pour Plate	Plate count agar, also known as tryptone glucose yeast agar	7.0 ± 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) 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.

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

7) (For SimPlate Method) Unit Dose (for a single sample): A 10.0 ~~10~~ mL volume of test sample shall be 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, 9.0 ~~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 1.0 ~~1~~ mL sample. Then the procedure indicated for the 10.0 ~~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 10.0 ~~10~~ mL sample is used, the Unit Dose MPN/mL shall be read directly. If a 1.0 ~~1~~ mL sample is used, then the MPN/mL value shall be corrected by multiplying it by 10.

8) (For SimPlate Method) Multiple Dose (for 10 samples of 1.0 ~~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 ~~1.0~~ mL test sample shall be pipetted to the center of a plate containing 84 small wells, followed by 9.0 ~~9~~ mL of the reconstituted medium. The plate shall be gently swirled to mix the sample and medium, and the mixture shall be distributed evenly to the 84 wells on the plate. Then the procedure indicated in subsection (t)(7) 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 the MPN/mL value shall be multiplied by the dilution factor.

9) (For Pour Plate Methods) 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 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) 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 39 Ill. Reg. _____, effective _____)