CITY OF CAPE CORAL
REQUEST FOR SOLE SOURCE OR SINGLE SOURCE PURCHASE

Requesting Department: Public Works - ERD

Vendor Name: IDEXX Laboratories

Address: One IDEXX Drive, Westbrook, MA 04092

Phone: 1-207-556-4919 **E-Mail:** Christina-Lee@Idexx.com

Price: $49,010.36

Description of item to be procured:

Consumables parts (Chemical reagents, fluorescent testing kits, sample containers/racks, quality control confirmation test kits) for microbiological surface, ground, wastewater, and drinking water testing.

1.) Uniqueness of vendor's item/service. How is this vendor the only vendor uniquely qualified to provide the product or service:

This vendor has developed alternative microbiological testing methods that are much faster than historical culturing and enumeration of bacteria colonies methods approved under the Clean Water Act. This vendor's methods and consumables have been approved by the US Environmental Protection Agency (EPA) and followed by the Florida Department of Health as regulatory methods. There are no alternative methods and consumables available at this time that are approved for regulatory testing. See attached EPA document.

The City Laboratory has been accredited by the FL Department of Health to run methods developed by IDEXX (see attached certification). Those methods provide much faster (24h turn around time instead of 48-72 h) results and are less labor-intensive. These are critical elements to be able to monitor water quality efficiently.

2.) Market Research. Describe other, similar sources or products available in the market, if any, and why they are not acceptable:

The City Laboratory has used other methods (filtration) in the past before this method (enzyme substrate) was approved by federal and state agencies and is able to compare the older method. The older method is much more cumbersome, lengthy, and prone to more errors. There is currently no other comparable method available on the market and approved by federal and state agencies that can provide the same testing capabilities that the City Laboratory requires.

3.) Proposed Actions. Describe the actions the department will take to overcome the present barriers to competition for any future acquisition of this product or service:

Wait for patent to expire, wait for the EPA to approve alternative methods, and look for competitors.

Department Director's Signature: NR [signature] **Date:** 11/29/23

Approval: Procurement Manager Wanda Koop (not to exceed $50,000.00) **Date:** 11/30/2023

Approval: City Manager _____ (not to exceed $100,000.00) **Date:** _____

Council authorization required if exceeding $100,000.00

Revised 1/6/2020- Per Ordinance #52-19, Article VII: Purchase and Sale of Real and Personal Property. Adopted 12/02/2019

Date: 11/15/2023

Please accept this letter as confirmation that IDEXX Distribution, Inc. (FEIN # 35-2186625) is a wholly owned subsidiary of IDEXX Laboratories, Inc. and is the ***sole supplier*** of the following products to the Water Market:

Product	Sole Manufacturer	Sole Supplier in U.S. and Canadian Water Testing Markets**
Colilert* reagent	Yes	Yes
Colilert Comparator	Yes	Yes
Colilert*-18 reagent	Yes	Yes
Colisure* reagent	Yes	Yes
Enterolert* reagent	Yes	Yes
Pseudalert* reagent	Yes	Yes
Legiolert* reagent	Yes	Yes
IDEXX Vessels	Yes	Yes
Quanti-Tray* Sealer PLUS	Yes	Yes
Quanti-Tray*	Yes	Yes
All Colilert Starter Kits	Yes	Yes
All 20-pack, 100-pack, and 200-pack Combo Packs	Yes	Yes
IDEXX-QC kits[1]	Yes	Yes
Quanti-Cult™ QC kit	Yes	Yes
SimPlate* for HPC test kit	Yes	Yes
HPC for Quanti-Tray* reagent	Yes	Yes
EasyDisc* HPC tests	Yes	Yes
IDEXX Water SARS-CoV-2 RT-PCR Test	Yes	Yes
IDEXX Water DNA/RNA Magnetic Bead Kit	Yes	Yes
IDEXX Water Matrix and Fecal Control Kit	Yes	Yes
IDEXX Water Internal Control	Yes	Yes
Filta-Max* *xpress* Filter modules	Yes	Yes
Filta-Max *xpress* pressure Elution station	Yes	Yes
Filta-Max manual wash station	Yes	Yes
Filta-Max filter modules	Yes	Yes
Tecta* B4	Yes	Yes
Tecta B16	Yes	Yes
Tectalert tests (EC/TC, EC, FC, ENT)	Yes	Yes

** Utility, Public Health and Private labs performing environmental testing

Please note that IDEXX Distribution, Inc. was formed as a wholly owned subsidiary of IDEXX Laboratories, Inc.

I hope this information is of assistance. If you have any questions, please contact me at 1-800-321-0207

Sincerely,

Chun-Ming Chen VP General Manager

1) IDEXX-QC kits: 98-29000-01, 98-29001-01, 98-29002-01, 98-29003-01, 98-29004-01, 98-29006-01, 98-29007-01, 98-0009287-01

IDEXX LABORATORIES

FY QUOTE

Number / Date
20260567 / 09/20/2023

Ship to Address
CITY OF CAPE CORAL ERD LAB
ATTN: Eloisa Moreno
815 NICHOLAS PKWY EAST
CAPE CORAL FL 33990
UNITED STATES
UNITED STATES

Sold to Address
CITY OF CAPE CORAL ERD LAB
ATTN: Eloisa Moreno
815 NICHOLAS PKWY EAST
CAPE CORAL FL 33990
UNITED STATES
UNITED STATES

Bill-to Customer 332766

Net weight : 316.314

Pricing valid 10/1/23-9/30/24

Material ID / *Commodity/COO*	*Description* / *Batch* / *Exp.Date* / *Backorder item*	*Quantity*	*UnitPrice*	*Total Value*
98-09221-00 3926909910/CN	WV120SBST-200,VESSELS W/ST AND SB, 200PK	20	141.48	2,829.60
98-21675-00 3926909910/US	WQT2K QUANTI-TRAY 2000 DISPOSABLE 100/BX	40	213.76	8,550.40
98-08877-00 3822190080/US	WP200I-18 GAMMA IRAD COLILERT-18 200PACK	17	1,274.63	21,668.71
98-21375-00 3822190080/US	WENT200 ENTEROLERT 100ML 200-PACK	5	1,512.65	7,563.25
98-20745-00 7326908630/US	WVR20 VESSEL RACK	4	65.81	263.24
98-09227-00 3822190080/US	WQT2KC, PRE-DISP.QT 2000 COMPARATOR	3	35.58	106.74
98-11682-00 3822190080/US	WP104 COLI P/A COMPARATOR	3	18.63	55.89
98-0018012-00 3822190080/CA	TECTA-CCA-48, TECTALERT ECTC 48PK	10	605.00	6,050.00

All local taxes at customer charge

IDEXX
LABORATORIES

Date
09/20/2023

Number
20260567

Items Total		47,087.83
Freight Value		1,922.53
Total Amount	USD	49,010.36

All local taxes at customer charge

EPA United States Environmental Protection Agency

Analytical Methods Approved for Compliance Monitoring under the Long Term 2 Enhanced Surface Water Treatment 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 for *Cryptosporidium* are listed at 40 CFR 141.704, the methods for enumeration of *E. coli* in source water are listed in Table 1H at 40 CFR 136.3(a) and the methods for turbidity are listed at 40 CFR 141.74. Additional approved methods are listed in Appendix A to Subpart C of Part 141.

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

Contaminant

Cryptosporidium: Systems must analyze at least a 10 L sample or a packed pellet volume of at least 2 mL. Systems unable to process a 10 L sample must analyze as much sample volume as can be filtered by two filters approved by EPA for the methods listed, up to a packed pellet volume of at least 2 mL.

Method	Organization	Reference Title	Date	EPA Publication Number
1622	EPA	*Cryptosporidium* in Water by Filtration/IMS/FA	December 2005	EPA-815-R-05-001
1623	EPA	*Cryptosporidium* and Giardia in Water by Filtration/IMS/FA	December 2005	EPA-815-R-05-002
1623.1	EPA	*Cryptosporidium* and Giardia in Water by Filtration/IMS/FA	January 2012	EPA-816-R-12-001

Contaminant

Escherichia coli: The time from sample collection to initiation of analysis may not exceed 30 hours. The State may approve on a case-by-case basis the holding of an *E.coli* sample for up to 48 hours between sample collection and initiation of analysis if the State determines that analyzing an *E.coli* sample within 30 hours is not feasible. *E. coli* samples held between 30 to 48 hours must be analyzed by the Colilert reagent version of Standard Method 9223B as listed in § 136.3 (a) Table 1H of this title.

Systems must maintain samples between 0°C and 10°C during storage and transit to the laboratory.

Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample.

To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

Method	Organization	Reference Title	Date	Notes
9221B.2 F-2006	Standard Methods Online	Online version. Approval year is designated by the last 4 digits. Only online versions cited in the regulations or in Appendix A to Subpart C of Part 141 are approved.	2006	Samples shall be enumerated by the multiple-tube or multiple-well procedure. Using multiple-tube procedures, employ an appropriate tube and dilution configuration of the sample as needed and report the Most Probable Number (MPN). The multiple-tube fermentation test is used in 9221B.2-2006. Lactose broth may be used in lieu of lauryl tryptose broth (LTB), if at least 25 parallel tests are conducted between this broth and LTB using the water samples 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. No requirement exists to run the completed phase on 10 percent of all total coliform-positive tubes on a seasonal basis. After prior enrichment in a presumptive medium for total coliform using 9221B.2-2006, all presumptive tubes or bottles showing any amount of gas, growth or acidity within 48 ± 3 h of incubation shall be submitted to 9221 F-2006. Commercially available EC-MUG medium or EC medium supplemented in the laboratory with 50 µg/mL of MUG may be used.
9223 B-2004 Colilert®	Standard Methods Online	Online version. Approval year is designated by the last 4 digits. Only online versions cited in the regulations or in Appendix A to Subpart C of Part 141 are approved.	2004	Multiple tube or multiple well These tests are collectively known as defined substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli* Descriptions of the Colilert®, Colilert-18®, and Quanti-Tray® may be obtained from IDEXX Laboratories Inc.

Method	Organization	Reference Title	Date	Notes
9223 B-2004 Colilert-18®	Standard Methods Online	Online version. Approval year is designated by the last 4 digits. Only online versions cited in the regulations or in Appendix A to Subpart C of Part 141 are approved.	2004	Multiple tube or multiple well These tests are collectively known as defined substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli* Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and *E.coli* that provides results within 18 h of incubation at 35° C, rather than the 24 h required for the Colilert® test, and is recommended for marine water samples. Descriptions of the Colilert®, Colilert-18®, and Quanti-Tray® may be obtained from IDEXX Laboratories Inc.
991.15 Colilert®	AOAC International	Official Methods of Analysis of AOAC International, 16th Edition, Volume I, Chapter 17	1995	Multiple tube or multiple well These tests are collectively known as defined substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli* Descriptions of the Colilert®, Colilert-18®, and Quanti-Tray® may be obtained from IDEXX Laboratories Inc.
991.15 Colilert-18®	AOAC International	Official Methods of Analysis of AOAC International, 16th Edition, Volume I, Chapter 17	1995	Multiple tube or multiple well These tests are collectively known as defined substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli* Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and *E.coli* that provides results within 18 h of incubation at 35° C, rather than the 24 h required for the Colilert® test, and is recommended for marine water samples. Descriptions of the Colilert®, Colilert-18®, and Quanti-Tray® may be obtained from IDEXX Laboratories Inc.

Method	Organization	Reference Title	Date	Notes
1103.1	EPA	EPA Method 1103.1: *Escherichia coli* (*E.coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC), EPA-821-R-10-002, March 2010.	2010	A 0.45-µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth. Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies. Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample. When the MF method has not been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results. To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

Method	Organization	Reference Title	Date	Notes
9222 B-2006/9222 G-2006	Standard Methods Online	Online version. Approval year is designated by the last 4 digits. Only online versions cited in the regulations or in Appendix A to Subpart C of Part 141 are approved.	2006	A 0.45-µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth. Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies. Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample. When the MF method has not been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results. To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines. Subject total coliform positive samples determined by 9222B-2006 or other membrane filter procedure to 9222G-2006 using NA-MUG medium.

Method	Organization	Reference Title	Date	Notes
9222 D/9222 G	Standard Methods	*Standard Methods for the Examination of Water and Wastewater*, 20th edition	1998	A 0.45-µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth. Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies. Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample. When the MF method has not been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results. To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines. Subject total coliform positive samples determined by 9222B-2006 or other membrane filter procedure to 9222G-2006 using NA-MUG medium.

Method	Organization	Reference Title	Date	Notes
9213 D-2007	Standard Methods Online	Online version. Approval year is designated by the last 4 digits. Only online versions cited in the regulations or in Appendix A to Subpart C of Part 141 are approved.	2007	A 0.45-µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth. Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies. Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample. When the MF method has not been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results. To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

Method	Organization	Reference Title	Date	Notes
D5392-93	ASTM International	Annual Book of ASTM Standards – Water and Environmental Technology. Section 11.02.	1996	A 0.45-µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth. Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies. Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample. When the MF method has not been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results. To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

Method	Organization	Reference Title	Date	Notes
D5392-93	ASTM International	Annual Book of ASTM Standards – Water and Environmental Technology. Section 11.02.	1999	A 0.45-µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth. Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies. Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample. When the MF method has not been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results. To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

Method	Organization	Reference Title	Date	Notes
D5392-93	ASTM International	Annual Book of ASTM Standards – Water and Environmental Technology. Section 11.02.	2000	A 0.45-µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth. Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies. Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample. When the MF method has not been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results. To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.
1603	EPA	EPA Method 1603: *Escherichia coli (E. coli)* in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC), EPA-821-R-14-010, September 2014.	2014	
1604	EPA	EPA Method 1604: Total Coliforms and *Escherichia coli (E.coli)* in Water by Membrane Filtration by Using a Simultaneous Detection Technique (MI Medium), EPA 821-R-02-024, September 2002.	2002	Preparation and use of MI agar with a standard membrane filter procedure is set forth in the article, Brenner et al. 1993. New Medium for the Simultaneous Detection of Total Coliform and *Escherichia coli* in Water. Appl. Environ. Microbiol. 59: 3534-3544

Method	Organization	Reference Title	Date	Notes
mColiBlue-24®	Hach Company			A description of the mColiBlue24® test may be obtained from Hach Company.

Water Quality Parameters

Turbidity: §141.704(c) Systems must use methods for turbidity measurement approved in 141.74 (a)(1).

Method	Organization	Reference Title	Date	Notes
2130 B	Standard Methods	*Standard Methods for the Examination of Water and Wastewater*, 18th Edition	1992	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
2130 B	Standard Methods	*Standard Methods for the Examination of Water and Wastewater*, 19th Edition	1995	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
2130 B	Standard Methods	*Standard Methods for the Examination of Water and Wastewater*, 20th Edition	1998	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
2130 B	Standard Methods	*Standard Methods for the Examination of Water and Wastewater*, 21st Edition	2005	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
2130 B	Standard Methods	*Standard Methods for the Examination of Water and Wastewater*, 22nd Edition	2012	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
180.1	EPA	Methods for the Determination of Inorganic Substances in Environmental Samples, EPA/600/R-93/100, August 1993	1993	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
Method 2	Great Lakes Instruments	Great Lakes Instruments Method 2, Turbidity, November 2, 1992	1992	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin

Method	Organization	Reference Title	Date	Notes
10133	Hach	Hach FilterTrak Method 10133 Determination of Turbidity by Laser Nephelometry January 2000 Revision 2.0	2000	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
M5271	Leck Mitchell	Mitchell Method M5271, Revision 1.1, Determination of Turbidity by Laser Nephelometry, March 5, 2009	2009	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
M5331	Leck Mitchell	Mitchell Method M5331, Revision 1.1, Determination of Turbidity by LED Nephelometry, March 5, 2009	2009	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
AMI Turbiwell	Swan Analytische Instrumente AG	Continuous Measurement of Turbity Using A SWAN AMI Turbiwell Turbidimeter, August 2009	2009	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
AQ4500	Thermo Scientific	Orion Method AQ4500, Revision 1.0, Determination of Turbidity by LED Nephelometry, May 8, 2009	2009	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
M5331, Rev. 1.2	Leck Mitchell	Mitchell Method M5331, Revision 1.2, Determination of Turbidity by LED or Laser Nephelometry, February 2016	2016	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin
10258	Hach Company	Hach Method 10258, Determination of Turbidity by 360° Nephelometry, January 2016	2016	Styrene divinyl benzene beads (e.g. AMCO-AEPA-1 or equivalent) and stablilized formazin (e.g. Hach StablCal™ or equivalent) are acceptable substitutes for formazin

IDEXX Summary 15B

Topic	Evaluation of Colilert*-18/Quanti-Tray* versus *Standard Methods*[1] 9222D for the detection of fecal coliforms in wastewater samples
Title	***"Evaluation of Colilert-18® for Detection and Enumeration of Fecal Coliform Bacteria in Wastewater Using the U.S. Environmental Protection Agency Alternative Test Procedure Protocol"***
Author(s)	Paul S. Warden, Monique S. DeSarno, Sarah E. Volk, and Bradley J. Eldred; Analytical Services, Inc., 130 Allen Brook Lane, Williston, VT 05495
Date	September 2011

Highlights:

- An Alternative Test Protocol[2] (ATP) study was performed in order to validate the use of Colilert-18/Quanti-Tray for measuring fecal coliforms in wastewater samples at 44.5°C versus *Standard Methods* 9222D
- **Recovery:** Recovery of fecal coliforms by Colilert-18 was significantly higher or statistically equivalent to the recovery by the reference method (*Standard Methods* 9222D)
- **False Positive / False Negative rates:** Both methods had low false-positive rates (<2%); however, the false-negative rate observed with *Standard Methods* 9222D (21.5%) was substantially higher than that observed with Colilert-18 (7%)
- **Accuracy:** The accuracy rates of the two methods were calculated as 96.5 and 88.9% for Colilert-18 and *Standard Methods* 9222D, respectively
- A copy of the article is attached

1. *Standard Methods for the Examination of Water and Wastewater*, 21st Ed, 2005, APHA, AWWA & WEF, Washington, DC
2. EPA Microbiological Alternate Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Method, United States Environmental Protection Agency, Washington, D.C., April 2004

MICROBIOLOGICAL METHODS

Evaluation of Colilert-18® for Detection and Enumeration of Fecal Coliform Bacteria in Wastewater Using the U.S. Environmental Protection Agency Alternative Test Procedure Protocol

PAUL S. WARDEN, MONIQUE S. DESARNO, SARAH E. VOLK, and BRADLEY J. ELDRED
Analytical Services, Inc., 130 Allen Brook Lane, Williston, VT 05495

This study compared recovery of fecal coliform bacteria from sewage by Colilert-18® and Standard Methods 9222D (membrane-Fecal Coliform medium) in accordance with the U.S. Environmental Protection Agency (EPA) Alternative Test Protocol (ATP). Samples were collected from 10 different wastewater treatment plants in the northeastern United States and tested in a single laboratory. Twenty replicates of each sample were analyzed by each method, and 200 positive and 200 negative responses were confirmed for each method. Recovery of fecal coliforms by Colilert-18 was significantly higher than (8 of 10 sites) or statistically equivalent to (1 of 10 sites) recovery by the reference method (Standard Methods 9222D) for samples from all but one site. Both methods had low false-positive rates (<2%); however, the false-negative rate observed with Standard Methods 9222D (21.5%) was substantially higher than that observed with Colilert-18 (7%). The accuracy rates of the two methods were calculated as 96.5 and 88.9% for Colilert-18 and Standard Methods 9222D, respectively. The results of this study demonstrate that Colilert-18 meets the acceptance criteria for alternative methods specified in the EPA ATP.

The term "fecal coliform" has no clear definition but is generally accepted to be that part of the total coliform group that is thermotolerant and largely composed of the genera *Escherichia, Klebsiella, Enterobacter,* and *Citrobacter.* Commonly used methods for detection of fecal coliform bacteria use method-derived definitions based on the performance of organisms in a few simple physiological tests. Although the role of this group of organisms has been largely displaced by the use of *Escherichia coli* as an indicator of potential fecal contamination in drinking water, the fecal coliform group is still widely used as an indicator in wastewater, biosolids, and shellfish harvest monitoring in the United States. Consequently, laboratories continue to use traditional membrane filtration methods, which utilize fermentation of lactose and incubation at 44.5°C to differentiate fecal coliforms from other organisms.

The examination of drinking water for the presence of total coliforms and *E. coli* is most frequently performed in the United States using methodologies that detect the presence of the enzymes β-D-galactosidase and β-D-glucuronidase as markers of these organisms. Because many laboratories test both drinking and wastewater, this has resulted in many laboratories running two different methods, which is inefficient and increases the QA, training, and demonstration of competency testing that laboratories are required to perform. Furthermore, the membrane filtration-based methods require confirmation using physiological tests, which increases the cost of the tests and delays the availability of final results.

The study reported here examined the ability of a defined substrate technology® (DST®)-based method to detect fecal coliforms in wastewater and compared the results to the standard membrane filtration procedure of incubation on membrane-Fecal Coliform (m-FC) agar, with confirmation using lauryl tryptose broth (LTB) and *E. coli* (EC) broth. The defined substrate method is typically incubated at 35 ± 0.5°C and simultaneously detects total coliforms and *E. coli* (1). Several workers have previously used enzymatic methods for detection of fecal coliforms, with varying degrees of success (2–4). Warren et al. (2) and Berg and Fiksdal (3), were able to detect a single fecal coliform within 20 and 6 h, respectively. Interestingly, in a later study, the second group (3), using the same approach, found 2.9% false positives and 7.8% false negatives. Colilert-18® is approved by the U.S. Environmental Protection Agency (EPA) for the detection and enumeration of total coliforms and *E. coli* in source and drinking water samples. In addition, this method is EPA-approved for detection and

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enumeration of *E. coli* in wastewater. Advantages of DST methods for the above uses include simplicity, speed, and accuracy, and this study was designed to determine if the use of DST could be expanded to include detection and enumeration of fecal coliforms in wastewater.

The purpose of this study was to compare recovery of fecal coliforms from diluted sewage samples using Colilert-18 with Quanti-Tray® and incubation at 44.5°C to the recovery using the reference method Standard Methods 9222D with m-FC medium (5). The design of this study was based on the EPA's Microbiological Alternative Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater (6).

Method

Sample Sources

Ten effluent samples were collected from 10 different sewage treatment facilities in the New England area for the comparison study. Samples were collected in sterile 500 mL plastic bottles (no sodium thiosulfate) and delivered to the laboratory at 2–8°C. All samples were initially analyzed for fecal coliform concentration within 6 h of collection.

Analytical Methods

A defined substrate technology procedure, Colilert-18/Quanti-Tray/2000 (IDEXX Laboratories, Westbrook, ME) was compared to the reference method SM 9222D in this study.

Samples were delivered to the laboratory by express courier, checked for temperature and hold time, assigned unique identification numbers, and allowed to equilibrate to room temperature. Aliquots of each sewage sample were used to prepare duplicate serial dilutions (10^{-2} through 10^{-6}) in sterile deionized water and analyzed to determine the concentration required to obtain one set of dilutions that contained 20–50 CFU/100 mL. These diluted sewage samples were screened using the Colilert-18/Quanti-Tray procedure to estimate the fecal coliform concentration. Based on these results, the volumes of sewage required to be spiked into 100 mL deionized water to target the upper and lower ends of the desired range (20–50 CFU/100 mL) were determined. Fresh dilutions of sufficient volume were prepared from the original sewage sample (stored at 2–8°C) to allow analysis of 20 replicates of each by both methods.

The reference procedure consisted of concentration of each sample by membrane filtration, placing the membrane on a plate of m-FC agar (Northeast Laboratory Services, Waterville, ME), followed by incubation of the plates in a water bath at 44.5 ± 0.2°C for 24 ± 2 h. Plates were examined for typical blue colonies, which were counted as presumptive fecal coliforms.

Each sample (100 mL) for analysis using Colilert-18 was poured into one sterile plastic container, and the medium was added. Samples were gently agitated to allow dissolution of the medium. Each 100 mL aliquot was then poured into one Quanti-Tray (IDEXX Laboratories) and sealed using a heat sealer. The trays were incubated in an incubator at 44.5 ± 0.2°C (Binder KB720, Binder Inc., Great River, NY) for 18–22 h. After incubation, the trays were read by comparing each potentially positive well to a comparator, and the number of positive (yellow) wells was counted and recorded.

Confirmation Procedures

After incubation and enumeration, the dilution that yielded fecal coliform recovery within the target range (20–50 CFU/mL), as measured by the reference method (SM 9222D), was selected for the confirmation step. Using the chosen dilution, a 10 µL aliquot was taken from one positive and one negative well from each Quanti-Tray, inoculated into 10 mL LTB (Difco, Becton Dickinson, Sparks, MD), and processed as described below. Similarly, one positive and one negative colony from each m-FC plate was inoculated into LTB and processed as described below. For archiving purposes, 800 µL of the same positive and negative wells from the Colilert-18 wells was mixed with 200 µL sterile glycerol, and the samples were frozen at –80°C for later use, if necessary. After incubation, 800 µL of each LTB inoculated from m-FC plates was mixed with 200 µL glycerol, and the resulting suspension was frozen at –80°C.

To confirm results from initially positive response samples, one positive plate or well was selected from the set of replicates, and a colony or 10 µL aliquot was inoculated into LTB and incubated at 35 ± 0.5°C for 48 ± 3 h. An aliquot (10 µL) from LTB was inoculated into EC broth (Difco) and incubated (44.5 ± 0.2°C for 24 ± 2 h). Tubes with gas and growth were considered true positives, while tubes with no gas and growth were considered presumptive false positive (PFP). A 10 µL aliquot from each PFP tube was streaked onto Eosin Methylene Blue agar (EMB, Northeast Laboratory Services) and incubated (35 ± 0.5°C for 21 ± 3 h). Typical fecal coliform-type colonies were inoculated onto Nutrient Agar slants (Northeast Laboratory Services) and incubated (35 ± 0.5°C for 21 ± 3 h). These aliquots were uniquely coded and stored at 2–8°C for subsequent identification using the Vitek® 2 semiautomated bacterial identification system (bioMérieux, Inc., Durham, NC). Vitek 2 results of the genera *Escherichia, Klebsiella, Enterobacter,* or *Citrobacter* (fecal coliforms) with confidence ≥90% were considered true positives. Vitek 2 results of other genera, or <90% confidence, were considered PFPs, and further testing was performed using archived aliquots (Figure 1).

Similarly, to confirm results from initially negative response samples, one negative plate or well was selected

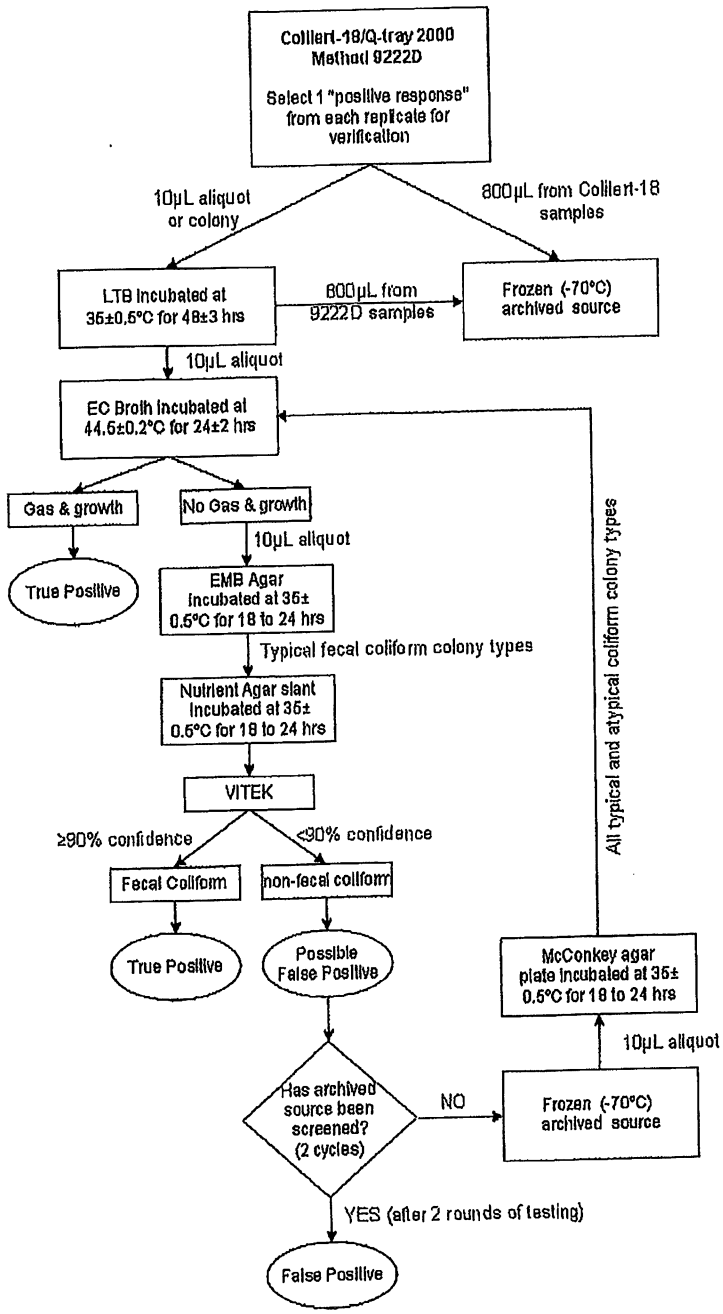

Figure 1. Confirmation procedure for positive response samples.

from the set of replicates, and a negative (non-coliform) colony or 10 µL aliquot was inoculated into LTB and incubated at 35 ± 0.5°C for 48 ± 3 h. LTBs displaying no growth (not turbid) were considered true negatives. If the LTB was positive, the original result was suspect, and the sample was considered a presumptive false negative (PFN). An aliquot (10 µL) from each PFN tube was inoculated into EC broth and incubated in a water bath (44.5 ± 0.2°C for 24 ± 2 h). Tubes with no gas and growth were considered true negatives, while tubes with gas and growth were considered false negatives. The latter were plated on MacConkey agar (Northeast Laboratory Services) and incubated (35 ± 0.5°C for 21 ± 3 h), after which typical fecal coliform-type colonies were harvested and processed as described above for subsequent identification using the Vitek 2 (Figure 2).

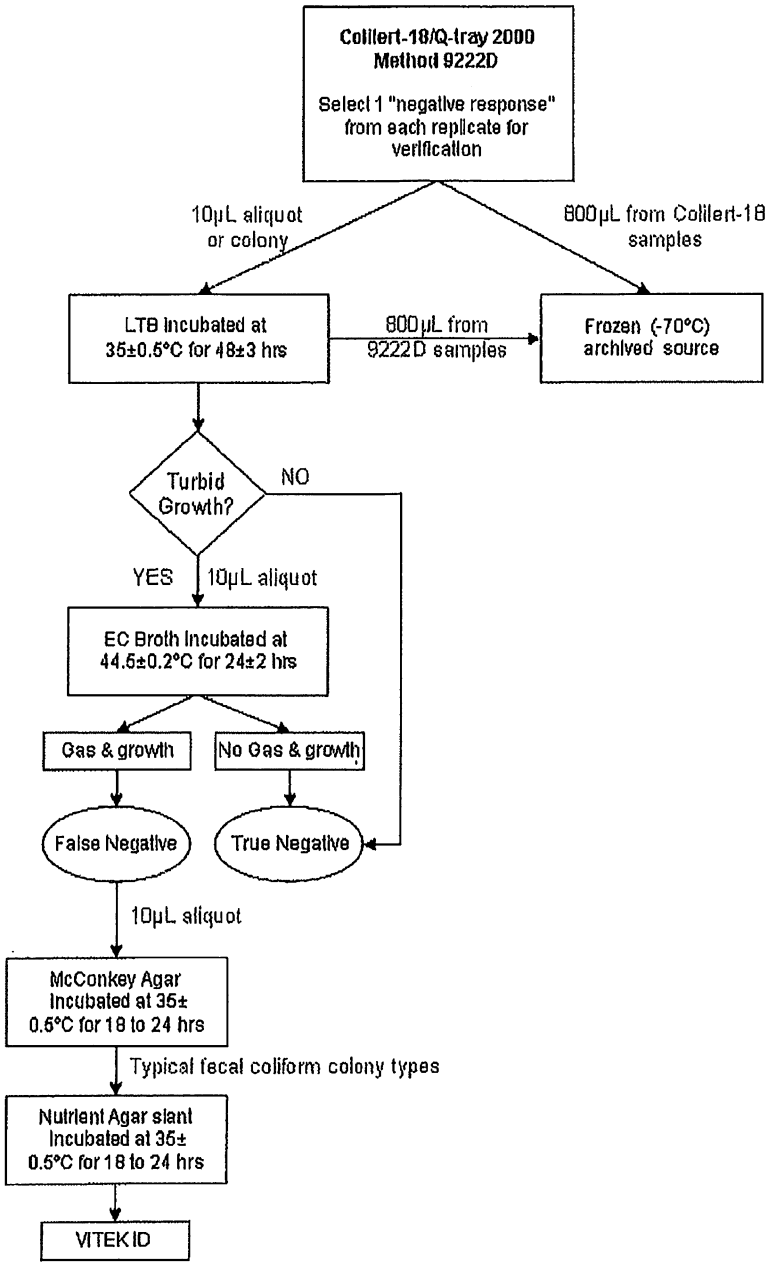

Figure 2. Confirmation procedure for negative response samples.

QA/QC

All media were prepared in-house according to the manufacturer's instructions, incubated at use conditions, and evaluated for contamination before use. All purchased media were accompanied by QA/QC documentation. Temperatures of incubators, water baths, and refrigerators were read twice daily, Monday through Friday, using National Institute of Standards and Technology-traceable thermometers, and were recorded in bound logbooks. The Binder incubators were new, and before the study were validated to maintain specified temperature throughout the chamber when full of test samples. All data were recorded in bound logbooks or controlled access spreadsheets and checked by a second analyst trained in the assay procedures.

Table 1. Summary statistics of fecal coliform recovery from primary sewage effluent from 10 sites by Colilert-18 (CL-18) and the reference method SM 9222D (m-FC)

	Mean		Range		SD		RSD	
Sample	CL-18	m-FC	CL-18	m-FC	CL-18	m-FC	CL-18	m-FC
1	53.6	27.9	31–68	18–42	9.76	7.50	18.25	26.89
2	40.5	23.6	23–57	16–34	10.43	5.16	25.74	21.88
3	58.0	37.1	41–79	28–44	10.46	4.76	18.02	12.82
4	50.9	43.5	33–75	31–57	10.57	6.97	20.75	16.02
5	37.2	44.6	26–47	26–58	6.00	8.26	16.07	18.52
6	58.0	46.0	40–73	35–61	8.80	6.25	15.18	13.61
7	58.4	40.8	39–75	31–55	10.24	6.85	17.57	16.79
8	65.8	39.6	52–93	17–61	10.68	11.23	16.07	28.40
9	52.1	31.9	33–72	9–42	9.73	7.97	18.64	25.02
10	42.0	43.4	27–50	16–59	7.28	9.84	17.31	22.70
Mean	51.7	37.8						

Statistical Procedures

The fecal coliform recovery data generated using Colilert-18 and SM9222D for each site were tested for normality with each effluent sample using the Kolmogorov-Smirnov test. The homogeneity of variance of the fecal coliform recovery for each test, across all matrixes, was tested using the Bartlett's test. Precision within each method was measured using Levene's test. A two-way analysis of variance (ANOVA) was performed across all 10 matrixes to determine whether significant interaction between method and matrix occurred. Because the ANOVA results indicated significant interaction between samples and methods, the fecal coliform recovery comparison was made between methods with each independent effluent sample using a two-sample *t*-test.

Results and Discussion

The recovery of fecal coliforms using both test methods for the 10 different samples is shown in Table 1 and includes mean, range, SD, and RSD for each method. Even without the application of statistical methods, it is clear that the defined substrate method recovered more fecal coliforms than the membrane filtration-based reference method. In fact, the SM9222D procedure recovered only 73% of the total number of fecal coliforms recovered by the Colilert-18 procedure.

Assessment of Normality

The recovery data for Colilert-18 and SM9222D was tested for normality with each effluent sample using the Kolmogorov-Smirnov test (data not shown). Analysis of these data indicated fecal coliform recovery by both methods was consistent with a normal distribution, with *P*-values in excess of 0.366 for most samples. An exception to this was one effluent tested by Colilert-18 method, which had a *P*-value of 0.119. However, this *P*-value is greater than the usual threshold of 0.10. These results confirm that the assumption of normality was met for both test methods.

Assessment of Precision Within Each Method

The homogeneity of variance in fecal coliform recovery across all matrixes for each test method was tested using the Bartlett's and Levene's tests (data not shown). For the Colilert-18 method, the variances across the 10 sites were similar with a *P*-value of 0.308. For SM9222D, the variances across the 10 sites were not homogeneous with a *P*-value of 0.006. Additional analysis using Levene's test showed good internal consistency ($P > 0.05$) with Colilert-18 but not with SM9222D. The low internal consistency with SM9222D seems to underscore the difficulty in interpreting the results of this method with true environmental samples.

Assessment of Precision Between Methods

The variance equality in fecal coliform recovery between Colilert-18 and SM9222D was tested using the *F*-test, assuming a normal distribution in recovery with each effluent sample (data not shown). The variability of fecal coliform recovery by the Colilert-18 method was statistically equivalent ($P > 0.05$) to SM9222D with 80% of the effluent samples tested. SM9222D showed higher precision with two of the effluent samples (sites 2 and 3). As noted above, SM9222D had the lower overall precision across the 10 matrixes as measured by Levene's test. This complicates the comparison of precision

Table 2. Comparison of recovery of fecal coliforms from diluted sewage from 10 sites using Colilert-18 and SM9222D (m-FC)

Effluent site	Mean recovery Colilert-18	Mean recovery m-FC	*T*-value	*P*-value
1	53.6	27.9	9.38	0.000
2	40.5	23.6	6.47	0.000
3	58.0	37.1	8.06	0.000
4	50.9	43.5	2.61	0.013
5	37.2	44.6	–3.23	0.003
6	58.0	46.0	4.99	0.000
7	58.4	40.8	6.37	0.000
8	65.8	39.6	7.60	0.000
9	52.1	31.9	7.20	0.000
10	42.0	43.4	–0.50	0.623

between the methods because such an analysis assumes internal consistency within each method.

Fecal Coliform Recovery

A two-way ANOVA across all 10 matrixes revealed that a significant interaction ($P = 0.00$) was present between the test methods and effluent samples. As a result, the fecal coliform recovery comparison was made between methods with each independent effluent sample using a two-sample *t*-test, rather than across all samples and sites.

Recovery of fecal coliforms by Colilert-18 and SM9222D was compared (Table 2), and clearly Colilert-18 was more effective at recovering fecal coliforms than was SM9222D. Of the 10 effluents tested in this study, Colilert-18 recovered a statistically larger population of fecal coliform bacteria ($P < 0.05$) than did SM9222D in 80% of the effluents tested. Colilert-18 and SM9222D recovered an equivalent number of fecal coliform bacteria ($P = 0.623$) from site 10 and Colilert-18 recovered a statistically smaller number of fecal coliforms ($P = 0.003$) than did SM9222D from site 5.

Table 3. False-positive reactions detected using Colilert-18 and SM9222D (m-FC)

Description	Colilert-18	m-FC
No. of colonies tested	200	200
No. confirmed by EC broth	175	166
No. confirmed by Vitek	25	33
False-positive results	0	1
Percentage false positive	0	0.5

Table 4. False-negative reactions detected by each method: Colilert-18 and SM9222D (m-FC)

Description	Colilert-18	m-FC
No. of colonies tested	200	205
No. confirmed by EC broth	186	161
No. confirmed by Vitek	0	0
False-negative results	14	44
Percentage false negative	7.0	21.5

Assessment of False-Positive and False-Negative Rates

A minimum of 200 presumptive positive responses from each test method were confirmed using both EC broth and Vitek identification to verify the accuracy of each test. For the purposes of this study, a fecal coliform was defined as any member of the genera *Escherichia*, *Klebsiella*, *Enterobacter*, or *Citrobacter* capable of growing at 44.5 ± 0.2°C. This definition was applied equally to the DST and SM9222D methods, and the results are shown in Table 3. The majority of the presumptive positive samples were confirmed as being true fecal coliform bacteria by the EC broth method. However, a substantial number of isolates (12.5–22.5% of presumptive positives, depending upon the method) required further characterization by the Vitek bacterial identification system because they failed to generate gas in the EC medium when grown at 44.5 ± 0.2°C (Table 3). The identity of these anaerogenic fecal coliform bacteria was primarily either *E. coli* or *K. pneumoniae*.

A minimum of 200 presumptive negative colonies (i.e., non-blue) or wells (i.e., non-yellow) were confirmed using EC broth and, where necessary, Vitek identification. The results are shown in Table 4. The confirmed false-negative rates for Colilert-18 method was 7.0 %, considerably lower than the 21.5% seen with SM9222D (Table 4).

Table 5. Genera of fecal coliforms recovered from false-negative wells or colonies from Colilert-18 and SM9222D (m-FC), respectively

Test method	False-negative responses	Genera	Occurrence	Frequency, %
Colilert-18	14	*Escherichia*	4	29
		Klebsiella	10	71
		Enterobacter	0	0
		Citrobacter	0	0
m-FC	44	*Escherichia*	33	75
		Klebsiella	10	23
		Enterobacter	1	2
		Citrobacter	0	0

Interestingly, 75% (33 of 44) of the false-negative isolates for the reference method (SM9222D) belonged to the genera *Escherichia*; almost all of the remainder were *Klebsiella* (Table 5). In contrast, approximately one quarter (4 of 14, or 29%) of the Colilert-18 false-negative responses belonged to the genus *Escherichia*; the remainder (10 of 14, or 71%) were *Klebsiella* (Table 5).

The confirmed false-negative rate of SM9222D was 21.5%, which far exceeded the rate for Colilert-18. The genera identified from false-negative m-FC samples included *Escherichia*, *Klebsiella*, and *Enterobacter*; however, the majority (75%) of the confirmed false-negative responses for m-FC belonged to the genus *Escherichia* (Table 5).

Determination of Accuracy

Various measures of method accuracy have been reported in the literature. In this study, the following equation was used:

$$\text{Accuracy (\%)} = 100 \times \frac{(TP + TN)}{(TP + FP + TN + FN)}$$

where TP = number of true positives; TN = number of true negatives; FP = number of false positives; and FN = number of false negatives).

Using this equation, and the data from the confirmed colonies/wells shown in Tables 3 and 4, the respective accuracy rates for each method were calculated as 96.5 and 88.9% for Colilert-18 and SM9222D, respectively.

The data generated during this study are consistent with those generated by many other studies showing that enzyme-based methods consistently detect more coliforms than lactose-based methods (1, 7). The results presented here also show that direct comparison of methods can demonstrate differences in performance between two methods (1, 7). Differences in the performance of microbiological methods can be caused by many factors, including formulation of the medium (particularly the compounds used to inhibit nontarget organisms), temperature of incubation, and the use of membrane filtration as opposed to inoculation directly into liquid media. Of particular note, however, is the plethora of recent studies (1, 7) comparing methods that use media containing substrates for the enzyme β-D-galactosidase with methods that rely upon fermentation of lactose (with or without the production of gas). There are many strains of coliforms, including those belonging to the so-called fecal coliforms (i.e. *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter*) that fail to ferment lactose within 48 h but give a positive reaction for β-D-galactosidase (8, 9). This factor alone can result in differences of approximately 20% between methods (1, 7).

Membrane filters can often be difficult to read, particularly when large numbers of nontarget organisms are present. The presence of large numbers of nontarget organisms can result in a wide diversity of colonial morphologies for the target organism. During this study, membranes incubated on m-FC were sometimes difficult to interpret consistently, and this has been our experience with routine samples submitted to our laboratory. In contrast, methods such as Colilert-18 produce results that are simple to read with considerably less subjectivity.

Colilert-18 outperformed SM9222D at recovering fecal coliform bacteria from at least 80% of the effluent samples tested. One factor that may account for these differences in fecal coliform recovery is that SM9222D tended to underestimate the concentration of fecal coliforms that were present due to its much higher false-negative rate than the other method studied here. Some fecal coliforms were present on the m-FC medium, but did not display the typical blue colony morphology specified in the method; therefore, they were not included in the presumptive fecal coliform count.

The lack of a distinctive blue colony formation can be due to the presence of nontarget bacteria, but also because some target bacteria do not form typical colonies on m-FC medium. In particular, it is also likely that some strains that caused the false-negative reaction were weak-to-moderate lactose-fermenting organisms (at 44.5°C) and did not produce sufficient acid to react with the m-FC indicator and produce the typical blue color. Organisms that ferment lactose slowly at 44.5°C are often encountered in environmental samples. Such strains would not generally confirm with EC broth, which uses lactose fermentation for identification. Both methods examined in this study, Colilert-18 and SM9222D, showed very low false-positive rates (<2%); however, Colilert-18 yielded fewer false-negative results (7.0 versus 21.5%). Comparison of calculated accuracy rates (96.5 and 88.9% for Colilert-18 and SM9222D, respectively), indicate Colilert-18 to be the superior method.

Conclusions

The results of this study indicate that the Colilert-18 method meets the EPA ATP acceptance criteria for alternative methods. Fecal coliform recovery by Colilert-18 exceeded that achieved by SM9222D with at least 80% of the individual effluent samples when the site-specific recovery was compared.

The relatively high false-negative rate of SM9222D (21.5%) did not fully explain the differences between the methods studied here. Of particular concern is the use of confirmation procedures for SM9222D that require production of gas during fermentation. Many anaerogenic strains of the four genera that comprise the fecal coliform group exist, and these are of no less health significance than aerogenic strains. Similarly, non-lactose-fermenting strains of some of these four genera occur frequently (8, 9).

It is clear that m-FC medium significantly underestimates the number of true fecal coliforms present in sewage effluents. Thus, the use of SM9222D with m-FC medium to measure fecal coliform concentration as an indicator of the microbiological quality of effluent waters, biosolids, and ambient waters must be called into question.

In conclusion, this study has demonstrated that Colilert-18 meets the acceptance criteria outlined by the EPA ATP for alternative methods. The use of this DST-based method can be recommended for examination of environmental samples for the presence of fecal coliforms.

Acknowledgments

This study was supported by IDEXX Corporation, Westbrook, ME.

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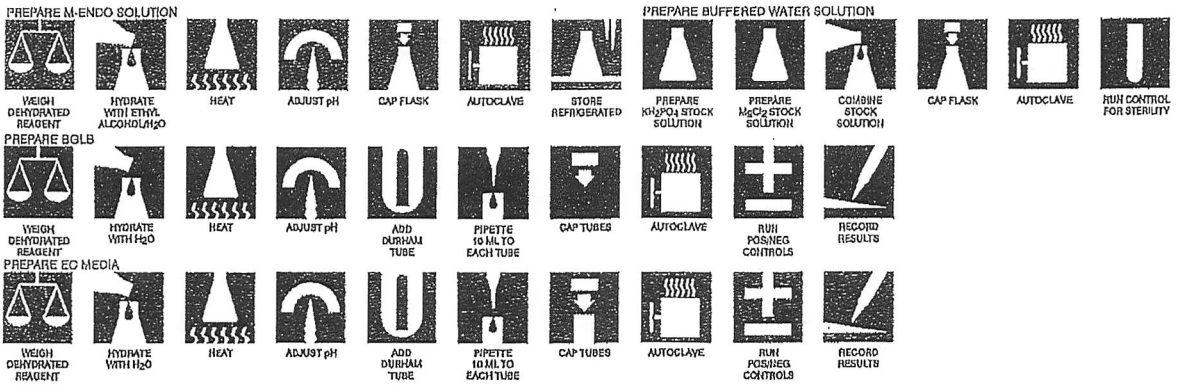
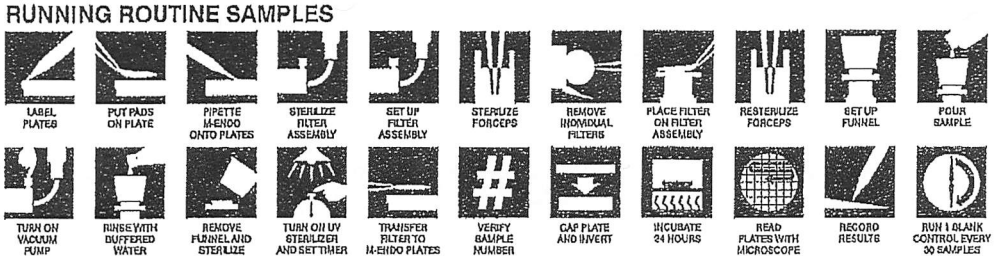


RUNNING ROUTINE SAMPLES



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MEMORANDUM

CITY OF CAPE CORAL
PUBLIC WORKS DEPARTMENT

TO: Wanda Roop, Procurement Manager
Milagros Rosario, Senior Buyer

FROM: Persides Zambrano, Interim Public Works Director
Maya Robert, Environmental Resources Manager

DATE: November 28, 2023

SUBJECT: IDEXX Consumables Sole Source Purchase Order

The FL Department of Environmental Protection and Department of Health are following suite with the US Environmental Protection Agency (US EPA), using enzyme substrate tests for bacteriological testing of all types of waters. These tests provide faster, more reliable results that are less labor intensive, have less chance for human error, do not require a confirmation step that, and reduces audit findings during the City Laboratory's accreditation process.

In events such as septic/sewage spills and first responders diving testing, when public health is of the upmost importance and concern, it is essential to be able to report results in the most reliable and quickest way possible. Eliminating the need for confirmation testing enables the City Laboratory to report results in as little as 18 hours, compared 48-72 hours for the previous method.

IDEXX is the sole source provider for enzyme substrate style testing as they hold the patent for the technology and are approved by the US EPA and State agencies. There are no other similar sources or products available in the market.

The Environmental Resources Division is requesting to open a Sole Source Purchase Order with IDEXX, for a total of $49,010.36, using Stormwater Funds, budgeted in account 440-30408-552199.

PZ:mr (IDEXX Consumables Sole Source Purchase Order)

Attachments: Sole Source Letter
Supporting Documentation
Quote

C: Lindsey Arnaud, Laboratory Quality Control Officer
Eloisa Moreno, Laboratory Supervisor