# 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:** $67,615.08, including $53,563.56 supplies, $10,775 maintenance, $2,276.52 regular shipping, $1,000 provisional expedited emergency shipping.

**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. Service Agreement for annual maintenance of the Tecta B16 unit and IDEXX Sealer unit to comply with state audit requirements.

**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 turnaround 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.

APS **Department Director's Signature:** MA Date: 12/10/24

**Approval: Procurement Manager** Wanda Rapp (not to exceed $50,000.00) **Date:** 12/18/24

**Approval: City Manager** Connie Barron (not to exceed $100,000.00) **Date:** 12/19/24

**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*

November 20, 2024

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 and Sealer Care | 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 and Tecta service plans | Yes | Yes |
| Tecta B16 and Tecta service plans | 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.

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

# QUOTE 20271411

Number / Date
20271411 / 07/22/2024

**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 : 322.112

Valid 10/1/24-9/30/25 Line 12: 2-year Service PLUS

Line 15: 2-year Sealer PLUS Plan

| *Material ID* / *Commodity/COO* | *Description* / *Batch* | *Exp.Date* | *Backorder item* | *Quantity* | *UnitPrice* | *Total Value* |
|---|---|---|---|---|---|---|
| 98-0018012-00 / 3822190080/CA | TECTA-CCA-48, TECTALERT ECTC 48PK | | | 18 | 653.40 | 11,761.20 |
| 98-08877-00 / 3822190080/US | WP200I-18 GAMMA IRAD COLILERT-18 200PACK | | | 16 | 1,376.60 | 22,025.60 |
| 98-09221-00 / 3926909910/CN | WV120SBST-200,VESSELS W/ST AND SB, 200PK | | | 30 | 152.80 | 4,584.00 |
| 98-09227-00 / 3822190080/US | WQT2KC, PRE-DISP.QT 2000 COMPARATOR | | | 1 | 38.42 | 38.42 |
| 98-11682-00 / 3822190080/US | WP104 COLI P/A COMPARATOR | | | 1 | 20.12 | 20.12 |
| 98-21375-00 / 3822190080/US | WENT200 ENTEROLERT 100ML 200-PACK | | | 5 | 1,633.66 | 8,168.30 |
| 98-21675-00 / 3926909910/US | WQT2K QUANTI-TRAY 2000 DISPOSABLE 100/BX | | | 22 | 230.86 | 5,078.92 |
| 98-0018016-00 / 9027905695/CA | TECTA-PDS-VALC40708, TECTA VALIDATION | | | 1 | 972.00 | 972.00 |
| 95-0018307-00 | Tecta Service PLUS Agreement | | | 1 | 9,775.00 | 9,775.00 |
| 98-09588-00 / 3926909910/CN | WV290SBST-100, 290 ML VESSEL W/NA 100-PK | | | 3 | 305.00 | 915.00 |
| 95-21376-02 | IDEXX Sealer Care | | | 2 | 500.00 | 1,000.00 |

**All local taxes at customer charge**

IDEXX
LABORATORIES

Date
12/04/2024

Number
20271411

| | | |
|---|---|---|
| **Items Total** | | **64,338.56** |
| **Freight Value** | | **2,276.52** |
| **Total Amount** | **USD** | **66,615.08** |

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All local taxes at customer charge

## Agreement on Services for IDEXX Tecta Instrument Products

This Agreement on Services for IDEXX Tecta Instrument Products ( "Agreement") incorporates IDEXX's General Terms and Conditions of Sale ("General Terms", available at https://www.idexx.com/en/about-idexx/terms-of-sale/) and applies to the various service plans offered by IDEXX Laboratories, Inc. or one of its affiliates, referred to as IDEXX, we, us and our, for IDEXX Tecta B4 and/or IDEXX Tecta B16 instrument. By signing an order form, placing an order, or accepting the services identified below, you agree to the General Terms and the terms of this Agreement.

**Covered Products; Modifications:** This Agreement covers the IDEXX Tecta instrument identified by the serial number(s) specified on your order form and/or invoice. We reserve the right to modify our service terms in this Agreement from time to time upon not less than 30 days' notice to you.

### A. Service Plans

Described below are the various service plans that you may purchase under this Agreement. Not all service plans may be available in your region. Please refer to your order form or invoice for the service plan(s) that you have elected.

**1.Tecta Start-up Service:**

- Description: If you purchase this service, a trained professional will travel to your location for up to two days to install the instrument(s) you purchased from us and provide new-user training.
- This service plan will include installing (if applicable) and commissioning the Tecta instrument, installing any required software updates, performing initial quality testing, and training people who will use the instrument. Other services may be performed as we deem appropriate. This is not intended to be a preventative maintenance or repair visit.
- Schedule and duration of service: This service event is limited to two consecutive days of on-site service. It will be performed during Monday to Friday, excluding holidays. Scheduling will be arranged by our account manager. An additional fee would apply for any extra days.
- Twelve-month term: This service must be used within twelve months of the purchase date of the covered instrument under this Agreement.

**2. Tecta Basic Service Plan (1-year term):**

- Description: For each Tecta instrument, if you purchase this service, you will receive a one-year basic warranty service as described herein, beginning upon the expiration date

of the manufacturer's standard warranty period (twelve months).

- Under this service, we will pay for the cost of replacement parts and provide remote (virtual) support to you while you repair the instrument. We will pay for the shipping charges of these replacement parts.
- We will ship these parts to you in an expedient manner, using reasonable efforts to make sure you have timely access to the replacement parts.
- No on-site service; no loaner instrument: This service plan does not include an on-site visit from a trained professional or access to a loaner instrument(s).
- Eligibility period: This service plan is available for purchase any time during the twelve-month period following the purchase of the instrument.
- IDEXX may, in its sole discretion, elect to offer this service option to you after the expiration of the manufacturer's warranty, but is not obligated to do so. In this circumstance, the coverage period would begin on the date of your payment for the service.

**3. Tecta Service Plus Plan (1-year term)**

- Description: For each Tecta instrument, if you purchase this service, you will receive the one-year basic service plan (as specified in Sub-Section 2 above) for such instrument, as well as access to a loaner instrument if the original instrument needs to be sent to IDEXX for repair, and one optional on-site service visit (two day maximum). Each of these are described in further detail below:
    - Loaner instrument:
        - Description: For any instrument failure that cannot be diagnosed and/or repaired remotely, we will supply a loaner instrument to be used while the original instrument is returned to us for repair. You will be responsible for shipping the original instrument to an address to be provided by us after you receive the loaner. We will pay all shipping costs and make reasonable efforts to deliver the loaner instrument in an expedient manner.
        - A loaner instrument will not be supplied until it is determined by us that the original instrument cannot be diagnosed and/or repaired remotely. This may include IDEXX sending replacement parts to you so you can attempt a remote-guided repair. We will make reasonable efforts to reach this conclusion as quickly as possible, but this may mean that you have a period of time without a loaner instrument while the situation is assessed. IDEXX may, in its sole discretion, elect to send a loaner instrument before a remote repair is attempted, but is not obligated to do so.
        - Single swaps and double swaps: If your original instrument can be repaired, IDEXX will offer to return that original instrument to you after the repair is completed. After receiving the original instrument, you would be required to return the loaner instrument to IDEXX. If the original instrument cannot be

repaired, you will be offered a new instrument (or you will be offered to keep the loaner, if the loaner was a new instrument when you received it). All shipping costs will be paid by IDEXX.

- On-site service visit ("Visit"):
  - Description: This Visit will typically include cleaning, preventative maintenance, system validation and quality control, installation of software updates, and user training. Other services may be performed as we deem appropriate.
  - Scheduling and duration of Visit: The Visit is limited to two consecutive days of on-site service. It will be performed during Monday to Friday, excluding holidays. Scheduling will be arranged by our account manager. An additional fee would apply for any extra days.
  - The Visit should be scheduled with reasonable advance notice. This Visit is intended to be for routine preventative maintenance and training for already-installed instruments, and is not intended to be for emergency repair or instrument installation. If circumstances allow, IDEXX may, in its sole discretion, elect to perform this Visit as an emergency visit, but is not obligated to do so. If you have already used your Visit under this Agreement and require a subsequent emergency repair, an additional charge would apply.
  - This is an optional visit at your discretion. This means that you are ultimately responsible for notifying IDEXX of the request to have the Visit. If IDEXX does not perform the Visit during the 1-year term of the Tecta Service Plus Plan, you will not be entitled to a full or partial refund. IDEXX will make reasonable accommodations for Visits that are scheduled but later canceled due to events beyond your control, such as illness or weather.

- Term, eligibility period, and effective date:
  - This is a one-year-term service, which shall be purchased any time during the twelve-month period following the purchase of the covered instrument.
  - All services covered by this service plan shall be effective on the date of the purchase of the service. As such, the basic service plan included in this service option will not be delayed to begin upon the expiration of the manufacturer's standard warranty period (which will be 12 months starting from your purchase of the covered instrument).
- Tecta Service Plus Plan with a 2-year Term. You may choose to purchase the Tecta Service Plus plan for a 2-year term. In that case, all the terms specified above will apply except that the term for the service will be two years effective as of the date of your purchase of the service and two on-site service Visits at your option will be provided.

- Add instrument(s) to current Tecta Service Plus Plan:
    - Description: If you have an existing Tecta Service Plus plan purchased under this Agreement, you may choose to add additional instrument(s) ("Added Instrument") to such Service Plus plan, provided that such Added Instrument is located at the same site where the instrument(s) already covered under the current Tecta Service Plus plan is located and that an additional fee (as specified in the order form or invoice) is paid on a per-instrument basis. You can only choose to add instrument(s) to the current Tecta Service Plus plan within 12 months of the purchase date of such instrument(s).
    - The current Tecta Service Plus plan (including the starting and end date of the plan) will apply to the Added Instrument, except that there will be no separate and additional on-site service visit for the Added Instrument. So, if you choose to add an instrument after the on-site service visit has already occurred under the current Tecta Service Plus plan, you will not be entitled to an additional on-site service visit for the Added Instrument. However, in such case, IDEXX may, in its sole discretion, elect to adjust the service price for the Added Instrument (but is not obligated to do so).

**4. On-site Service Visit:**

- Description: This is a stand-alone service in addition to what you may have under any other service plan(s). With this stand-alone on-site service visit plan, you will receive one on-site service visit of up to two days to service or repair such instrument. The cost of replacement parts is not included and will be invoiced to you separately. This service does not cover any services beyond the single two-day visit.
- Scheduling and duration of service: This service event is limited to two consecutive days of on-site service. It will be performed during Monday to Friday, excluding holidays. Scheduling will be arranged by our account manager. We will make reasonable efforts to perform this service in a timely manner, understanding that delays may cause disruption to your testing. An additional fee would apply for any extra days beyond the second day.

**B. General Terms (for all service plans)**

**1. Hours of Service; Service Returns:** We will provide service in accordance with our normal procedures and during our normal business hours, except holidays. In case of malfunction, you must first contact IDEXX Customer Support by telephone at our telephone number provided in your product documentation.

Our telephone support is available during our normal business hours, Monday through Friday, except holidays. Our support personnel will guide you to attempt to correct reported problems

yourself. If telephone support is not successful, we will give you further instructions. Unless specifically indicated in this Agreement, we have no obligation to provide on-site service. If it is necessary to return the product, you must do so to our designated facility for examination. If we authorize a return, we will pay shipping costs to and from our repair facility except in cases of improper use or mistreatment, etc. as provided below in "Your Obligations, Exclusions for Improper Use, Etc." All exchanged parts and products become our property. Delayed returns are subject to daily rental charges at our then-current rate.

**2. Your Obligations; Exclusions for Improper Use, Etc.:** You must take reasonable care of the product, maintain it in a clean and appropriate environment and carry out the routine maintenance recommended by us in the applicable user guide, instructions or other documentation or otherwise communicated to you from time to time. You must provide reasonable supporting data to help identify reported problems.

We cannot assure you of the performance of our products if you use them other than in strict accordance with our product instructions or if you use them on or in conjunction with products or services not provided and configured by us. FAILURE TO USE ONLY OUR AUTHORIZED PRODUCTS OR SERVICES IN OR ON OUR PRODUCTS VOIDS THIS AGREEMENT AND OUR OBLIGATIONS TO YOU. In addition, if your equipment is not under warranty and is not currently covered by our service agreement, we may at our option inspect your equipment before we agree to provide coverage. We may charge you our then-standard rates for such inspection, and if repairs are required, we may either charge you for such repairs and replacement parts at our then-standard rates, exclude repairs to parts that have exceeded their reasonable life from service coverage, or vary your service fee accordingly. If for any period you are not covered by our service agreement and wish to start or resume such coverage, resumption will be at our discretion, and we may charge you the service fee for any period you were not covered.

Our service plan coverage does not cover damage resulting from any causes external to our products (which if repairable will be repaired at your expense), such as negligence or improper use or handling; casualty; external electrical fault; failure to follow packing or shipping instructions; use of unauthorized products in conjunction with our products; or repairs or modifications made by anyone other than us or our authorized service providers. We will repair normal wear-and-tear damage only to the extent required for proper functioning of equipment; cosmetic damage to equipment is not covered. If we determine that the reported problem is not covered by this Agreement, you must reimburse us for the costs of equipment shipping, and we will attempt to repair / replace the product at your cost, at our then-standard rates for such work, or return it as you instruct and at your cost; in such case you will also return any loaner or replacement equipment to us at your cost.

**3. All-inclusive Fee:** The fee you pay for the service plan in this Agreement is an all-inclusive price. Travel costs for the IDEXX team member are included in such fee and thus will not be invoiced separately. Cost of the service will not be itemized by component (e.g., installation vs. training cost).

**4. Renewal; Renewal Fees**: For Tecta Basic Service and Service Plus plans, you may renew the service plan by notifying us prior to the expiration of your current service plan ("Renewal Term"). The service fee for any Renewal Term will be IDEXX's then-current fee. IDEXX may invoice you for Renewal Term(s) before the current Term expires. If IDEXX does not receive payment of the service fee in accordance with IDEXX's invoices to you, or before the beginning of the Initial Term or any Renewal Term, then IDEXX reserves the right to terminate the service plan(s) immediately, including any IDEXX warranty or support obligations to you. If for any period of time you are not covered by our service plan and wish to start or resume such coverage, we may charge you the service fee for any period you were not covered.

**5. Termination:** The service plan may be terminated by either party upon 60 days written notice to the other. If it is terminated by you without cause or by us due to your breach of the Agreement, you shall not be entitled to any refund of any fee paid by you. If the service plan is terminated by us without cause, IDEXX shall refund to you a pro rata portion of any fee paid with respect to the initial service period or current renewal, as the case may be. We reserve the right to refuse to provide service to you if you are in breach of this Agreement or if your account with any IDEXX company for any product or service is delinquent.

Revised April 2023

# IDEXX Sealer Care* Agreement

Contact Name_____ Laboratory Name_____

Address _____

City_____ State/Province_____ ZIP/Postal Code_____

Country_____ Telephone _____ Fax_____

Sealer Serial Number_____ Purchase Order Number_____

## Sealer

☐ **Quanti-Tray* Sealer PLUS**

## Coverage Options (check one):

**IDEXX Sealer Care***
**Point-of-sale purchase:**

☐ 1 year: $375 ($490 CAD)
☐ 2 years: $600 ($780 CAD)
☐ 3 years: $875 ($1,140 CAD)
☐ 4 years: $975 ($1,270 CAD)

**Renewal:**

☐ 1 year: $500 ($650 CAD)

## Additional Options (check one):

**IDEXX Sealer Care with Loaner**
**Point-of-sale purchase**

☐ 1 year: $900 ($1,170 CAD)

**Renewal:**

☐ 1 year: $1,000 ($1,300 CAD)

This IDEXX Sealer Care agreement (this "Agreement") applies to the extended service plan offered by IDEXX Laboratories, Inc. or its nominee, referred to as we, us and our, to the customer signing below, referred to as you, for the IDEXX Quanti-Tray* Sealer. By signing below, you agree to the terms of this Agreement.

**Covered Products; Modifications:** This Agreement covers only the IDEXX sealer identified by serial number above (and any replacement sealer provided under this Agreement). We will perform repair services under this Agreement at no further cost to you (and we will pay shipping costs to and from our repair facility), except in cases of improper use or mistreatment, etc. as provided below. We reserve the right to modify our service terms from time to time upon not less than 30 days notice to you.

**Our Extended Service Commitment**: Provided you have paid the service fee for the current service period, **if your equipment does not conform to our published specifications during the service period elected by you above, and unless you elect and pay for the "IDEXX Sealer Care with Loaner" option, we will replace your equipment with quality recertified equipment that is functionally equivalent or superior to the replaced equipment in performance. If you elected and paid for the "IDEXX Sealer Care with Loaner" option, we will repair your sealer with new parts or quality recertified parts that are equivalent or superior to new parts in performance.**

**Hours of Service; Service Returns:** We will provide service in accordance with our normal procedures and during our normal business hours at our service locations, except holidays. In case of malfunction, you must first contact IDEXX Customer Support by telephone at our number provided in your product documentation. Our telephone support is available during our normal business hours, which are from 8:00 a.m. to 5:00 p.m. (ET), Monday through Friday, except holidays. Our support personnel will guide you to attempt to correct reported problems yourself. If telephone support is not successful, we will give you further instructions. We have no obligation to provide on-site service; if it is necessary to return the product, you must do so to our designated facility for examination. If we authorize a return, we will pay shipping costs to and from our repair facility except in cases of improper use or mistreatment, etc. as provided below in "Your Obligations; Exclusions for Improper Use, Etc." All exchanged parts and products become our property.

If you elected the standard IDEXX Sealer Care option, and we determine that you need to exchange the equipment for quality recertified equipment, we will ship you the replacement equipment by overnight delivery (if available) within 24 hours, during normal business hours, Monday-Friday, excluding holidays. You shall pack and return-ship us the malfunctioning equipment the next business day after your receipt of replacement equipment. Delayed returns are subject to daily rental charges at our then-current rate.

If you have elected the IDEXX Sealer Care with Loaner option above, we will ship you a loaner unit by overnight delivery (if available) within 24 hours, during normal business hours, Monday-Friday, excluding holidays. You shall pack and return-ship us the malfunctioning equipment the next business day after your receipt of the loaner equipment. We will ship you the

repaired equipment and you shall pack and return-ship the loaner equipment to us within two business days after your receipt of the repaired equipment. Delayed returns are subject to daily rental charges at our then-current rate.

**Your Obligations; Exclusions for Improper Use, Etc.:** You must take reasonable care of the equipment, maintain it in a clean and appropriate environment and carry out the routine maintenance recommended by us in the applicable user guide, instructions or other documentation or otherwise communicated to you from time to time. You must provide reasonable supporting data to help identify reported problems.

We cannot assure you of the performance of our products if you use them other than in strict accordance with our product instructions or if you use them on or in conjunction with products or services not provided and configured by us. FAILURE TO USE ONLY OUR AUTHORIZED PRODUCTS OR SERVICES IN OR ON OUR PRODUCTS VOIDS THIS AGREEMENT AND OUR OBLIGATIONS TO YOU. In addition, if your equipment is not under warranty and is not currently covered by our service plan, we may at our option inspect your equipment before we agree to provide coverage. We may charge you our then-standard rates for such inspection, and if repairs are required, we may either charge you for such repairs and replacement parts at our then-standard rates, exclude repairs to parts that have exceeded their reasonable life from service coverage, or vary your service fee accordingly. If for any period you are not covered by our service plan and wish to start or resume such coverage, resumption will be at our discretion and we may charge you the service fee for any period you were not covered.

Our service plan coverage does not cover damage resulting from any causes external to our products (which if repairable will be repaired at your expense), such as negligence or improper use or handling; casualty; external electrical fault; failure to follow packing or shipping instructions; use of unauthorized products in conjunction with our products; or repairs or modifications made by anyone other than us or our authorized service providers. We will repair normal wear-and-tear damage only to the extent required for proper functioning of equipment; cosmetic damage to equipment is not covered. If we determine that the reported problem is not covered by this Agreement, you must reimburse us for the costs of equipment shipping, and we will attempt to repair/replace the product at your cost, at our then-standard rates for such work, or return it as you instruct and at your cost; in such case you will also return any loaner or replacement equipment to us at your cost.

**Service Period; Renewal; Renewal Fees:** If you purchased IDEXX Sealer Care at the time you purchased the equipment, the service period begins upon the expiration of the relevant product warranty period and ends after the elapse of the number of years of coverage you selected on the first page of this Agreement. For other service plan purchases, including renewals, the service period beings when we receive your signed Sealer Care Agreement and ends after elapse of the number of years of coverage you selected on the first page. You may renew the service plan by notifying us prior to expiration of your current service period and paying the then current fee. We may invoice you for renewal of the service period before the current period expires. If we do not receive payment of the service fee in accordance with our invoices to you, then we reserve the right to terminate this Agreement immediately, and we will not have any further obligations to you.

**Limitation of Damages:** We are not liable for failure to provide services due to circumstances beyond our reasonable control. UNDER NO CIRCUMSTANCES WILL WE OR OUR LICENSORS BE LIABLE TO YOU OR ANY OTHER PERSON FOR LOSS OF PROFIT OR USE, SPECIAL, INCIDENTAL, CONSEQUENTIAL, INDIRECT, EXEMPLARY, PUNITIVE OR MULTIPLE DAMAGES, INCLUDING WITHOUT LIMITATION FOR LOSS OF GOODWILL, DATA OR EQUIPMENT OR FOR BUSINESS INTERRUPTION, ARISING OUT OF THE MANUFACTURE, SALE, SUPPLY OR USE OF OUR PRODUCTS OR SERVICES OR FAILURE OR DELAY IN DELIVERING SUCH PRODUCTS OR SERVICES, WHETHER BASED ON WARRANTY, CONTRACT, TORT OR OTHERWISE, EVEN IF WE WERE ADVISED OF THE POSSIBILITY OF SUCH DAMAGES OR LOSSES.

OUR ENTIRE LIABILITY FOR A PRODUCT WHETHER BASED ON CONTRACT, TORT OR OTHERWISE, SHALL NOT EXCEED THE SERVICE FEE FOR THE APPLICABLE PRODUCT PAID BY YOU FOR THE MOST RECENT SIX MONTHS OF THE SERVICE PERIOD (OR THE LAST SUCH PERIOD, FOR ANY CLAIMS ARISING AFTER ALL SERVICE PERIODS).

EXCEPT AS STATED IN THIS AGREEMENT, WE AND OUR LICENSORS MAKE NO OTHER WARRANTY, REPRESENTATION OR CONDITION, EXPRESS OR IMPLIED, WRITTEN OR ORAL, AND THERE IS NO WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, CARE AND SKILL, TITLE OR NONINFRINGEMENT.

**Termination:** This Agreement may be terminated by either party upon 60 days written notice to the other. If this Agreement is terminated by you, you shall not be entitled to any refund of any fee paid by you. If this Agreement is terminated by us, IDEXX shall refund to you a pro rata portion of any fee paid with respect to the initial service period or current renewal, as the case may be.

We reserve the right to refuse to provide service to you if you are in breach of this Agreement or if your account with any IDEXX company for any product or service is delinquent.

**Miscellaneous; No Assignment:** This Agreement is our entire agreement with respect to its subject matter, and it supersedes all prior oral or written agreements or statements. Any term of any purchase order or other document that you provide us that is in any way inconsistent with or in addition to the terms set forth in this Agreement will not become a part of the contract between the parties or be binding on us. Except as provided above for our right to modify service terms from time to time upon not less than 30 days' notice to you, neither party can modify this Agreement (including this paragraph) except in a written document signed by authorized representatives of both parties. You may not assign any duties, rights or claims hereunder without our prior written consent, even if you sell your equipment to another. Any such attempted assignment is void, and we will not have any obligations to you or your assignee.

**Governing Law; Venue; Waiver of Jury Trial:** This Agreement and the transactions contemplated hereby, and all related disputes between the parties under or relating to this Agreement, whether in contract, tort or otherwise, shall be governed by the laws of the State of Maine (or the Province of Ontario, for Canadian sales), without reference to conflict of laws principles, and any related legal actions must be brought in the court of appropriate jurisdiction in the State of Maine (Province of Ontario, for Canadian sales), which shall have exclusive jurisdiction (except that either of us may bring an action for an injunction or similar equitable relief against the other in any proper jurisdiction). You hereby waive any claim of lack of jurisdiction or inconvenient forum.

YOU AND WE WAIVE TRIAL BY JURY IN ANY LEGAL ACTION BY OR AGAINST US IN SUCH LEGAL ACTIONS. We each further waive any claims against the other for multiple, punitive or exemplary damages in any legal actions relating to this Agreement. The prevailing party in any such legal actions shall be entitled to an award of its reasonable legal fees and costs.

**English Language (Québec only):** The parties confirm that it is their wish that this Agreement and any other documents delivered or given pursuant to this Agreement, including notices, have been and shall be in the English language only. Les parties aux présents confirment leur volonté que cette convention de même tous les documents, y compris tous avis, s'y rattachant, soient rédigés en anglais seulement.

---

**I acknowledge that I have received and reviewed the IDEXX Sealer Care* Agreement and accept and agree to its conditions.**

**Company Name:** _____

**By (signature):** _____

**Name (printed):** _____

**Title:** _____

**Date:** _____

**IDEXX Representative:** _____

Fax the Agreement to 1-207-556-4630, email water@idexx.com, or mail to:

IDEXX Sealer Care
Attn: Technical Services
IDEXX Laboratories
One IDEXX Drive
Westbrook, Maine 04092 USA

# Service Plans for IDEXX Tecta Instruments

IDEXX Tecta® instruments offer the benefits of automation to water microbiology testing. IDEXX is pleased to offer a suite of enhanced service, protection, and training plans to meet your needs. These options allow laboratories to receive enhanced support services in the event of an instrument failure, as well as on-site installation and maintenance services (included in certain options).

Each service offering is described briefly in the table below. Visit **idexx.com/TectaServiceAgreement** or contact your IDEXX representative to review the full terms and conditions for each service plan.

## Installation and training

| Service plan | Description | Price (USD) | Other notes<br>(review terms and conditions for full coverage details) |
|---|---|---|---|
| Tecta Start-up Service<br>(95-0018304-00) | Service to install and commission a Tecta instrument. | $4,500 | • Includes an on-site installation and training visit of up to two days. Travel costs included.<br>• The on-site visit will include installation and commissioning of the Tecta instrument, as well as performing any required software updates, quality testing, and user training.<br>• Must be purchased at time of instrument sale. Must be used within 12 months following instrument purchase. |

IDEXX

## Ongoing maintenance, repair, and training

| Service plan | Description | Price (USD) | Replacement part coverage | On-site service and training | Access to loaner instrument | Other notes (review terms and conditions for full coverage details) |
|---|---|---|---|---|---|---|
| Tecta® Basic Service Plan (95-0018306-00) | Provides replacement for failed parts and remote support for instrument service and repair. | $1,250 | ✓ | | | • Coverage begins after expiration of manufacturer's warranty (12 months).<br>• 1-year coverage; can be renewed annually. Must be purchased prior to manufacturer's warranty expiration.<br>• Coverage is for a single instrument. |
| Tecta Service Plus Plan (95-0018307-00) | Includes replacement for failed parts, access to a loaner instrument, and one optional preventative service and support visit per year. | $5,750 (1-year)<br>$9,775 (2-year) | ✓ | ✓ | ✓ | • Available as 1-year or 2-year term; can be renewed annually. Must be purchased within 12 months of instrument purchase.<br>• Additional instruments can be added for $1,750 (1-year) or $2,975 (2-year) per instrument (95-0018308-00).<br>• Coverage begins immediately after purchase.<br><br>**What's included:**<br>• Provides replacement for failed parts and remote support for instrument service and repair.<br>• For any failure that cannot be diagnosed and/or repaired remotely, IDEXX will supply a loaner instrument to be used while the original instrument is returned to IDEXX for repair. Related shipping costs are included.<br>• The on-site visit may include services such as cleaning, preventative maintenance, system validation, installing software updates, and additional user training.<br>• On-site visit is 2 days maximum, at customer's request. Visit expires at end of coverage term. Travel costs included. (2-year option includes 2 separate on-site visits over the 24 months.) |
| On-Site Service Visit (No Coverage) (95-0018305-00) | Any on-site service event not covered by a Service Plus Plan (no loaner or parts coverage). | $6,500 | | ✓ (service and repair only) | | • Covers one on-site service or repair event of up to 2 days. Travel costs included.<br>• Does not include replacement for failed parts (charged separately).<br>• Does not include the use of a loaner instrument during repair. |

IDEXX

## General notes

- All prices and descriptions above are specific for customers in the U.S. or Canada. Service plans may not be available for all customers or situations. Speak with your IDEXX representative to learn more.
- All service options cover the Tecta® B16 and/or Tecta® B4 instruments. Service features and pricing are the same for each instrument type.
- Combinations: The Tecta Start-up Service may be combined with the Tecta Basic Service Plan or the Tecta Service Plus Plan. Discounts may apply for combined plans - contact your representative to learn more. The Tecta Basic Service Plan and Tecta Service Plus Plan may not be combined.
- Eligibility period: Tecta Basic Service Plan and Tecta Service Plus Plan must be purchased within first 12 months after purchase of the instrument.
- Added instruments: Additional instruments located at the same site may be added to a Tecta Service Plus Plan for a discounted rate. In this scenario, the beginning and end dates of coverage will be the dates related to the original instrument. Review the program terms or contact your IDEXX representative to learn more.
- The prices and descriptions in this table are accurate as of the time of publication, but are subject to change over time. Check with your IDEXX representative to learn more. The enclosed order form must be used to purchase this service plan.

## Order form: Coverage and Instrument Information

Contact Name _____ Laboratory Name _____

Address _____

City _____ State/Province _____ ZIP/Postal Code _____ Country _____

Telephone _____ Email _____ Fax _____

Tecta Instrument Serial Number(s) _____ Purchase Order Number _____

Review program terms and conditions at idexx.com/TectaServiceAgreement. By placing an order or accepting services, you confirm that you have reviewed and agreed to these terms and conditions.

## To be completed by an IDEXX representative (optional information for customer records)

| Serial number and instrument type | Service plan (include part number) | Initial purchase or renewal | PO number | Coverage dates (Tecta Basic Service Plan and Tecta Service Plus Plan only) |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |



IDEXX

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).<br>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.<br><br>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<br><br>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*<br><br>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<br><br>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*<br><br>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.<br><br>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<br><br>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*<br><br>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<br><br>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*<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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.<br><br>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 stabilized 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 stabilized 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 stabilized 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 stabilized 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 stabilized 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 stabilized 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 stabilized 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

* Colilert and Quanti-Tray are trademarks or registered trademark of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries

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
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**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

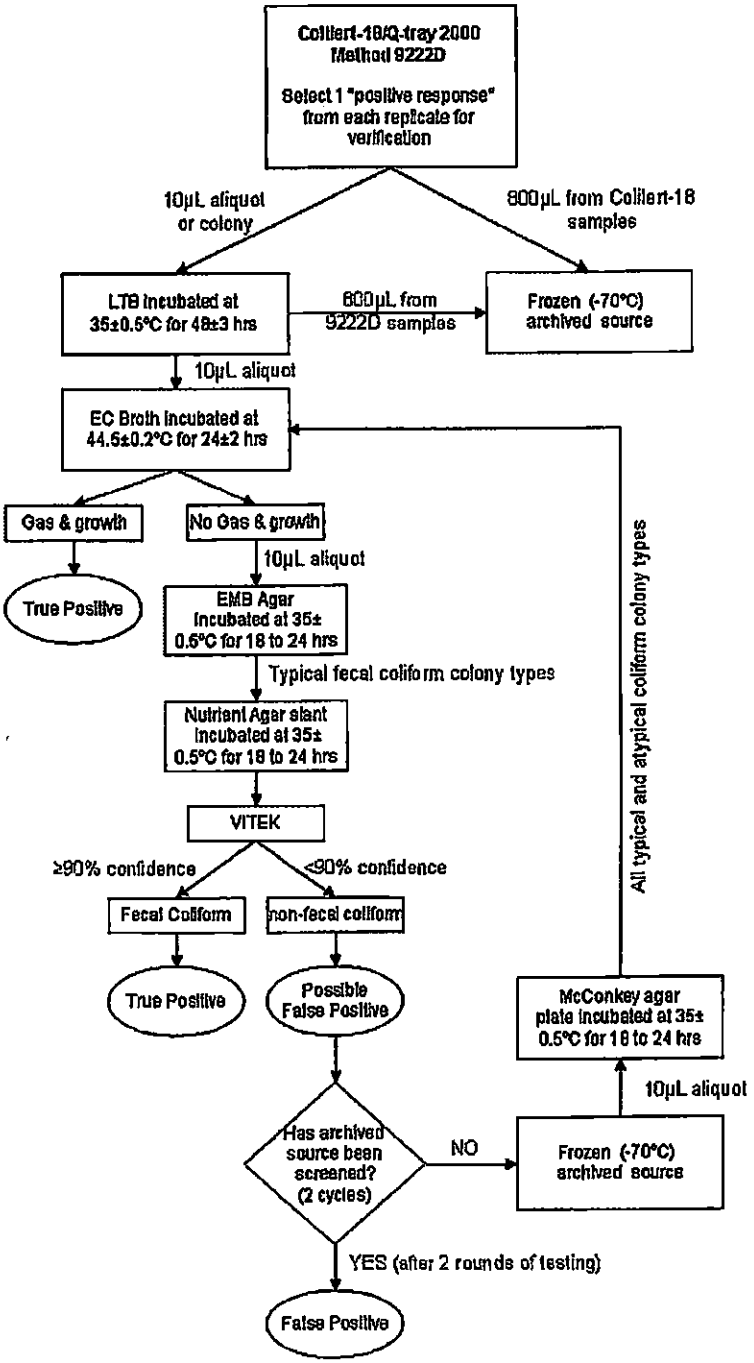


**Figure 1. Confirmation procedure for positive response samples.**

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).

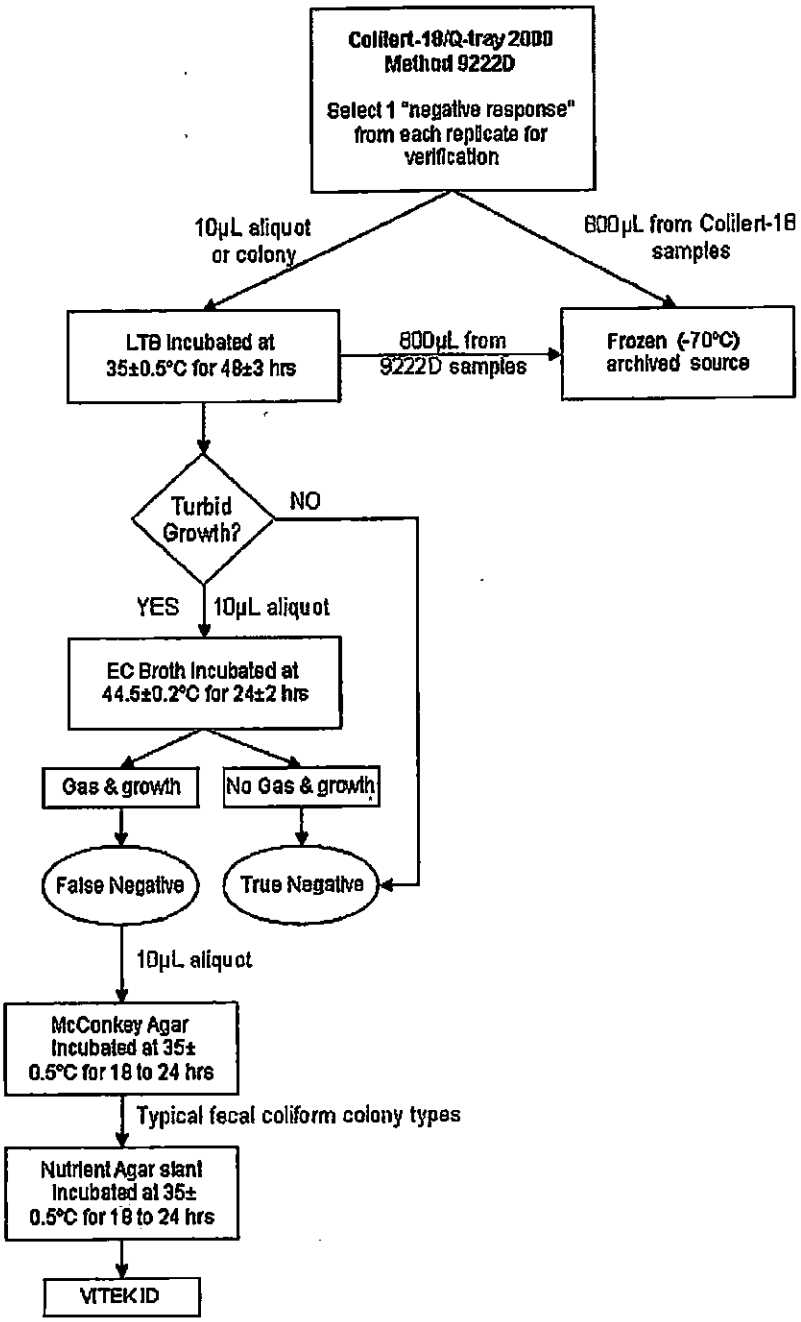


**Figure 2. Confirmation procedure for negative response samples.**

*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 | 10.58 | 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 | 16–69 | 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

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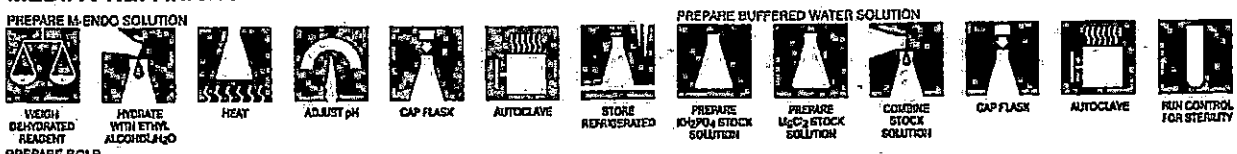
# Count the steps.
# Then choose an easier way to test.

## Membrane filtration

### MEDIA PREPARATION

PREPARE M-ENDO SOLUTION

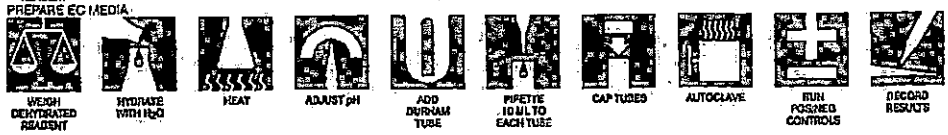



PREPARE BUFFERED WATER SOLUTION




PREPARE BGLB


PREPARE EC MEDIA

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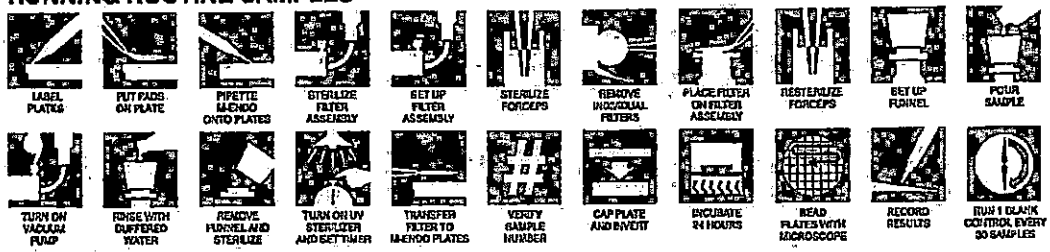
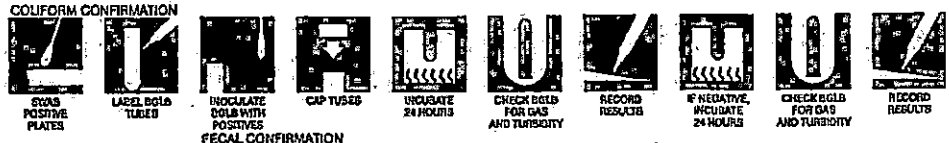
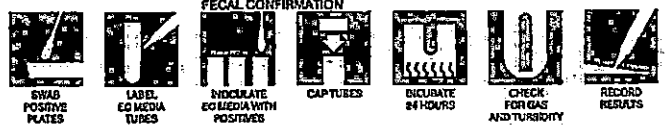



### CONFIRMATION TESTING

COLIFORM CONFIRMATION




FECAL CONFIRMATION




## The IDEXX Quanti-Tray® System

### PRESENCE/ABSENCE


### QUANTIFICATION


IDEXX Water **1-800-321-0207** ∘ **idexx.com/water**

IDEXX



ISO **9001:2008** CERTIFIED

TO: Michael Ilczyszyn, City Manager
Crystal Fest, Interim Finance Director
Wanda Roop, Procurement Manager

FROM: Matt Williams, P.E., CFM, Public Works Director MW
Maya Robert, Environmental Resources Manager MR

DATE: December 6, 2024

SUBJECT: IDEXX Sole Source Purchase Order

**Background**

The City Laboratory performs bacteriological tests for regulatory compliance of city operations on drinking water, surface water, groundwater, and wastewater products. Few methodologies are approved both by the US Environmental Protection Agency and the Fl Department of Environmental Protection. Only IDEXX methods are adapted to the wide range of tests the City Laboratory performs, reliable, and fast. IDEXX is the sole source provider for enzyme substrate style testing, holding the patent for the technology. There are no other similar sources or products available in the market.

Staff reached out to the Sole Source vendor IDEXX to receive pricing for their annual consumables and maintenance needs.

**Recommendation**

Many of those consumables have specific shelf life, so the orders will be shipped throughout the year. In the event of increased line break testing, staff might need supplies rapidly in volume exceeding the regular workflow of the lab operations. Staff recommends opening a Purchase Order with IDEXX for a total of $67,615.08, including $53,563.56 for consumables supplies, $10,775.00 for annual maintenance requirements, $2,276.52 for regular shipping, and add a provision of $1,000.00 for expedited shipping in the event of emergency responses' testing. All shipping charges to be organized and the charges invoice by the Sole Source vendor.

**Fund Availability**

The funds for this purchase have been budgeted in the Stormwater Enterprise Funds and are available in account 440-30408-552199 for the Consumables and 440-30408-546300 for Maintenance.

MW/MR:mr (IDEXX Sole Source Purchase Order)

Attachment: Sole Source Form
Sole Source Letter
Quote
Supporting Documentation

C: Alicia Pearce Smith, Public Works Business Manager APS