



Department of Environmental Protection

One Winter Street Boston, MA 02108 • 617-292-5500

Charles D. Baker
Governor

Karyn E. Polito
Lieutenant Governor

Matthew A. Beaton
Secretary

Martin Suuberg
Commissioner

Water Quality Monitoring Grant Program Request For Grant Proposals

1.0 GRANT OPPORTUNITY SUMMARY

Proposals Sought: Massachusetts Department of Environmental Protection (MassDEP) is seeking grant proposals from eligible entities outside of the agency for bacteria data collection, and/or associated capacity-building in order to perform data collection in the future, with the goal of increasing the amount of bacteria data available for MassDEP's assessment of primary and secondary contact recreation activities in surface waters of the Commonwealth.

Overview of the Challenge and Grant Program Goals: The mission of MassDEP's Watershed Planning Program (WPP) is to implement a comprehensive surface water monitoring strategy for the Commonwealth that serves all water quality management needs and addresses all waterbody types. However, with over 3,000 lakes and ponds and 12,000 miles of streams and rivers in the state, MassDEP can only sample a small fraction of these surface waters in any given year. Therefore, MassDEP's goal in presenting this grant opportunity is to supplement its own surface water dataset ("internal dataset") with data collected by groups outside of the agency ("external dataset").

To help meet this goal, MassDEP is soliciting grant proposals for the 2019 summer sampling season (i.e., from April 1 to October 15, 2019) to support eligible entities in the collection of bacteria data in surface waters. This external dataset will enhance the amount and spatial coverage of data that MassDEP can utilize to determine whether Massachusetts' waters are safe for swimming and boating activities (as well as other forms of primary and secondary contact recreation).

The intent of this grant opportunity is to expand the capabilities of eligible entities to collect bacteria data and increase the availability of this external dataset for MassDEP's use in water quality assessments. MassDEP's internal data, and credible external data collected by others, will be used as the basis for assessing surface water quality in accordance with the requirements set forth in §305(b) and §303(d) of the federal Clean Water Act (CWA). In addition to the data collected by MassDEP staff, reliable data obtained from other state and federal agencies, local governments, drinking water utilities, volunteer organizations and other sources are also considered in MassDEP's surface water quality assessments. Use-attainment determinations (i.e., is the waterbody able to support swimming and boating?) are made for each waterbody for which adequate data and information are available. However, many waters are not assessed for one or both of these uses in any given 2-

year assessment cycle. Therefore, many surface waters that were assessed in the past are in need of updated information to determine the current condition of the waterbody, and many small and/or unnamed streams and ponds have never been monitored or assessed. For more information on MassDEP's water quality assessment process, the CALM, please see: <https://www.mass.gov/media/1838926/>.

The quality of the data used by MassDEP reflects project organization, extensive planning, the application of rigorous data quality objectives (DQO), and training in standardized field and laboratory procedures, metadata collection and data verification. Collectively, the result of these actions is a scientifically-sound and legally-defensible dataset that can help MassDEP determine if a waterbody is meeting CWA water quality standards (for additional information, see <https://www.mass.gov/regulations/314-CMR-4-the-massachusetts-surface-water-quality-standards>; for more information on writing a Quality Assurance Project Plan (QAPP), see <https://www.mass.gov/guides/water-quality-monitoring-for-volunteers>).

External data can be submitted to MassDEP's WPP data portal. Guidelines for submission to MassDEP's web site are located here: <https://www.mass.gov/guides/external-data-submittals-to-the-watershed-planning-program>. This site was designed to guide users on data format protocols.

Alternatively, external datasets can be submitted through the national Water Quality Portal (WQP). The WQP is a cooperative service sponsored by the United States Geological Survey (USGS), the Environmental Protection Agency (EPA), and the National Water Quality Monitoring Council (NWQMC). It serves data collected by over 400 states, federal, tribal and local agencies. For access to the user's guide see this link: <https://www.waterqualitydata.us>. MassDEP staff can access external water quality data submitted through the National WQP.

Eligible Entities and Projects: Eligible Entities for this Grant Opportunity are limited to legally-recognized non-profit organizations, and can include watershed groups, lake and pond associations, academic institutions and other non-profit organizations with the ability to conduct bacteria monitoring and data collection, or who wish to develop this capacity. To be eligible for this Grant, all non-profit organizations must provide MassDEP with documentation of the organization's non-profit status (i.e., 501(c)(3)) in their grant application package.

Eligible Entities must describe through their proposals how the grant funding will support one or more of the following or similar activities for external surface water bacteria monitoring data collection and/or to build capacity for surface water data collection. Examples of grant-eligible capacity building expenditures for this grant include, but are not limited to, the following:

- monitoring project design and planning (e.g., QAPP and Sampling and Analysis Plans (SAP));
- purchase of monitoring equipment and supplies (e.g., enzyme-substrate analytical systems, incubators, reagents, sample containers);
- salary for a monitoring program coordinator;
- training in monitoring support areas (e.g., use of enzyme-substrate analytical systems, probe calibration, QA, data management and review);
- field program for surface water bacteria monitoring;
- technical assistance in data analysis and reporting; and/or
- technical assistance in formatting data submissions to MassDEP's external water quality data portal or the National Water Quality Portal (WQP).

Ineligible Applicants and Projects: Entities considering submitting a Grant proposal should note that the following Entities and/or projects are ineligible for this Grant funding:

- Applicants cannot propose projects that would be undertaken to comply with local or governmental enforcement actions such as State or Federal Administrative Orders or Consent Orders.
- Applicants cannot propose projects to implement specific requirements of National Pollutant Discharge Elimination System (NPDES) storm water permits.
- Primary applicants or sub-contractors that are not registered as a non-profit organization.

MassDEP Priority Data Needs: Eligible entities should be aware that MassDEP has identified the following waters with Priority Bacteria Data Needs:

- Waters that have never been assessed for pathogens;
- Waters that have been assessed for pathogens historically, but for which there are no recent bacteria data;
- Waters assessed as impaired for pathogens (Category 5 of the *Massachusetts Integrated List of Waters*, <https://www.mass.gov/media/1638446/>); and
- Waters for which MassDEP has specifically identified a need for recent bacteria data in the *Massachusetts Integrated List of Waters*, <https://www.mass.gov/media/1638446/> (see Appendix B of the Application Form).

It should be noted that *E. coli* and *Enterococci* are the indicators of pathogenic contamination in freshwaters and *Enterococci* is the indicator in marine waters.

Application Deadline: Applications are due by 5:00 P.M. on February 27, 2019.

Page Limit: Grant applications must not exceed fifteen (15) pages in length.

Funding Availability:

MassDEP's FY19 operating budget allocates \$200,000 for water monitoring grants. MassDEP plans to award grants of \$5,000 to \$15,000 per eligible entity. Additional funding could be made available in FY19 if the quality and quantity of the proposals exceeds the current allocation.

Applicants may submit proposals for projects that will start in FY19 (April 2019) and end in FY20 (October 2019). The \$200,000 allocated to the FY19 grants expires June 30, 2019 and is therefore available to reimburse awardees for costs incurred on or before June 30, 2019. Applicants are encouraged to develop project proposals that maximize incurred costs prior to June 30, 2019. The amount of grant funds that will be available for FY20 (which begins July 1, 2019) cannot be determined until the Commonwealth's FY20 budget has been finalized (on or after July 1, 2019). While MassDEP anticipates continued funding for the water monitoring grant program in FY20, reimbursement for costs incurred after June 30, 2019 are contingent upon appropriation of funds. MassDEP will notify all grant awardees of the status of FY20 funds as soon as possible after the FY20 budget is finalized. In the event that FY20 funds are not available for this grant program, awardees will need to proceed with any remaining commitments utilizing an alternate source of funding.

Data collection and submittal is expected throughout the 2019 sampling season. Completed datasets must be submitted to MassDEP following the conclusion of the monitoring season and not later than December 31, 2019.

Match Requirement: While Eligible Entities are not required to provide matching funds, a match is preferred, and projects with a matching source of funding, including in-kind services (e.g., training, technical assistance) and/or external funding will be weighed more favorably. There are no restrictions on how matching funds are provided (e.g., corporate, municipal government etc.). Eligible Entities are encouraged to identify other known sources of matching funding for their proposed project on the Application Form, from both secured and anticipated sources.

Total Anticipated Duration of Grant(s): The contract duration will be from the time of announcement of the grant recipients through December 31, 2019.

Applicable Procurement Law: Grants-- MGL c. 7A, § 7; St. 1986 c. 206, § 17; 815 CMR 2.00

On Thursday, December 6, 2018 at 10:00 AM, MassDEP held a pre-grant solicitation information meeting at the Central Regional Office, 8 New Bond Street, Worcester, Massachusetts. The purpose of the meeting was to provide potential applicants and other interested parties with the opportunity to ask questions about the Grant program, including project eligibility and administrative procedures, in a public setting. During the meeting, a number of questions were raised by the audience, which MassDEP answered on an informal basis. These questions, with MassDEP's official written answers, and a copy of the power point presentation and list of attendees, are all posted on MassDEP's website: <https://www.mass.gov/info-details/grants-financial-assistance-watersheds-water-quality>.

Grant Contact: For all questions regarding this grant, contact:

Therese Beaudoin

Massachusetts Department of Environmental Protection
Watershed Planning Program
8 New Bond Street
Worcester, MA 01606
(508) 767-2742
therese.beaudoin@mass.gov

2.0 INSTRUCTIONS FOR APPLICATION SUBMISSION:

Evaluation Criteria (general): Eligible Entities must submit a completed application that includes all of the required supporting materials, agreement to the program conditions, and the ability to meet the stated eligibility requirements, in order to be considered for a grant award. A MassDEP review committee will evaluate proposed projects based upon the criteria listed below. The review committee reserves the right to reject any or all proposals.

Evaluation Criteria Components: The review committee will evaluate and score the grant applications from Eligible Entities (as defined previously in this Grant Opportunity), based upon the following criteria:

A. Organizational Capacity (20 points)

The review committee will evaluate this criterion based upon the following information:

All applicants must provide a description of your organization, the year it was founded, your mission statement, scope of your watershed(s), (number of towns, waterbodies covered) and key staff resumes. All applicants must also provide documentation of the organization's non-profit status (i.e., 501(c)(3) status).

All applicants must explain how this grant will support your organization's efforts to collect bacteria data and/or develop capacity for the collection of bacteria data. Proposals will be evaluated based on the organization's current and/or future capacity to plan and execute a bacteria monitoring data collection program, on a level that will support MassDEP in assessing swimming and boating uses (i.e., primary and secondary contact recreation).

If applicable, applicants must provide a summary description for your organization's bacteria monitoring program for data collected in the last 3 years. This description must provide details on the number of locations, the time period in which samples were collected, bacterial indicators and frequency of monitoring efforts. Please identify any of your stations that overlap with locations for which MassDEP has identified the need for bacteria data, as shown in Appendix B. If applicable, Applicants should also provide documentation of a Department-approved QAPP and records of Data Submission to MassDEP (or National WQP) within the last three years.

For all Eligible Entities, please provide a description of how this grant will support your existing programs and identify any challenges to meeting the goal of expanding your existing data collections. Specifically, the organization should identify any needs that are key to building capacity, including but not limited to planning support (QAPP and sampling and analysis planning), training in data management, QA, data review, assistance in formatting the submission of data (i.e., to either the MassDEP state water quality data (WQD) portal or the federal WQP) and/or required purchase(s) of analytical equipment (e.g., enzyme-substrate analytical system, and or assistance in data analysis and reporting).

B. Description of How Funding will be Utilized (20 points)

Applicants must describe the proposed project, and its projected benefits and goals. The review committee's focus will be upon the following: How well does the applicant describe the proposed project and the work to be covered by this funding? This includes project tasks, personnel, and desired outcome(s) for the project.

C. Priority Data Needs (20 points)

Applicants must describe their project in relation to MassDEP's priority data needs. The review committee's focus will be upon the following: How well does the applicant understand the context for data collections? Does the applicant's program contribute to the collection of bacteria data in priority waters (see the description provided in **MassDEP Priority Data Needs** above)?

D. Financial Need (10 points)

Applicants must describe how the proposed grant funding will impact the organization's financial needs. The review committee's focus will be upon the following: How well does the applicant demonstrate the need for financial assistance to implement the proposed project? Does the applicant identify and describe other anticipated or secured funding sources that will support portions of the project? Is there any in-kind service(s) or monetary match that can be committed to the project?

E. Project Budget and cash flow schedule (10 points)

The applicants must present a project budget for the expenditure of the grant funds. The review committee's focus will be upon the following: How well does the applicant estimate and describe project costs? Does the applicant demonstrate a realistic understanding of project costs by providing a budget with detailed and credible cost estimates? What costs will be incurred in FY 2019 (which ends on June 30, 2019) and what costs will be incurred in FY2020 (which commences on July 1, 2019?)

F. Project Timeline (5 points)

Applicants must submit a timeline which specifies the tasks and deliverables for the project, including when these activities commence and when the activities will be completed. The review committee's focus will be upon the following: How well does the applicant specifically describe the timeline for the proposed work? Is that a reasonable amount of time for the described tasks to be accomplished? Can the project be feasibly completed and relevant deliverables submitted in accordance with the proposed timeline?

G. Project Benefit (15 points)

MassDEP will evaluate the proposals based on the benefits to meeting the agency's strategic monitoring goals of assessing primary and secondary contact recreation activities in the Commonwealth's surface waters. Specifically, the Department, through the review committee, will evaluate how and to what extent the proposed project furthers the goals outlined below:

- Enhancing the availability of credible bacteria data that can be utilized to support MassDEP's CWA Programs.
- Providing information for unassessed waters (including those waters that have never been assessed, as well as waters that have not been assessed recently), and waters known to be impaired for bacteria (see Category 5 waters in the draft *Massachusetts Year 2016 Integrated List of Waters*, available at <https://www.mass.gov/media/1638446/>, and those waters for which MassDEP has identified a specific need for current bacteria data (see Appendix B).
- Improvements in planning for capacity building (e.g., QAPP, SAP, formatted data submission through MassDEP's external data portal or the federal WQP portal, and/or purchase of supplies and equipment).

Application Completion and Submission Instructions: All Grant applicants must complete the attached application form. Once the application form is complete, the document can be submitted electronically in Microsoft Word, or to reduce file size, the document may be converted and submitted as an Adobe PDF file.

Applications must be submitted electronically, via email to:

Therese Beaudoin
Massachusetts Department of Environmental Protection
Watershed Planning Program
8 New Bond Street
Worcester, MA 01606
therese.beaudoin@mass.gov
(508) 767-2742

In the subject line of the email, please write the applicant name and "MassDEP Water Quality Monitoring Grant Program."

All applications must be received electronically by MassDEP by 5:00 P.M. on February 27, 2019.

Applications received after the deadline will be rejected automatically. MassDEP reserves the right to reject any and all proposals or request additional information if needed.

A. Additional Required Documentation:

Additional Forms: If selected for a grant award, the applicant will be required to submit the following forms to complete the contracting process. Forms with an asterisk * need not be submitted, if they have been completed previously and are already on file with the Commonwealth:

- [Commonwealth Standard Contract Form](http://www.mass.gov/comptroller/docs/forms/contracts/standard-contract-frm.doc), filled out and signed by the applicant

- [Commonwealth Terms and Conditions](http://www.mass.gov/comptroller/docs/forms/contracts/comm-termsconditions.doc)* filled out and signed by the applicant
<http://www.mass.gov/comptroller/docs/forms/contracts/comm-termsconditions.doc>
- [Commonwealth W-9 tax information form](http://www.mass.gov/comptroller/docs/forms/vendorcustomer/newmass-w9.doc) filled out and signed by the applicant with DUNS number and Federal Tax ID * <http://www.mass.gov/comptroller/docs/forms/vendorcustomer/newmass-w9.doc>
- Completed Contractor Authorized Signature Verification Form
<http://www.mass.gov/comptroller/docs/forms/contracts/casl-form.docx>
- Electronic Funds Transfer (EFT) form* <https://massfinance.state.ma.us/VendorWeb/eftRegisterfrm.asp>

Applicants are encouraged to review these forms prior to submission of an application.

ESTIMATED PROCUREMENT CALENDAR FOR GRANT APPLICATION/AWARD:

Procurement Activity	Date	Time
Information Session and submission of questions: MassDEP 8 New Bond Street Worcester, MA 01606	December 6, 2018	10:00-12:00
Question and Answer period ends	December 28, 2018	5:00 PM
Notice of Grant Opportunity (posted on COMMBUYS And MassDEP website)	January 30, 2019	
Grant Opportunity and Application Release Date (Posting Date) on MassDEP website	January 30, 2019	
Information Session Power point, Official Answers for Q&A published on MassDEP website	January 30, 2019	
GRANT APPLICATION DEADLINE; ELECTRONIC SUBMISSION TO therese.beaudoin@mass.gov	February 27, 2019	5:00 PM
Notification of Grant Award(s) (Estimated) (POSTED ON COMMBUYS AND MASSDEP WEBSITE)	March 20, 2019	
Estimated Contract Start Date	March 27, 2019	



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Appendix A

Water Quality Monitoring Grant Program Application

Overview: The Massachusetts Department of Environmental Protection (MassDEP) is soliciting grant proposals from eligible entities outside the agency to assist in the collection of bacteria data (*E. coli*, Enterococci) for use in the Department's assessment of primary and secondary contact recreation activities in the Commonwealth's surface waters. The purpose of this grant is to support these groups' monitoring program activities and/or assist in the development/expansion of bacteria monitoring capacity. This funding is available to non-profit organizations, including but not limited to watershed organizations, academic institutions, and other eligible entities with the capacity for surface water quality monitoring. The funding is expected to enhance MassDEP strategic partnerships with data collection organizations which are external to state and federal government. The overarching goal of this program is to enhance the amount and usability of reliable external bacteria data for MassDEP's use in assessing primary and secondary contact recreation activities.

Section 1: Applicant Information (NOTE: Applicant must be authorized by the specified organization to submit this application and commit to the proposed project).

Applicant Organization: _____

Contributing Partners (if applicable): _____

Name of person completing form: _____ Title: _____

Address: _____

Phone: _____ Email: _____

Section 2: Qualifications/Ability to Perform Proposed project

Ability to Use Funds: Please describe your entity's eligibility for an award of the Water Quality Monitoring Grant. If awarded a Water Quality Monitoring Grant, the awardee must be able to enter into a contract with

MassDEP by the date specified in the Estimated Procurement Calendar for Grant Application/ Award. Please discuss your entity's ability to enter into the contract within this timeline, including specific steps that must be taken to accept a state grant. Please identify any potential constraints.

Section 3: Application

A. Organizational Capacity (20 points)

All applicants must explain how this grant will support your organization's efforts to collect bacteria data, and/or expand data collection capacity. Proposals will be evaluated based on the organization's capacity to plan and execute a bacteria monitoring program, and/or develop capacity for a bacteria data collection program. All applicants must provide a description of your organization, the year it was founded, your mission statement, scope of your watershed(s), (number of towns, waterbodies covered) and key staff resumes. All applicants must also provide documentation of the organization's non-profit status (i.e., 501(c)(3) status).

B. Description of How Funding will be Utilized (20 points)

Applicants must describe the proposed project, and its projected benefits and goals to be covered by the grant. Descriptions should include proposed project tasks, personnel, and desired outcome(s) for the project.

C. Priority Data Needs (20 points)

Applicants should demonstrate how their proposed project will contribute to the future pool of reliable bacteria data for utilization in MassDEP's assessment of recreation activities in the surface waters of the Commonwealth. The applicant should describe the context for data collections and how their proposed project will contribute to the collection of bacteria data in unassessed or Appendix B waters.

D. Financial Need (10 points)

All applicants must explain the need for financial assistance to implement the proposed project. Applicant should identify and describe other anticipated or secured funding sources (including in-kind or monetary matching funds) that will support portions of the project.

E. Project Budget and cash flow schedule (10 points)

The applicants must present a project budget for the expenditure of the grant funds. The applicant should provide an estimate and description of proposed project costs. Specifically, the applicant must demonstrate a realistic understanding of project costs by providing a budget with detailed and credible cost estimates. Applicant must include a cash flow projection schedule for costs expected to be incurred for FY 2019 (which ends on June 30, 2019) and those costs expected to be incurred during FY 2020 (which commences on July 1, 2019).

F. Project Timeline (5 points)

Applicants must submit a timeline which specifies the tasks and deliverables for the project. The applicant must describe their strategy for utilizing funds during FY 2019 (which ends on June 30, 2019) and during FY 2020 (which commences on July 1, 2019).

G. Project Benefit (15 points)

The applicant must describe how the proposed project will supplement the agency's strategic monitoring goals of assessing primary and secondary contact recreation activities in the Commonwealth's surface waters as follows:

- Enhancing the availability of credible external bacteria data that can be utilized to support MassDEP's CWA Programs.
- Providing information for unassessed waters, and waters known to be impaired for bacteria (see Category 5 waters in the *Massachusetts Year 2016 Integrated List of Waters*, available at <https://www.mass.gov/media/1638446/> and those waters for which MassDEP has identified a specific need for current bacteria data (see Appendix B).
- Improvements in planning for capacity building (e.g., QAPP, SAP, formatted data submission through MassDEP's external data portal or the federal WQD portal, and/or purchase of supplies and equipment).

Section 4: Application Submission All grant applicants must complete the attached application form. Once the Application Form is complete, the document can be submitted electronically in Microsoft Word or, to reduce the file size, the document may be converted and submitted as an Adobe PDF file.

Applications must be submitted electronically, via email to:

Therese Beaudoin
Massachusetts Department of Environmental Protection
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8 New Bond Street
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In the subject line of the submittal email, please write the applicant name and "MassDEP Water Quality Monitoring Grant Program."

All applications must be received electronically by MassDEP by 5:00 P.M. on February 27, 2019

Applications received after the deadline will be rejected automatically. MassDEP reserves the right to reject any and all proposals or request additional information if needed.



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APPENDIX B: WATERS WITH IDENTIFIED BACTERIA DATA NEEDS

SEGMENT	WATER_NAME	WATER_TYPE	2016 Use
MA83-11	Long Meadow Brook	RIVER	Primary Contact Recreation
MA83-13	Sandy Brook	RIVER	Primary Contact Recreation
MA83-19	Shawsheen River	RIVER	Primary Contact Recreation
MA83-15	Unnamed Tributary (aka Pinnacle Brook)	RIVER	Primary and Secondary Contact Recreation
MA83-22	Webb Brook	RIVER	Primary and Secondary Contact Recreation
MA95-31	Acushnet River	RIVER	Primary Contact Recreation
MA95-32	Acushnet River	RIVER	Primary Contact Recreation
MA95-32	Acushnet River	RIVER	Secondary Contact Recreation
MA97-05	Chilmark Pond	Estuary	Primary Contact Recreation
MA94-14	Jones River	Estuary	Primary and Secondary Contact Recreation and Aesthetics
MA91-10	Ox Pasture Brook	RIVER	Primary and Secondary Contact Recreation
MA91010	Pentucket Pond	Lake	Primary Contact Recreation
MA84A-39	East Meadow River	RIVER	Primary Contact Recreation
MA84A-40	Fish Brook	RIVER	Primary Contact Recreation

SEGMENT	WATER_NAME	WATER_TYPE	2016 Use
MA81-56	Asnebumskit Brook	RIVER	Primary Contact Recreation
MA81-21	Nissitissit River	RIVER	Primary Contact Recreation
MA81-54	Unnamed Tributary	RIVER	Primary Contact Recreation
MA81-72	Wekepeke Brook	RIVER	Primary Contact Recreation
MA74-02	Weir River	RIVER	Primary Contact Recreation
MA73-03	Neponset River	RIVER	Secondary Contact Recreation
MA73-24	Purgatory Brook	RIVER	Primary and Secondary Contact Recreation
MA36-17	Quaboag River	RIVER	Primary Contact Recreation
MA36-39	Unnamed Tributary	RIVER	Secondary Contact Recreation
MA51-11	West River	RIVER	Primary Contact Recreation
MA53-13	Clear Run Brook	RIVER	Secondary Contact Recreation
MA61-01	Lee River	RIVER	Primary Contact Recreation



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APPENDIX C

The following serves as a general guidance for organizations' survey planning, sampling and analysis for bacteria. More detail will be needed in the organization's draft Quality Assurance Project Plan (QAPP) submitted to MassDEP for approval (or in an existing previously-approved QAPP). The link to the generic MassDEP QAPP is located on our website here: <https://www.mass.gov/guides/water-quality-monitoring-quality-management-program>.

- Water Resources to sample: rivers, streams, lakes, ponds (pipe discharges can also be sampled but will not be used in assessments of ambient waters). See Appendix B for suggested waters to sample; other waters currently lacking assessment information are also a priority.
- Freshwater Indicator: *E. coli* and or Enterococci bacteria
- Marine Indicator: Enterococci bacteria
- MassDEP-approved monitoring QAPP required prior to sampling & analysis? YES
- Safety considerations for sampling crews: YES (describe in QAPP). Sampling teams of two persons are recommended.
- Training documentation required? YES (describe in QAPP and keep records)
- Data Quality Objectives? YES (describe in QAPP)
- Sampling Period: Bathing and contact season (April 1-October 15; June-September is preferred)
- Sampling Frequency: weekly for all waters within a 90 day period, from June-Sept., preferred. These frequencies will ensure an adequate sample number within the 90 day averaging period.
-
- Number of sampling locations: As many as can be accomplished within budget
- Sampling Methodology: simple grabs, wade-in, representative of waterbody (describe specific sampling process in QAPP)
- Sample Containers: sterile (w/ dechlorination as appropriate)
- Analytical Methods: SM 9223B, EPA 1603 (*E. coli*); EPA 1600, SM 9223B, ASTM D6503-99 (Enterococci)
- Analytical holding Times: 8 hours. To be met for all sample results.
- QC sampling required? YES. Collect sequential or simultaneous field duplicates at a rate of one minimum per lab batch of samples and at least once per season for each sampling crew. Also, collect field blanks using sterile deionized water or an appropriate source of reagent water at a rate of 10% of total samples.



- Analytical lab services: in-house or private lab is acceptable. MA certification in the specific bacteria method is preferred but not required. In any case, MassDEP-approval of lab-specific protocols (standard operating procedures) for analysis is required as part of QAPP approval.
- Analytical reagents: All reagents used in lab analyses shall be unexpired and from a reputable source
- Lab QC sampling?: YES. Minimum of one lab duplicate and one lab blank per batch of samples
- Lab proficiency testing? Optional, but preferred (using appropriate QC testing materials; describe in QAPP)
- Data review and validation: Grantees are expected to critically review the resulting data and qualify or censor data as needed that do not meet project specifications, prior to submitting final data to MassDEP. MassDEP to provide training on data review in upcoming 2019 workshops.
- Data submittal to MassDEP or through National Water Quality Portal? YES, not required before June 30, 2019 (also include QC data with submittal so that MassDEP can evaluate the quality of the data)
- Project Report to MassDEP? YES, a summary of deliverables and expenditures.



Appendix D

The following is the Standard Operating Procedure that MassDEP uses to analyze E. coli and Enterococci bacteria using an enzyme substrate test method (ColiLert®).

MassDEP

Massachusetts Department of Environmental Protection

Division of Watershed Management

STANDARD OPERATING PROCEDURE

ANALYTICAL QUANTIFICATION OF *ESCHERICHIA COLI* AND ENTEROCOCCI BACTERIA IN AMBIENT SURFACE WATERS USING AN ENZYME SUBSTRATE TEST (STANDARD METHODS 9223B)

CN 198.0

February, 2007



Prepared by:

Date:

Richard Chase, Quality Assurance Analyst
Joan Beskenis, Environmental Analyst

Approved by:

Date:

Arthur Johnson, Monitoring Coordinator

Approved by:

Date:

Dennis Dunn, Program Supervisor

** see pdf version for valid signatures*



Acknowledgements: Several existing SOP documents for enzyme substrate procedures were used in developing this SOP, including that from the MADEP Wall Experiment Station, Massachusetts Water Resource Authority, the USEPA NE Regional Laboratory and the Ohio River Sanitation Commission.



LIST OF REVISIONS

Rev. #	Date	Description of Revision(s)	Page #s
0	4/2004	Original draft	19
1	6/4/2004	Mainly Section 10 changes, misc. minor revisions	19
2	7/2006	Minor, misc changes throughout	---
3	9/2006	Added dilution requirement for marine Enterolert samples	5, 12
4	2/2007	Clarified the applicability of different Idexx reagents for waters of varying conductivities/salinities	4-5, 10-11
5	3/2009	Revised media-batch and sample batch QC	8,9



1.0 SCOPE & APPLICATION

This SOP describes the detection and enumeration of total coliform, *Escherichia coli* (*E-coli*) and Enterococci bacteria in surface water samples using the enzyme substrate test, as detailed in **Standard Methods 9223B**. These methods use hydrolyzable substrates for the detection of target bacteria.

The commercially-available supplies developed by IDEXX Laboratories, Inc. were chosen to perform this procedure. The IDEXX “Colilert” and “Colilert-18” methods are employed to detect and enumerate both total coliform and *E-coli* bacteria. The IDEXX “Enterolert” method is used to detect and enumerate Enterococci (fecal streptococci) bacteria. These defined enzyme substrate methods were recently included in an EPA Final Rule for Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Ambient Water (July, 2003).

This SOP is applicable for samples collected by the DWM (or its agents) and analyzed by DWM staff at DWM's offices/laboratory in Worcester, MA. The main target analytes for DWM waterbody assessments, TMDL determinations and bacteria source tracking are *E. coli* and Enterococci (total coliform is of lesser importance).

2.0 SUMMARY OF METHODS

Because pathogenic (disease-causing) organisms are difficult to isolate and identify, fecal coliform and Enterococci bacteria are used to indicate the potential presence of pathogens in water. Coliform and fecal streptococci bacteria are widely distributed in nature and are present in the intestines of warm-blooded animals, including humans. Their presence in surface waters may indicate human or animal fecal contamination. They can be relatively easily identified and enumerated.

Colilert (-24) and Colilert-18:

The Colilert reagent is added to a 100-mL volume of freshwater sample, the sample is poured into a multi-well tray, the tray sealed and then incubated for 24 hours (Colilert) at $35^{\circ} \pm 0.5^{\circ}\text{C}$. The tray is then checked for color (total coliform) and fluorescent (*E-coli*) reactions. The most probable number (MPN) technique utilizing a multiple well system format is used to determine the number of total coliform and *E. coli* per unit volume (100 mls.). The Colilert-18 reagent provides results in only 18-22 hours.

Colilert (-24) and Colilert-18 are US EPA-approved for drinking water and source waters. Unlike Colilert (-24) (mainly freshwater use), Colilert-18 can also be used in salt water, but for *E. coli* only--- not total coliform. NOTE:



do at least a 1:10 dilution on Colilert-18 samples if they've been collected in salt/brackish water.

<http://www.idexx.com/water/refs/060202711C18.pdf>

The method detection limit (MDL) for total coliform and *E. coli* using Colilert (-24) and Colilert-18 is a MPN of 1 colony forming unit (CFU) per 100 mls.

The total coliform group is defined as all bacteria possessing the enzyme B-D-galactosidase. The Colilert reagent contains the nutritive ortho-nitrophenyl-B-D-galactopyranoside (ONPG), which is used to detect the enzyme B-D-galactosidase of total coliforms. The hydrolyzation of the ONPG by the enzyme produces a color change (positive=yellow) at 35.0 ° +/- 0.5° C after an incubation period of 24 to 28 hours. The Colilert method also simultaneously detects the presence of *E. coli* through the hydrolyzation of the fluorogenic, nutritive substrate, 4-methyl-umbelliferyl-B-D-glucuronide (MUG) by the *E. coli* enzyme B-glucuronidase. This reaction produces a fluorescent product detectable when viewed under a long-wavelength (365-nm) ultraviolet (UV) light. Non-coliform bacteria cannot metabolize the indicator nutrients.

Enterolert:

Similarly, the Enterolert reagent is added to a 100-mL volume of fresh or marine water (diluted 10X) sample, the sample is poured into a multi-well tray, the tray sealed and then incubated for 24 hours at 41° ± 0.5°C. The tray is then checked for blue fluorescent wells indicating enterococci presence. A MPN table is used to determine the most probable number of Enterococci per 100 mls. The MDL for Enterococci is also a MPN of 1 CFU/100 mls.

The Enterococci, a sub-group of the fecal streptococci, contain the enzyme B-glucosidase. The Enterolert reagent contains 4-methyl-umbelliferyl-B-D-glucoside, which reacts with the enterococci-produced enzyme B-glucosidase to produce a fluorescent blue color.

Enterolert is most applicable for marine/brackish water samples (using the required minimal 10X dilution), but can also be used for fresh water (Note: MA. freshwater surface water quality standard is based on *E. coli* (Colilert), not *Enterococcus*). Enterolert is approved by the US EPA as a method for enterococci detection in ambient waters, including fresh, marine or estuarine surface water. It is also an ASTM-approved method (#D 6503-99).

3.0 DEFINITIONS

RESERVED.

4.0 INTERFERENCES



The following situations represent potential interferences or complications in achieving accurate and precise results when using the Colilert, Colilert-18 and Enterolert methods.

Colilert and Colilert-18:

- 4.1 Non-coliform bacteria, particularly *Aeromonas*, and *Pseudomonas* species, may produce small amounts of the enzyme β -D-galactosidase, but are suppressed and generally will not produce a positive response within the incubation time unless more than 104 colony-forming units (CFU)/mL are present.
- 4.2 *Serratia* species may turn the medium yellow after 24 hours of incubation but the yellow color is typically brighter than that represented by the color comparator.
- 4.3 Some strains of *Shigella* species may produce a positive fluorescence response. This is not considered a detriment for testing the sanitary quality of water due to the pathogenic nature of *Shigella*.
- 4.4 Some water samples containing humic material may have an innate color. If a water sample has some background color, compare inoculated Colilert® sample to a control blank of the same sample.
- 4.5 Incubation beyond 28 hours may yield a false positive Colilert result, due to cessation of suppression of non-coliform heterotrophic bacteria. The same is true beyond 22 hours for Colilert-18.
- 4.6 Presence of free chlorine in the sample may result in a transient blue color upon addition of the Colilert reagent. To avoid this, all bacteria samples should be taken using sample bottles containing sodium thiosulfate.
- 4.7 Dilute brackish/marine water samples with sterile fresh water at least ten fold for Colilert-18 testing.

Enterolert:

- 4.8 Dilute marine water samples with sterile fresh water at least ten fold, per IDEXX Enterolert procedure.

5.0 LAB SAFETY

- 5.1 All applicable elements of DWM's Lab Safety Plan must be adhered to at all times.



- 5.2 Samples (and positive controls) may contain organisms that are pathogenic to humans, and often the analyst works with water samples of different ranges of contamination. Handle all samples and cultures as if they are infectious. All precautions are to be taken to minimize exposure. These include the use of personal protective equipment (lab coats, safety glasses, and protective gloves), keeping the lab work area clean and organized, working at a reasonable pace and using good judgment at all times.
- 5.3 It is recommended that all bacteria lab personnel must be immunized against the hepatitis A and B viruses.
- 5.4 All bacteria lab personnel must receive on-the-job laboratory safety training.
- 5.5 The sealer device is a burn hazard if not properly used and maintained. See 11.1.
- 5.6 Material Safety Data Sheets (MSDS) for all chemicals used in this SOP are included in Room 229 and in Room 228.
- 5.7 Adherence to the waste storage and disposal procedures in Section 15 shall be maintained at all times at DWM and at WES.
- 5.8 Additional safety issues with regard to performance of this SOP shall be addressed by DWM's Lab Safety Officer and/or senior staff on an as needed basis.
- 5.9 NOTE: *Pseudomonas* sp. and *Klebsiella pneumoniae* used for media batch QC (see page 8-9) are categorized as Class 2 bio-safety hazards, whereas *Enterococcus faecium* and *E. coli* are categorized Class 1. While Class 2 organisms can be used in this SOP, their use presents greater potential risk to analysts should an accident occur, if poor lab techniques were used or if safety protocols were not followed.

6.0 EQUIPMENT AND SUPPLIES

The following lab equipment and supplies are needed to employ the multi-well tray Colilert and Enterolert methods. It is assumed that the necessary field supplies, such as sterile, 120 ml. bacteria sample bottles containing sodium thiosulfate, will be available for use in the field.

Colilert and Colilert-18:

- 6.1 Lab disinfectant (e.g., Roccall II 10%, Conflit detergent) and paper towels (supply room)
- 6.2 Disposable plastic gloves and "hot" gloves (incubator and sealer) and lab coats
- 6.3 Lab notebook
- 6.4 Lab fridge and storage areas for reagents and supplies
- 6.5 Model 2X Quanti-tray sealer
- 6.6 Quanti-tray 2000 97-well sample trays **[Store in cabinet]**
- 6.7 Quanti-tray 2000 tray rubber inserts (minimum 2)



- 6.8 Colilert reagent **[Store in dark at 2-30 deg. C; use within 12 months of manufacture]** and Colilert-18 reagent **[Store in dark at 2-25 deg. C; use within 12 months of manufacture]**
- 6.9 Sterile lab sample bottles for raw and diluted samples. Preferably, these bottles should be high clarity (to see end points clearly), graduated for 100 mls. (accurate to within 2.5% or approx. 2-3 mls.), and be non-fluorescing. IDEXX vessels are preferred.
- 6.10 Sterile dilution bottles (90 and 99 ml for 10X and 100X dilutions) pre-filled with sterile, buffered and/or non-buffered dilution water. **(NOTE: IDEXX, Inc. stipulates the use of non-buffered, sterile dilution water, since their reagents are already buffered. Use of non-buffered dilution water avoids potential analytical complications due to the presence of additional buffering agents. In practice, a suitable buffered dilution water source may be found that does not cause analytical error or imprecision.) [Store as directed by manufacturer]**
- 6.11 Sterile pipettes (1, 10 and 50-100 ml sizes)
- 6.12 Sterile water (non-buffered). Autoclaved sterile water available from WES in 120-250 ml. bottles. DWM's reagent water system (Barnstead deionization with 0.2u filtration) in Room 226 serves as a backup supply (see Table 1). Another source may be purchased water from IDEXX. **[If sterile-bottled by WES/DWM, use within 1-2 months. If purchased from IDEXX in sealed, 100 ml bottles, shelf life is 2 years. Store at 2-8 deg. C or in cabinet]**
- 6.13 Anti-foam solution (as needed; usu. used with P/A test) **[Store in cabinet]**
- 6.14 Positive (*E. coli*, *Enterococcus faecium*) and negative (*Pseudomonas*, other) control cultures (for QC). Difco Bactrol discs are acceptable. **[Store at 2-8 deg. C. Discs have 1 year shelf life]**
- 6.15 Two-shelf, bench-top, mechanical-convection incubator (5-65 deg. C; +/- 1.5 deg. C uniformity; +/- 0.5 deg. C stability) with thermometer. If also running Enterolert, two incubators (at each temperature) are preferred. Incubators should have a temperature log sheet attached to track internal temperatures several times/day.
- 6.16 NIST-certified (or traceable) thermometer
- 6.17 365 nm UV lamp (6 watt)
- 6.18 UV viewing cabinet
- 6.19 Colilert color comparator NOTE: The color comparator is the lowest color and fluorescence level at which a result can be considered positive. A typical positive result is much more intense. **[Store in cabinet]**
- 6.20 MPN tables
- 6.21 Quanti-cult QC kit **[Store at 2-8 deg. C until use; use within 18 months of manufacture]**
- 6.22 Labeled, plastic bio-waste disposal bags, step-on can and labeled 32 gallon can with cover for temporary waste storage.
- 6.23 Three-clock timer
- 6.24 Autoclave (to disinfect bio-waste in bags). This function to be performed by WES on an as-needed basis.

Enterolert: (in addition to those listed above for Colilert)

- 6.25 Enterolert reagent **[Store in dark at 2-30 deg. C]**



Table 1: DWM Reagent Water Quality (for backup; based on recent testing)

Parameter	Criteria for Microbiological Analyses	Test Date	Results
Specific Conductance	< 2 uS/cm	Weekly (2004)	1 uS/cm
pH	Approx. 6-7 is generally preferred	Weekly (2004)	6-7 (for "new" DI H ₂ O; if allowed to equilibrate to air, pH drops below 6)
Metals (total) for Cd, Cr, Cu, Ni, Pb and Zn	< 50 ug/l	October, 2002 (Ten Mile Watershed Metals Study)	For trip and field blanks: All DWM reagent water samples < MDL (.02-1 ug/l), except Cu which showed some "hits" from .5-4 ug/l)
Metals (total, combined)	< 100 ug/l	October, 2002 (Ten Mile Watershed Metals Study)	Total metals in DWM reagent water samples (mostly < MDL) were estimated to be <10 ug/l.
Kjeldahl Nitrogen (TKN)	< 100 ug/l	Monthly in 2003	Invariably less than the method detection limit of 20 ug/l for ambient field blanks
Total Residual Chlorine	< 10 ug/l	No recent testing. (Current WES Lab MDLs (SM 4500-Cl-F) may not be able to achieve an MDL of 10 ug/l).	DWM's Barnstead DI water system has a dedicated carbon adsorption cartridge to adsorb chlorine, and is designed based on feedwater quality to meet Type I reagent water specifications (assuming system maintenance and feedwater consistency).
Fecal coliform	NA	Approx. weekly in 2003 (SM 9222D)	Rarely exceeding method detection limits (e.g., <6, <2) for ambient field blanks
E. coli	NA	Approx. weekly in 2003 (EPA 1603)	Rarely exceeding method detection limits (e.g., <6, <2) for ambient field blanks
HPC	< 100,000 CFU/100 mls.	No recent testing	---

7.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

For a detailed description of field sample collection for bacteria, refer to DWM Sampling Techniques for Surface Water Quality Monitoring (CN 1.21).

Briefly, new, plastic, disposable, sterile, pre-labeled, 120-mL sample bottles (usually containing a sodium thiosulfate tablet/powder for chlorine neutralization) are used to collect surface water bacteria samples.



Examples include Corning locking “flip-top” bottles (preferred) and IDEXX clear, polystyrene “vessels”. The collection method (opening, filling and capping underwater) is consistent with the DWM SOP (CN 1.21). After the sample is collected, a slight amount is poured off to allow at least 1/2” of headspace in the bottle. This is required to allow for sufficient mixing of the sample at the lab prior to analysis. Samples are placed in a clean plastic bag, which is then placed in a cooler half-filled with wet ice immediately or soon as possible after collection. Samples (at 4 deg. C) are delivered to the lab within 6 hours from collection with the sample tracking/chain-of-custody form filled out by the collector. At the lab, sample analysis must be initiated as soon as possible and within 8 hours of collection and two hours of receipt.

8.0 QUALITY CONTROL

Colilert and Colilert-18:

8.1 Media Lot-Specific Quality Control: DWM’s main intent in running the following media lot QC samples is to verify that the Colilert and Colilert-18 media does not provide false positives or false negatives for *E.coli*.

Each lot is tested using **Quanti-cult** (see Appendix D) cultures or lypholyzed microorganism pellets as follows:

- Negative Control: sterile water alone, AND
- Negative Control: sterile water inoculated with non-fluorescent *Pseudomonas* sp. or *Enterococcus faecium* (i.e., total coliform & *E. coli* negative) or with *Klebsiella pneumoniae*, *Enterobacter aerogenes* or *Enterobacter cloacae* (i.e., total coliform positive & *E. coli* negative), AND
- Positive Control: sterile water inoculated with *E. coli* (i.e., total coliform & *E. coli* positive) control cultures.

Alternatively, with each new lot of media reagents, perform the following QC sample analysis with specific regard to *E. coli* results (**using purchased QC media**):

- Negative Control: sterile water alone (lab blank), AND
- Positive Control: sterile water spiked with *E. coli*., OR
- Negative Control: sterile water spiked with an *E. coli*.-negative organism (see 8.1 above)

8.2 Upon receipt, check reagent packages integrity for proper seal, tears and lack of moisture. When using, inspect appearance of Colilert reagent; it should appear dry, free-flowing and white to off-white in color.

8.3 Each lot of medium can be checked before use with the UV light to detect auto-fluorescence. If any faint fluorescence is observed, then it should be recorded, discarded and replaced by a new lot.

8.4 If any media causes the sample to fluoresce prior to incubation, then another lot of medium should be used.



8.5 Sample Batch Specific QC: For each lab batch, run a minimum of one lab blank and one lab duplicate (@ approx. 10% of total samples).

8.6 For reading and interpretation of results, all analysts should participate in group QC consisting of inter-analyst comparisons to confirm acceptable precision among different readers. This is especially important for the *E. coli* fluorogenic end-point, for which the comparator seems to be less useful.

Enterolert: (in addition or changed from that above for Colilert)

8.7 Media Lot-Specific Quality Control: Similarly, run the following media lot QC samples to verify that the Enterolert media does not provide false positives or false negatives for Enterococci. With each new lot of media purchased, run the following controls:

- Negative Control: sterile water alone, AND
- Negative Control: sterile water inoculated with non-fluorescent *Serratia marcescens* (gram -) and *Aerococcus viridans* (gram +), or *E. coli* (gram -), AND
- Positive Control: sterile water inoculated with fluorescent *Enterococcus faecium*.

Reagent Water Testing:

8.8 Use of DWM reagent water (Barnstead deionization system) as a backup supply for bacterial analyses (dilutions, lab blanks) requires periodic testing to ensure adequacy. Test results are compared against criteria identified in Table 1 (excerpted from Standard Methods, Table 9020 II).

Specific conductivity and pH are measured weekly. TKN, Fecal coliform and *E. coli* have been typically tested monthly. Metals are measured as programmatic sampling dictates or annually (last tested in 11/2002; metals were below criteria). TOC and total residual chlorine have not typically been tested for by DWM. In general, past measurements have indicated that the DWM Barnstead deionization system, when operating normally (e.g., resistance of about 18.3, maintained correctly, etc.), may provide sufficient purity to perform bacterial analyses.

9.0 CALIBRATION AND STANDARDIZATION

9.1 Sample Containers: No testing of sample containers used to measure sample volume is planned. Containers are assumed to meet accuracy limits of $< \pm 2.5\%$.

9.2 Incubator: The incubator has an internal temperature monitoring device and maintains a temperature of $35^{\circ} \pm 0.5^{\circ}\text{C}$ for *E. coli*. Monitor temperatures both in air and “totally immersed” in water using total immersion incubator thermometers—should be the same.

Because pre-heated, air-type incubators may not bring water sample(s) to the specified incubation temperature of 35°C quickly, false-negative results could result. Therefore, the time it takes for water samples (or a set of 100-ml, water samples, depending on normal use) to reach 35°C must be accounted for



and should be known to ensure that the specified incubation period occurs (typ. >24 hrs.). See also 10.25, 10.29 discussing additional incubation periods.

Temperature should be recorded for days in use at least twice per day with readings separated by at least 4 hours.

10.0 DETAILED PROCEDURE (assumes reagent lot has been tested and passed; see 8.1)

Colilert and Colilert-18 (and Enterolert, subject to changes/additions, as specified herein; see 10.34-37):

- 10.1 Coordinate and plan analytical work with DWM field survey staff regarding the quantity and timing of sample delivery. The maximum number of trays allowed in a DWM-Worcester incubator at one time is about 60. For each DWM survey, one primary Colilert analyst and one backup analyst will be pre-identified and pre-scheduled to run the survey samples at DWM-Worcester. If the primary analyst is not available for any reason, the backup analyst must be available to run them. Also schedule the Colilert equipment and incubator to make sure that it is not being used at the scheduled time by anyone else. **NOTE:** Depending on timing, available reagents and logistics, decide on which reagent will be used (Colilert (24 hour) or Colilert-18).
- 10.2 At the proper time, turn on Quanti-Tray sealer to allow preheating (usually takes around 10 minutes). Sealer is ready to use when green light is on.
- 10.3 Prepare all work areas by cleaning with lab disinfectant.
- 10.4 Fill out lab notebook with required information, in anticipation of sample delivery. Include and verify the date that the media lot was tested and passed.
- 10.5 Check incubator to ensure it is ON, maintaining the proper temperature and has sufficient space for sample trays when they are ready. In order to minimize heat gain in the lab and provide adequate ventilation, make sure to turn ON the ceiling vent in Room 229 (switch is located behind Isotemp fridge in Room 227).
- 10.6 Record incubator temps to the nearest 0.5 deg. C by rounding (or 0.1 if thermometer allows). For example, if liquid level on a 1 deg. C graduated thermometer is less than but closer to 35.0 than to the midpoint between 34 and 35, round to 35.0, not 34.5.
- 10.7 Prepare counter work area with the necessary dilution bottles, reagents, trays, pipettes, etc., leaving sufficient space to prepare samples for incubation.
- 10.8 When samples arrive, transfer custody of the samples by removing them from the plastic bag in the ice chest and checking off each sample on the COC form. When all samples have been transferred, sign the COC form and record the time. Review the COC form and discuss with sample delivery staff for information regarding samples that are suspected of containing a high MPN value.
- 10.9 Optional: Depending on the amount of samples, perform sample tray preparation /incubation in groups resulting in approx. 30 minute incubation intervals. If not being used in the current 30 minute "sub-batch", keep samples on ice until needed. In all cases, keep the main incubation period the same for all samples.
- 10.10 Mark the Lab Notebook with date, sample ID, lab identification number, date and time of collection, date and time of receipt, date and time of analysis, volume of original sample analyzed (dilution rate),



lab QC sample information, start/end times of incubation, and the analyst's name. It is at this point that the dilution scheme for each sample is identified.

- 10.11 Use a Sharpie pen to mark all sample bottles with "HI" suspected of having high counts.
- 10.12 With a waterproof wax pencil (so as not to tear the paper on the back of the Quanti-Tray) gently mark the Quanti-Trays with the lab identification number of each sample.
- 10.13 For each sample (including dilutions), invert sample bottle 20-25 times to ensure complete mixing of the sample. DO NOT TOUCH THE INSIDES OF CONTAINERS OR SAMPLE WATER. Inspect each IDEXX vessel for inside cleanliness; if particles or other contamination is observed, place the container aside with other contaminated, new bottles (i.e., DO NOT USE), and discuss with DWM QC Analyst.
- 10.14 The preferred order for sample analysis is to start with the "oldest" samples first and proceed in the order in which they were collected. QC samples (e.g., lab blank, lab duplicates, QC samples) and dilutions can be done at any time during the analysis. If sample number is large and multiple samples are run at a time, be careful to put into its corresponding labeled tray.
- 10.15 **NO DILUTION SAMPLES:** If no dilutions are to be performed on the sample, mix sample (as above) and immediately pour the sample into a new, sterile IDEXX vessel up to the 100 ml line. If over the line, pour off to meet the 100 ml. meniscus, or use a sterile 1-10 ml pipette to pipette off to the 100 ml line. (If IDEXX vessel is used to collect sample in the field, simply mix and then pour off/pipette off to the 100 ml. line). Cap sample and proceed to 10.17. See Table 3 below for dilution summary.
(**NOTE:** Based on manufacturer specifications, the 100 ml line in the IDEXX vessel has a maximum measurement error of 2.5%. Due to potential inconsistencies between analysts or systematic error, this approach may result in volume errors of greater than 2-3 mls. Provided volume errors are minimized (<2.5 %), this level of measurement accuracy and precision is currently deemed acceptable.)
- 10.16 **10 ML DILUTION SAMPLES:** (e.g., for marine/brackish Colilert-18 samples; known or suspected "HOT" samples to be quantified at a level > 2420 org./100 ml.). Pour 100 mls. of sterile DI water from a new WES-DI container into a new, sterile IDEXX vessel to the 100 ml. line (and pipette off as needed to 100 mls using a sterile 10 ml pipette.). Now, using the same pipette, pipette off 10 mls to achieve 90 mls. of sterile water. Then use the same pipette to pipette 10 mls. of the original, mixed sample into the 90 mls. of sterile water. Cap and mix as above. See Table 3 below for dilution summary.
(**NOTE:** An alternative method is to use pre-filled dilution bottles containing 90 mls. of phosphate buffer is under investigation).
- 10.17 **100 ML DILUTION SAMPLES:** (e.g., for marine/brackish Colilert-18 samples; for known or suspected "VERY HOT" samples to be quantified at a level >> 2420 org./100 ml.). Pour 100 mls. of sterile DI water from a new WES-DI container into a new, sterile IDEXX vessel to the 100 ml. line (and pipette off as needed to 100 mls using a sterile 1 ml pipette.). Now, using the same pipette, pipette off 1 ml. to achieve 99 mls. Then use the same pipette to pipette 1 ml. of the original, mixed sample into the 99 mls. of sterile water. Cap and mix as above. See Table 3 below for dilution summary.
- 10.18 Tap each reagent packet before opening to ensure complete dispensing. Open Colilert reagent packet and add entire contents to the 100 ml. sample (or diluted sample). Cap and invert the bottle > 25 times until all the reagent is dissolved. Let any foam settle. Some stubborn particles may remain; these may dissolve during incubation.
- 10.19 After complete mixing as stated above, dispense sample into the Quanti-Tray. Use one hand to hold the Quanti-Tray upright with the well side facing the palm. Squeeze upper part of the Quanti-Tray so that it bends towards the palm. Open the Quanti-Tray by pulling the foil tab away from the well side.



Avoid touching the inside of the foil or tray. (**NOTE:** If Quanti-Tray is believed to have been contaminated at this point, discard the contaminated Quanti-tray and set up a new one, if possible, and note in notebook). Pour the Colilert-treated sample directly into the Quanti-Tray while avoiding contact with the foil tab. Tap the small wells to release any air bubbles.

- 10.20 Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with well side (plastic) of the Quanti-Tray facing down to fit into the carrier.
- 10.21 Seal the Quanti-Tray by moving the rubber tray into the sealer. The sealer will automatically take the rubber tray to dispense the sample into the wells and seal the Quanti-Tray. Remove the Quanti-Tray, assuring that it is fully sealed. (**NOTE:** If the Sealer stops in the middle of processing a Quanti-Tray sample, use the black button (with the arrows) on the front of the Sealer to reverse the direction of the Quanti-Tray in the Sealer. Allow the green light on the Sealer to go on before attempting to seal the sample again.) If the sealer does not easily “accept” the tray, lift up the end of the tray slightly while continuing to push it into the sealer, so that the leading edge clears the opening of the sealer.
- 10.22 **Reagent Lot QC samples (DWM QA Analyst only).** Run the following *E. coli* QC samples for each new lot of reagents when reagents are first received: 1) **Negative Control.** Prepare *P. aeruginosa* (or other) samples as for *E. coli* below. Cap/tray/seal/incubate. 2) **Positive Control.** Prepare a positive QC sample by pouring 100 mls into an IDEXX vessel (as above), then adding an *E. coli* pellet according to manufacturer’s directions, then adding Colilert reagent. Cap/tray/seal/incubate. Confirm correct analytical end-points.
- 10.23 **Batch QC samples (Lab analysts).** For each lab batch of samples, Prepare **LAB BLANK** as follows (minimum one per batch): Pour 100 mls. of sterile, deionized water into an IDEXX vessel, add reagent, cap and mix, pour into tray, seal and incubate. Prepare **LAB DUPLICATE** as follows (minimum one per batch): Using 250 ml. sample: Mix sample as above and pour two 100 mls. aliquots into two separate IDEXX vessels, add reagents, cap and mix, pour into trays, seal and incubate. Using a 120 ml. sample: Mix sample (each time) and pipette two 10 mls. aliquots into two separate IDEXX vessels containing 90 mls. of sterile, deionized water, add reagents, cap and mix, pour into trays, seal and incubate. Multiply results by 10. Provided 100 mls. is left in the sample container, run “raw”, 100 ml (undiluted) sample also. **NOTE:** Previous to 2011, lab analysts also ran positive and/or negative QC for each lab batch, but this was discontinued in 2011.
- 10.24 Incubate all prepared Colilert Quanti-Trays in the incubator at **35 ±0.5 °C** for 24 - 28 hours. If Colilert-18 is used, incubate for 18-22 hours at **35 ±0.5 °C**. Record the dates and times in the lab notebook. Set clock. Avoid any opening and closing the incubator during use in order to maintain stable proper temperature.
- 10.25 After sample trays have been placed in the incubator, thoroughly clean work area. First, place all “bio-waste” (items that have been in contact with sample water, including pipettes, sample bottles, dilution bottles, etc.) in pre-labeled, plastic bio-waste bags. Keep the plastic sample and reagent bottles separate from other waste (separate bags). Tie the bag(s) when only half-full and temporarily store in the designated bio-waste storage area in the lab. Recycle or dispose (as appropriate) other materials that have not touched the samples. Once the work area is clear, disinfect the work area.
- 10.26 After 24 hours (18 hours for Colilert-18), remove the Colilert trays from the incubator. Record the time in the lab notebook. Set clock. For each tray, count the number of wells that turn yellow (at least as strong as the yellow color of the comparator). A yellow color that is equal to or deeper than that of the color comparator verifies that the sample is positive for total coliforms. Note in the lab notebook



the number of positive (yellow) wells in the Quanti-Tray. Use the MPN table (Appendix A) to then record the confirmed total coliform value as MPN/100 ml sample in the lab notebook.

- 10.27 To determine the *E. coli* result, expose each sample to 365 nm UV light by placing them one-by-one into the UV light viewing cabinet (or in a dark room wearing UV-protective glasses with a hand-held UV lamp held within 5 inches of the sample. If the sample fluoresces (compare to comparator), then the sample is positive for *E. coli*. Count the number of fluorescent wells and record in the lab notebook. Use the MPN table (Appendix a) to record the confirmed E-coli value as MPN/100 mL sample in the lab notebook. *NOTE: Empty wells do not affect the test interpretation as long as the entire sample is in the tray. The effect on the Most Probable Number (MPN) table is statistically insignificant. An empty or partially filled well is interpreted the same way as a full well.*
- 10.28 For added QC or if the sample results are questionable after 24 hours of incubation (18 hours for Coli-18), the sample may be incubated for an additional 4 hours (total of 28 hours for Colilert; 22 for Coli-18), and rechecked for color and fluorescent reactions. If the color intensifies, the sample is total coliform positive; if it does not, the sample is negative. If an inoculated test is inadvertently incubated over 28 hours (22 for Coli-18), the following guidelines apply: 1) lack of yellow color is a valid negative test, and 2) a yellow color after 28 hours is not valid and must be repeated.
- 10.29 Colilert and Coli-18 results are definitive at 24-28 and 18-22 hours, respectively. Any positives for both total coliform and *E. coli* observed before the minimum time and negatives observed after the maximum time are also valid. Positive results after the maximum time are not valid.
- 10.30 After all MPN results have been recorded, review the lab notebook to ensure that all required data and metadata have been recorded completely and accurately.
- 10.31 Place used Colilert trays and other waste in “bio-waste” bags, tie securely when only ½ full and temporarily store in the lab in the designated, secured location.
- 10.32 Periodically, coordinate with WES for the delivery of the “bio-waste” to WES for autoclave sterilization and disposal at WES.

Enterolert: (in addition to or changes from that above for Colilert)

- 10.33 Incubate all prepared Enterolert Quanti-Trays in the incubator at **41 ±0.5 °C** for 24 - 28 hours.
- 10.34 Prepare negative and positive controls as indicated in Section 8.
- 10.35 Dilute marine (brackish to salt) water samples with sterile fresh water at least ten fold, per IDEXX Enterolert procedure.
- 10.36 There is no fluorescent end-point comparator for Enterolert. Compare fluorescence in sample trays to negative control.

General Dilution Schemes (depending on sampling bottle used and without pre-filled dilution bottles):

Table 2: Summary of General Dilution Procedures

Sample bottle used	Flip-top locking HDPE (or other)	IDEX vessel PS
	Procedures	



<i>Sample bottle used</i>	<i>Flip-top locking HDPE (or other)</i>	<i>IDEXX vessel PS</i>
Procedures		
0X dilution MIX bottle	Mix, pour 100 mls into clear PS IDEXX vessel, pour off/pipette off to 100 mls if necessary, add reagent	Mix, pour off/pipette off for necessary dilutions and to 100 mls, then add reagent
10X dilution bottle	Pour 100 mls. of sterile DI into IDEXX vessel and pour off/pipette off to 100 ml. line as necessary. Pipette off 10 mls. Pipette 10 mls. of mixed sample into the 90 mls. in the IDEXX vessel. Add reagent	Pour 100 mls. of sterile DI into a new IDEXX vessel and pour off/pipette off to 100 ml. line as necessary. Pipette off 10 mls. Mix sample and pipette off 10 mls into 90 ml IDEXX vessel. Add reagent
100 X dilution bottle	Same as above for 10X but using 1 ml. Add reagent	Same as above for 10X but using 1 ml. Add reagent
<i>* These procedures assume that the IDEXX vessel 100 ml line can be used as an accurate (+/- 2.5%) measure.</i>		

11.0 PREVENTATIVE MAINTENANCE

11.1 Sealer: Trained personnel (only) must inspect, clean and maintain the sealer according to the manufacturer's recommendations (Appendix C).

11.2 Rubber Inserts: Rinse with clean water; autoclave or clean with isopropyl alcohol or bleach.

12.0 DATA INTERPRETATION, ANALYSIS AND VALIDATION

For each sample batch analysis, draft sample and quality control data are reviewed, interpreted and validated using the following definitions and criteria.

Data Report: The lab analyst shall count the number of positive Quanti-Tray cells for each sample/tray, and use the MPN table to obtain the Most Probable Number (MPN) per 100 mls. MPN results will be multiplied by the dilution factor as needed to obtain the final draft result. The lab analyst shall then generate a brief draft results lab report for separate peer review.

Dilutions: Multiply MPN value (using IDEXX MPN table) by the dilution factor used for that sample to get the sample result.



Peer Review and Preliminary Approval: A separate lab report reviewer shall be designated to evaluate the accuracy and completeness of the lab report. The reviewer shall discuss any problems, concerns and issues with the lab analyst as needed, and if all data appear OK, preliminarily approve the MPN results as DRAFT data.

Data Validation: DWM's QA/QC Analyst shall perform more rigid and comprehensive data validation for larger data sets (e.g., annual projects), including compiled *E. coli* MPN data (by project), in relation to project-specific data quality objectives (DQOs).

Table 3: Colilert (and Colilert-18 Reactions)

Reaction	Result or interpretation
Yellow	Total Coliform positive
No color or indeterminant	Negative
Fluorescent (yellow)	<i>E. coli</i> positive
Yellow color intensity at or near the comparator color	Inconclusive; re-incubate an additional 4 hours
> 28 (22) hours, positive	Not valid
> 28 (22) hours, negative	Valid
< 24 (18) hours, positive	Valid
< 24 (18) hours, negative	Not valid

Table 4: Enterolert Reactions

Reaction	Result
Blue fluorescence in 24-28 hours	Enterococci positive
No fluorescence in 24-28 hours	Enterococci negative
Blue fluorescence < 24 hours	Enterococci positive



No fluorescence beyond 28 hours	Enterococci negative
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Table 5: QC Interpretations (Colilert and Enterolert)

Quality Control Organism	Yellow	Fluorescent
<i>Pseudomonas</i> sp. (Non-fluorescent strain)	No	No
<i>Klebsiella pneumonia</i> , <i>Enterobacter aerogenes</i> or <i>Enterobacter cloacae</i>	Yes	No
<i>E. coli</i>	Yes	Yes (yellow)
<i>Enterococcus faecium</i>	---	Yes (blue)
<i>Serratia marcescens</i>	---	No

Table 6: QC Validation Criteria

Quality Control	Frequency	Acceptance Criteria (control limits)	Corrective Action
Use of expired reagents and/or QC organisms	NA	Any and all >1 year passed exp date	Qualify data (m) Censor data (m)
Positive Culture Control (<i>E. coli</i>)	5%	Positive	If not ALL positive, retest reagent lot.
Negative Control (sterile water)	5%	Negative	If not ALL negative, retest reagent lot.
Sample storage	Every sample	Samples are analyzed within 8 hours of collection and are stored at $\leq 4^{\circ}\text{C}$ from time of collection to time of analysis	Qualify or censor data using H or P qualifiers (exceeded holding time or method of preservation not followed)
Lab duplicate (precision)	Min. one per batch per analyst and ~5% of samples	Range of logs = 0-TBD (based on mean range of logs + 3 SD for all lab duplicates , OR <30%RPD (<50 MPN); <20% (50-500 MPN); <10 % (500-5000 MPN); < 5% (>5000 MPN) for \log_{10} transformed data (this DQO can also be applied to field duplicate evaluation)	Discard batch results outside control limits
Lab blank (bias)	Min. one per batch per analyst and ~5% of samples	< MDL	Qualify or censor data



13.0 METHOD PERFORMANCE

13.1 The method detection limit for Colilert, Colilert-18 and Enterolert has been determined to be a MPN of 1 colony-forming unit (CFU) per sample volume or dilution tested. The DWM Lab minimum reporting limit (MRL) for data generated through use of this SOP has been chosen to be a MPN of 1 CFU per 100 mls.

13.2 Use of this method by DWM will be evaluated on a periodic basis to ensure consistent generation of quality-controlled data.

14.0 POLLUTION PREVENTION

14.1 In order to avoid or minimize waste due to non-use, the quantity of media and chemicals purchased should be based upon expected usage during its shelf life.

14.2 Actual preparation volumes should reflect anticipated usage and stability.

14.3 Recyclable materials, such as Type 6 polystyrene sterile sample bottles, should be used whenever possible.

14.4 Generation of wastewater will be minimized. Discharge of any wastewater from sample preparation and analysis to the sanitary sewer will be minimized and does not pose a pollution potential.

14.5 All solid waste and "bio-waste" will be disposed of properly.

15.0 WASTE MANAGEMENT

15.1 All materials that have come in contact with samples (trays, bottles, pipettes, etc.) shall be bagged as "bio-waste" and temporarily stored in 32 gallon, plastic cans in a designated, secured location at DWM, prior to transport to the MADEP WES Lab in Lawrence, MA. for proper disposal (autoclave sterilization, plastics recycling and solid waste disposal). See 15.3 also.

15.2 DWM's waste disposal procedures are consistent with State regulations (State Sanitary Code Title VIII, 105 CMR 480.00 and Solid Waste Regulations, 310 CMR 19.000) governing the disposal of "infectious waste". Infectious waste is considered a "special waste", not a regulated hazardous waste. It can be shipped to another location for treatment and disposal.

15.3 The used Colilert trays and other non-recyclable trash to be autoclaved shall be placed in separate, labeled "bio-waste" bags at DWM for transport to WES for sterilization and solid waste disposal. The sample bottles and reagent bottles (recycled at WES) shall be bagged as "bio-waste" separately from the trays. This will enable WES to autoclave and dispose of all materials properly.

15.4 DWM waste management procedures for Colilert shall adhere to all applicable aspects of DWM's Lab Safety Plan. This includes use of proper gloves, use of waste receipt forms when delivering waste to WES, and provisions for lab audits/inspections to ensure compliance and remedial training as necessary.

16.0 REFERENCES



- 16.1 *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, 1998. (SM 9323B)
American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC
- 16.2 <http://www.state.ma.us/dep/bwp/dhm/files/infwaste.htm>
- 16.3 <http://www.idexx.com/>
- 16.4 WES SOP for SM 9223: Enzyme Substrate Coliform Test, Presence-Absence Procedure for Analysis of Potable Water Samples, Revision 2.0, 2/2003.
- 16.5 Total Coliform and E. coli by the Enzyme Substrate SOP, Ma. Water Resource Authority, Southboro Lab, 5/2003
- 16.6 Standard Operating Procedure for Bacteria Surveys, Sample Collection and Analysis in the ORSANCO Mobile Water Quality Laboratory, undated

17.0 APPENDICES

- Appendix A: MPN Tables for Colilert and Enterolert (by reference)
- Appendix B: MSDS Sheets (by reference; in DWM Labs and at <http://www.idexx.com/>)
- Appendix C: Sealer User's Manual and Maintenance Instructions (by reference)
- Appendix D: Quanti-Cult Procedure (by reference)
- Appendix E: Lab Notebook Example
- Appendix F: Incubator Temperature Log
- Appendix G: Waste Receipt Form



Commonwealth of Massachusetts
Executive Office of Energy & Environmental Affairs

Department of Environmental Protection

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Secretary

Martin Suuberg
Commissioner

Appendix E: Lab Notebook Example

Analyst	Project	Test	Lab ID	OWM ID	Sample Date	Sample Time	Sample Receipt Date & Time	Analysis Date	Analysis Start Time	Media Lot #	Dilution	Incubator #	Incubation Start Time & Temp	Incubation End Time & Temp	UV Reader	# of large yellow wells	# of small yellow wells	Total Coliform MPN	# of large fluoress wells	# of small fluoress wells	E. coli MPN
J. Bush	Blackstone Source Tracking	Colilert	L04-01-01	51-587	2/12/04	12:45	2/12; 1:50	2/12/04	2:24	368-01	0	1	4:35; 35.5	4:45; 35.0	R. Chase	14	14	67	7	7	34
			L04-01-02	51-587	2/12/04	12:45		2/12/04	2:26	368-01	10		4:35; 35.5	4:45; 35.0		2	2	8	1	1	5
			L04-01-03	51-588	2/12/04	12:55		2/12/04	2:29	368-01	0		4:35; 35.5	4:45; 35.0		37	37	287	26	26	211
			L04-01-04	51-588	2/12/04	12:55		2/12/04	2:31	368-01	10		4:35; 35.5	4:45; 35.0		4	4	27	4	4	27
			L04-01-05	51-588	2/12/04	12:55		2/12/04	2:35	368-01	100		4:35; 35.5	4:45; 35.0		0	0	0	0	0	0
			L04-01-06	51-589	2/12/04	1:25		2/12/04	2:45	368-01	0		4:35; 35.5	4:45; 35.0		9	9	49	5	5	32
			L04-01-	51-590	2/12/04	1:25		2/12/04	2:47	368-01	10		4:35;	4:45;		2	2	8	2	2	8



			07										35.5	35.0							
			L04-01-07D	51-590 Lab Dup	2/12/04	1:25		2/12/04	2:48	368-01	10		4:35; 35.5	4:45; 35.0		3	3	15	2	2	8
			L04-01-08	Lab Blank	2/12/04	2:58		2/12/04	3:05	368-01	0		4:35; 35.5	4:45; 35.0		0	0	0	0	0	0
			L04-01-09	E. coli +	2/12/04	2:59		2/12/04	3:10	368-01	0		4:35; 35.5	4:45; 35.0		59	59	---	59	59	---



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Appendix F: Incubator Temperature Log
TEMPERATURE LOG

Date	Time	Incubator TEMP, deg. C	TEMP (other)	Temp Dial Setting	Initials	Comments
4/5/04	9:15	35.5	35.0	1.6	rc	



* Record before, during and after incubations. See Jeff Smith, Richard Chase or Joan Beskenis if adjustment needed.



Appendix G: Waste Receipt Form for “Bio-Waste”

WASTE RECEIPT FORM

GENERATOR _____ ID # M _____

RECEIVING FACILITY _____ ID # M _____

NAME OF WASTE DELIVERED: _____ AMOUNT: _____

SIGNATURE OF PERSON DELIVERING WASTE: _____ DATE: _____

SIGNATURE OF PERSON RECEIVING WASTE: _____ DATE: _____

GENERATOR COPY



WASTE RECEIPT FORM

GENERATOR _____ ID # M _____

RECEIVING FACILITY _____ ID # M _____

NAME OF WASTE DELIVERED: _____ AMOUNT: _____

SIGNATURE OF PERSON DELIVERING WASTE: _____ DATE: _____

SIGNATURE OF PERSON RECEIVING WASTE: _____ DATE: _____

Receiving Unit Copy



Appendix H: Colilert® QuickGuide (these procedures also generally apply to Enterolert)

- 1) Two hours prior to anticipated delivery of samples, turn on incubator and record temperature to verify 35.0 deg. C (\pm 0.5 C).
- 2) Prepare all work areas by cleaning with lab disinfectant.
- 3) When samples arrive, transfer custody of samples from crew to analyst using sign-off procedures (COC form)
- 4) Allow samples to warm up to near room temperature.
- 5) Turn on PC and open E.coli EDD template. Fill out electronic lab notebook with sample information and per instructions in the "read me" tab. This will generate the paper lab notebook page.
- 6) Prepare counter work area with the necessary dilution bottles, reagents, trays, pipettes, etc., leaving sufficient space to prepare samples for incubation. With a waterproof wax pencil (so as not to tear the paper on the back of the Quanti-Tray) gently mark the Quanti-Trays with the lab identification number of each sample. Inspect each IDEXX vessel for inside cleanliness; if particles or other contamination is observed, place the container aside with other contaminated, new bottles (i.e., DO NOT USE), and discuss with DWM QC Analyst.
- 7) Turn on Quanti-Tray sealer to allow preheating (usually takes around 10 minutes). Sealer is ready to use when green light is on.
- 8) For each sample (including dilutions), invert sample bottle 20-25 times to ensure complete mixing of the sample prior to taking aliquots (for dilutions). DO NOT TOUCH THE INSIDES OF CONTAINERS OR SAMPLE WATER. The preferred order for sample analysis is to start with the "oldest" samples first and proceed in the order in which they were collected. QC samples (e.g., lab blank, lab duplicates, QC samples) and dilutions can be done at any time during the analysis.
- 9) **NO DILUTION SAMPLES:** If no dilutions are to be performed on the sample, mix sample (as above) and immediately pour off sample water to the 100 ml line (bottom of meniscus). Tap each reagent packet before opening to ensure complete dispensing. Open Colilert reagent packet and add entire contents to the 100 ml. sample (or diluted sample). Mix thoroughly until no more reagent particles. Pour into pre-labeled tray. Seal in sealer.
- 10) **10 ML DILUTION SAMPLES:** Pour 100 mls. of sterile DI water (sterilized WES-DI water) into a new, sterile IDEXX vessel to the 100 ml. line (and pipette off as needed to 100 mls using a sterile 10 ml pipette.). Now, using the same pipette, pipette off 10 mls to achieve 90 mls. of sterile water. Then use the same pipette to pipette 10 mls. of the original, mixed sample into the 90 mls. of sterile water. Cap and mix. Add reagent. Mix thoroughly until no more reagent particles. Pour into pre-labeled tray. Seal in sealer.
- 11) **100 ML DILUTION SAMPLES:** Pour 100 mls. of sterile DI water into a new, sterile IDEXX vessel to the 100 ml. line (and pipette off as needed to 100 mls using a sterile 1 ml pipette.). Now, using the same pipette, pipette off 1 ml. to achieve 99 mls. Then use the same pipette to pipette 1 ml. of the original, mixed sample into the 99 mls. of sterile water. Cap and mix. Add reagent. Mix thoroughly until no more reagent particles. Pour into pre-labeled tray. Seal in sealer.
- 12) **Use of Trays.** After complete mixing as stated above, dispense sample into the Quanti-Tray. Use one hand to hold the Quanti-Tray upright with the well side facing the palm. Squeeze upper part of the Quanti-Tray so that it bends towards the palm. Open the Quanti-Tray by pulling the foil tab away from the well side. Avoid touching the inside of the foil or tray. Tap the small wells to release any air bubbles.
- 13) **Use of Sealer.** Place the sample-filled Quanti-Tray onto the rubber tray carrier of the Quanti-Tray Sealer with well side (plastic) of the Quanti-Tray facing down to fit into the carrier. Seal the Quanti-Tray by moving the rubber tray into the sealer. The sealer will automatically take the rubber tray to dispense the sample into the wells and seal the Quanti-Tray. Remove the Quanti-Tray, assuring that it is fully sealed. (NOTE: If the Sealer stops in the middle of processing a Quanti-Tray sample, use the black button (with the arrows) on the front of the Sealer to reverse the direction of the Quanti-Tray in the Sealer. Allow the green light on the Sealer to go on before attempting to seal the sample again.) If the sealer does not easily "accept" the tray, lift up the end of the tray slightly while continuing to push it into the sealer, so that the leading edge clears the opening of the sealer.
- 14) **Reagent Lot QC samples (DWM QA Analyst only).** Run the following *E. coli* QC samples for each new lot of reagents: 1) **Negative Control.** Prepare *P. aeruginosa* (or other) samples as for *E. coli* below. Cap/tray/seal/incubate. 2) **Positive Control.** Prepare a positive QC sample by pouring 100 mls into an IDEXX vessel (as above), then adding an *E. coli* pellet according to manufacturer's directions, then adding Colilert reagent. Cap/tray/seal/incubate. Confirm correct analytical end-points.



- 15) **Batch QC samples (Lab analysts).** For each lab batch of samples, Prepare **LAB BLANK** as follows (minimum one per batch): Pour 100 mls. of sterile, deionized water into an IDEXX vessel, add reagent, cap and mix, pour into tray, seal and incubate. Prepare **LAB DUPLICATE** as follows (minimum one per batch): Using 250 ml. sample: Mix sample as above and pour two 100 mls. aliquots into two separate IDEXX vessels, add reagents, cap and mix, pour into trays, seal and incubate. Using a 120 ml. sample: Mix sample (each time) and pipette two 10 mls. aliquots into two separate IDEXX vessels containing 90 mls. of sterile, deionized water, add reagents, cap and mix, pour into trays, seal and incubate. Multiply results by 10. Provided 100 mls. is left in the sample container, run "raw", 100 ml (undiluted) sample also. NOTE: Previous to 2011, lab analysts also ran positive and/or negative QC for each lab batch, but this was discontinued in 2011.
- 16) Incubate all prepared Colilert Quanti-Trays in the incubator at **35 ±0.5 °C** for 24 - 28 hours. If Colilert-18 is used, incubate for 18-22 hours at **35 ±0.5 °C**. Record the dates and times in the lab notebook.
- 17) After sample trays have been placed in the incubator, thoroughly clean work area. First, place all "bio-waste" (items that have been in contact with sample water, including pipettes, sample bottles, dilution bottles, etc.) in pre-labeled, plastic bio-waste bags. Keep the plastic sample and reagent bottles separate from other waste (separate bags). Tie the bag(s) when only half-full and temporarily store in the designated bio-waste storage area in the lab. Recycle or dispose (as appropriate) other materials that have not touched the samples. Once the work area is clear, disinfect the work area.
- 18) After 24 hours (18 hours for Colilert-18), remove the Colilert trays from the incubator (and shut off). Record the time in the lab notebook. For each tray, count the number of wells that turn yellow (at least as strong as the yellow color of the comparator). A yellow color that is equal to or deeper than that of the color comparator verifies that the sample is positive for total coliforms. Note in the lab notebook the number of positive (yellow) wells in the Quanti-Tray.
- 19) To determine the *E. coli* result, expose each sample to 365 nm UV light by placing them one-by-one into the UV light viewing cabinet (or in a dark room wearing UV-protective glasses with a hand-held UV lamp held within 5 inches of the sample. If the sample fluoresces (compare to comparator), then the sample is positive for *E. coli*. Count the number of fluorescent wells and record in the lab notebook.
- 20) Colilert and Colilert-18 results are definitive at 24-28 and 18-22 hours, respectively. Any positives for both total coliform and *E.coli* observed before the minimum time and negatives observed after the maximum time are also valid. Positive results after the maximum time are not valid.
- 21) After all MPN results have been recorded and entered into the electronic EDD, review the lab notebook to ensure that all required data and metadata have been recorded completely and accurately. Save (as lab batch ID) and print EDD sheet. Give printout to QC Analyst. Keep working printout with manually-recorded results in paper lab notebook.
- 22) Place used Colilert trays and other waste in "bio-waste" bags, tie securely when only ½ full and temporarily store in the lab in the designated, secured location.

Colilert (and Colilert-18 Reactions)

Reaction	Result or interpretation
Yellow	Total Coliform positive
No color or indeterminant	Negative
Fluorescent (yellow)	<i>E. coli</i> positive
Yellow color intensity at or near the comparator color	Inconclusive; re-incubate an additional 4 hours
> 28 (22) hours, positive	Not valid
> 28 (22) hours, negative	Valid
< 24 (18) hours, positive	Valid
< 24 (18) hours, negative	Not valid

QC Interpretations (Colilert and Enterolert)



Quality Control Organism	Yellow	Fluorescent
<i>Pseudomonas</i> sp. (Non-fluorescent strain)	No	No
<i>Klebsiella pneumonia</i> , <i>Enterobacter aerogenes</i> or <i>Enterobacter cloacae</i>	Yes	No
<i>E. coli</i>	Yes	Yes (yellow)
<i>Enterococcus faecium</i>	---	Yes (blue)
<i>Serratia marcescens</i>	---	No