Analytical Methods Approved for Drinking Water Compliance Monitoring under the Total Coliform Rule

Analysis for the following contaminants shall be conducted in accordance with the methods in the following table or their equivalent as determined by EPA. The methods and monitoring requirements for these contaminants are specified in 40 CFR 141.21. Additional methods are listed in Appendix A to Subpart C of Part 141.

The CFR is the legal reference for approved methods and takes precedent over this table. The table should accurately reflect the analytical methods information published in 40 CFR 141. If you find discrepancies, please notify The Safe Drinking Water Hotline (800-426-4791) so that EPA can correct the table.

Contaminant / Method	Organization	ReferenceTitle	Method Date	EPA Publication Number	Publication Order Number	Source of Method
Total Coliforms	The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10°C during transit. EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the methods(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for B-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent confirmation tests. False-positive and false-negative information is often available in published studies and/or from the manufacturer(s).					
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	Lactose broth, as commercially available, may be used in lieu of lauryl tryptose broth, if the system conducts at least 25 parallel tests between this medium and lauryl tryptose broth using the water normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliform, using lactose broth, is less than 10 percent. If inverted tubes are used to detect gas production, the media should cover these tubes at least one-half to two-thirds after the sample is added. No requirement exists to run the completed phase on 10 percent of all total coliform-positive confirmed tubes.					
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9223	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 21st Edition	2005			Standard Methods
	The ONPG-MUG Test is also known as the Autoanalysis Colilert System.					

Contaminant Method	Organization	ReferenceTitle	Method Date	EPA Publication Number	Publication Order Number	Source of Method
Total Coliforms	The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10°C during transit. EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the methods(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for B-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent confirmation tests. False-positive and false-negative information is often available in published studies and/or from the manufacturer(s).					
9223 B-97	Standard Methods Online	Online version of Standard Methods for the Examination of Water and Wastewater. Approval year by Standard Methods Committee is designated by last 2 digits. This is the only online version that is approved.				http://www.standardmethods.org/
	The ONPG-MUG Test is also known as the Autoanalysis Colilert System.					
Chromocult® Coliform Agar	EM Science	Chromocult® Coliform Agar Presence/Absence Membrane Filter Test Method for Detection and Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters, Version 1.0	November 2000			EMD Chemicals
Colisure Test	IDEXX Laboratories, Inc.	Colisure Test	February 28, 1994			IDEXX Laboratories, Inc.
Colitag®	CPI International, Inc.	Colitag® Product as a Test for Detection and Identification of Coliforms and *E. coli* Bacteria in Drinking Water and Source Water as Required in National Primary Drinking Water Regulations	August 2001			CPI International, Inc.
E*Colite® Test	Charm Sciences, Inc.	Presence/Absence for Coliforms and *E. coli* in Water	December 21, 1997			Charm Sciences, Inc

Contaminant Method	Organization	ReferenceTitle	Method Date	EPA Publication Number	Publication Order Number	Source of Method
Total Coliforms	The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10°C during transit. EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the methods(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for B-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent confirmation tests. False-positive and false-negative information is often available in published studies and/or from the manufacturer(s).					
m-ColiBlue24® Test	Hach Co.	m-ColiBlue 24 Test, "Total Coliforms and *E. coli* Membrane Filtration Method with m-ColiBlue 24 Broth," Method No. 10029, Revision 2.	August 17, 1999			Hach Company
Readycult® Coliforms 100 Presence/Absence Test	EM Science	Readycult® Coliforms 100 Presence/Absence Test for Detection and Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters," Verson 1.0	November 2000			EMD Chemicals
	Fluorocult® is approved as an acceptable version of Readycult® under the ATP program. It is available from EMD Chemicals (formerly EM Science).					

Contaminant / Method	Organization	ReferenceTitle	Method Date	EPA Publication Number	Publication Order Number	Source of Method
Escherichia coli	The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10°C during transit. EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the methods(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for B-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent confirmation tests. False-positive and false-negative information is often available in published studies and/or from the manufacturer(s).					
9222 G	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 19th Edition	1995			Standard Methods
	Alternatively, the 18th edition of Standard Methods may be used for *E. coli* detection if the following protocol is used: at least 10 mL of EC medium is supplemented with 50 µg/mL of 4-methylumbelliferyl-beta-D-glucuronide (MUG) (EC-MUG) before autoclaving. The inner inverted fermentation tube may be omitted. Transfer the total coliform-positive culture by one of the following methods: •remove the membrane containing the total coliform colonies from the substrate with a sterile forceps and carefully curl and insert the membrane into a tube of EC-MUG (the laboratory may first remove a small portion of selected colonies for verification), •swab entire membrane filter surface with a sterile cotton swab and transfer the inoculum to the EC-MUG (do not leave the cotton swab in the EC-MUG), or •inoculate individual total coliform-positive colonies into EC-MUG. Gently shake the inoculated tubes of EC-MUG to insure adequate mixing and incubate in a waterbath at 44.5 ± 0.2°C for 24 ± 2 hours. Following incubation, observe fluorescence with an ultraviolet light (366 nm) in the dark. If fluorescence is visible, *E. coli* are present. Alternatively, the 18th edition (1992) may be used if the membrane filter containing a total coliform-positive colony(ies) is transferred to nutrient agar, as described in Standard Method 9221 B.3 (18th edition), supplemented with 100 µg/mL of MUG. If the 18th edition is used, incubate the agar plate at 35°C for 4 hours and then observe the colony(ies) under ultraviolet light (366 nm) in the dark for fluorescence. If fluorescence is visible, *E. coli* are present.					
9222 G	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 20th Edition	1998			Standard Methods
	Alternatively, the 18th edition of Standard Methods may be used for *E. coli* detection if the following protocol is used: at least 10 mL of EC medium is supplemented with 50 µg/mL of 4-methylumbelliferyl-beta-D-glucuronide (MUG) (EC-MUG) before autoclaving. The inner inverted fermentation tube may be omitted. Transfer the total coliform-positive culture by one of the following methods: •remove the membrane containing the total coliform colonies from the substrate with a sterile forceps and carefully curl and insert the membrane into a tube of EC-MUG (the laboratory may first remove a small portion of selected colonies for verification), •swab entire membrane filter surface with a sterile cotton swab and transfer the inoculum to the EC-MUG (do not leave the cotton swab in the EC-MUG), or •inoculate individual total coliform-positive colonies into EC-MUG. Gently shake the inoculated tubes of EC-MUG to insure adequate mixing and incubate in a waterbath at 44.5 ± 0.2°C for 24 ± 2 hours. Following incubation, observe fluorescence with an ultraviolet light (366 nm) in the dark. If fluorescence is visible, *E. coli* are present. Alternatively, the 18th edition (1992) may be used if the membrane filter containing a total coliform-positive colony(ies) is transferred to nutrient agar, as described in Standard Method 9221 B.3 (18th edition), supplemented with 100 µg/mL of MUG. If the 18th edition is used, incubate the agar plate at 35°C for 4 hours and then observe the colony(ies) under ultraviolet light (366 nm) in the dark for fluorescence. If fluorescence is visible, *E. coli* are present.					

Contaminant Method	Organization	ReferenceTitle	Method Date	EPA Publication Number	Publication Order Number	Source of Method
Escherichia coli	The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10°C during transit. EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the methods(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for B-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent confirmation tests. False-positive and false-negative information is often available in published studies and/or from the manufacturer(s).					
9223/ONPG-MUG (Colilert)	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 18th Edition	1992			Standard Methods
	Edberg, *et.al.*, Applied and Environmental Microbiology, 55:1003-1008, 1989.					
	The Autoanalysis Colilert System is an MMO-MUG test.					
9223/ONPG-MUG (Colilert)	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 19th Edition	1995			Standard Methods
	Edberg, *et.al.*, Applied and Environmental Microbiology, 55:1003-1008, 1989.					
	The Autoanalysis Colilert System is an MMO-MUG test.					
9223/ONPG-MUG (Colilert)	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 20th Edition	1998			Standard Methods
	Edberg, *et.al.*, Applied and Environmental Microbiology, 55:1003-1008, 1989.					
	The Autoanalysis Colilert System is an MMO-MUG test.					
9223/ONPG-MUG (Colilert)	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 21st Edition	2005			Standard Methods
	Edberg, *et.al.*, Applied and Environmental Microbiology, 55:1003-1008, 1989.					
	The Autoanalysis Colilert System is an MMO-MUG test.					
9223-97/ONPG-MUG (Colilert)	Standard Methods Online	Online version of Standard Methods for the Examination of Water and Wastewater. Approval year by Standard Methods Committee is designated by last 2 digits. This is the only online version that is approved.				http://www.standardmethods.org/
	Edberg, *et.al.*, Applied and Environmental Microbiology, 55:1003-1008, 1989.					
	The Autoanalysis Colilert System is an MMO-MUG test.					

Contaminant Method	Organization	ReferenceTitle	Method Date	EPA Publication Number	Publication Order Number	Source of Method
Escherichia coli	The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10°C during transit. EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the methods(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for B-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent confirmation tests. False-positive and false-negative information is often available in published studies and/or from the manufacturer(s).					
Chromocult® Coliform Agar	EM Science	Chromocult® Coliform Agar Presence/Absence Membrane Filter Test Method for Detection and Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters, Version 1.0	November 2000			EMD Chemicals
Colisure Test	IDEXX Laboratories, Inc.	Colisure Test	February 28, 1994			IDEXX Laboratories, Inc.
Colitag®	CPI International, Inc.	Colitag® Product as a Test for Detection and Identification of Coliforms and *E. coli* Bacteria in Drinking Water and Source Water as Required in National Primary Drinking Water Regulations	August 2001			CPI International, Inc.
E*Colite® Test	Charm Sciences, Inc.	Presence/Absence for Coliforms and *E. coli* in Water	December 21, 1997			Charm Sciences, Inc
Filter Membrane Method with MI Medium	EPA	Brenner, K.P., *et.al*, Applied and Environmental Microbiology, 59:3534-3544	1993	EPA/600/J-99/225		http://www.epa.gov/nerlcwww/online.htm
	Coliscan® is approved as a modification of MI under the ATP program. It is available from Micrology Laboratories, P.O. Box 340, Goshen, IN 46527-0340.					

Contaminant Method	Organization	ReferenceTitle	Method Date	EPA Publication Number	Publication Order Number	Source of Method
Escherichia coli	The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10°C during transit. EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the methods(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for B-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent confirmation tests. False-positive and false-negative information is often available in published studies and/or from the manufacturer(s).					
m-ColiBlue24® Test	Hach Co.	m-ColiBlue 24 Test, "Total Coliforms and *E. coli* Membrane Filtration Method with m-ColiBlue 24 Broth," Method No. 10029, Revision 2.	August 17, 1999			Hach Company
Readycult® Coliforms 100 Presence/Absence Test	EM Science	Readycult® Coliforms 100 Presence/Absence Test for Detection and Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters," Verson 1.0	November 2000			EMD Chemicals
	Fluorocult® is approved as an acceptable version of Readycult® under the ATP program. It is available from EMD Chemicals (formerly EM Science).					

Contaminant / Method	Organization	ReferenceTitle	Method Date	EPA Publication Number	Publication Order Number	Source of Method
Fecal Coliforms	Public water systems need only determine the presence or absence of fecal coliforms; a determination of fecal coliform density is not required. When the MTF Technique or Presence-Absence (PA) Coliform Test is used to test for total coliforms, shake the lactose-positive presumptive tube or P-A vigorously and transfer the growth with a sterile 3-mm loop or sterile applicator stick into brilliant green lactose bile broth and EC medium to determine the presence of total and fecal coliforms, respectively. When EPA-approved total coliform membrane filtration methods are used, transfer the total coliform-positive culture by one of the following methods: •remove the membrane containing the total coliform colonies from the substrate with a steril forceps and carefully curl and insert the membrane into a tube of EC medium (the laboratory may first remove a small portion of selected colonies for verification), •swab the entire membrane filter surface with a sterile cotton swab and transfer the inoculum to the EC medium (do not leave the cotton swab in the EC medium), or •inoculate individual total coliform-positive colonies into EC medium. Gently shake the inoculated tubes of EC medium to insure adequate mixing and incubate in a waterbath at 44.5 ± 0.2 °C for 24 ± 2 hours. Gas production of any amount in the inner fermentation tube of the EC medium indicates a positive fecal coliform test. The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10°C during transit. EPA strongly recommends that laboratories evaluate the false-positive and negative rates for the methods(s) they use for monitoring total coliforms. EPA also encourages laboratories to establish false-positive and false-negative rates within their own laboratory and sample matrix (drinking water or source water) with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used. The Agency suggests that laboratories perform these studies on a minimum of 5% of all total coliform-positive samples, except for those methods where verification/confirmation is already required, e.g., the M-Endo and LES Endo Membrane Filter Tests, Standard Total Coliform Fermentation Technique, and Presence-Absence Coliform Test. Methods for establishing false-positive and negative-rates may be based on lactose fermentation, the rapid test for B-galactosidase and cytochrome oxidase, multi-test identification systems, or equivalent confirmation tests. False-positive and false-negative information is often available in published studies and/or from the manufacturer(s).					
9221 E	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 18th Edition	1992			Standard Methods
		The preparation of EC medium is described in paragraph 1a.				
9221 E	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 19th Edition	1995			Standard Methods
		The preparation of EC medium is described in paragraph 1a.				
9221 E	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 20th Edition	1998			Standard Methods
		The preparation of EC medium is described in paragraph 1a.				

Contaminant / Method	Organization	ReferenceTitle	Method Date	EPA Publication Number	Publication Order Number	Source of Method
Heterotrophic Bacteria	The time from sample collection to initiation of analysis may not exceed 30 hours. Systems are encouraged but not required to hold samples below 10°C during transit.					
9215 with R2A Medium	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 18th Edition	1992			Standard Methods
	Any method in Standard Methods Section 9215, Heterotrophic Plate Count, may be used with R2A medium, for enumerating heterotrophic bacteria in drinking water.					
	May be used if public water system operates under a variance to the Total Coliform Rule.					
9215 with R2A Medium	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 19th Edition	1995			Standard Methods
	Any method in Standard Methods Section 9215, Heterotrophic Plate Count, may be used with R2A medium, for enumerating heterotrophic bacteria in drinking water.					
	May be used if public water system operates under a variance to the Total Coliform Rule.					
9215 with R2A Medium	Standard Methods	Standard Methods for the Examination of Water and Wastewater, 20th Edition	1998			Standard Methods
	Any method in Standard Methods Section 9215, Heterotrophic Plate Count, may be used with R2A medium, for enumerating heterotrophic bacteria in drinking water.					
	May be used if public water system operates under a variance to the Total Coliform Rule.					

Contact information for methods that are not available on the Internet are summarized in the report titled "Sources of Approved Analytical Methods for National Drinking Water Regulations."