

QUALITY ASSURANCE PROJECT PLAN

for the

FRCOG/DRWA INTEGRATED DEERFIELD RIVER WATERSHED 2005/2006 WATER QUALITY MONITORING PROGRAM

DEP Project Number 2004-02/604

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4. Project/Task Organization

Key project personnel and their corresponding responsibilities:

| Name | Project Title/Responsibility |
|--|--|
| Christine Duerring, Sandy Shields, Marie-Françoise Walk, Marie Jose Iken | Technical Advisory Committee |
| Michael Cole, Robert May, Theodore Merrill, Marie-Françoise Walk, Christine Duerring | DRWA Water Quality Monitoring Committee: Program oversight |
| Marie Jose Iken | Project Coordinator/Lab Director/Field Coordinator: See Appendix B for description of responsibilities. |
| Marie-Françoise Walk | QC Officer: runs QC program |
| Kimberly Noake MacPhee | FRCOG Project Manager for the 604(b) project (2004-02/604) |
| DRWA Volunteer Monitors | Volunteer monitors will collect water samples for analysis of pH, alkalinity, and <i>E. coli</i> bacteria. |
| MaryJo Feuerbach | USEPA Project Contact: Oversees grant administration and ensures reporting requirements are met |
| Arthur Clark | USEPA QA Officer: Reviews QAPP |
| Arthur Screpetis | DEP QA Officer: Reviews QAPP |
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| Richard Chase | DEP Technical Reviewer |
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5. Problem Definition/Background

The Deerfield River Watershed encompasses approximately 347 square miles in Massachusetts. The Deerfield River is widely regarded as one of the coldest and cleanest rivers in Massachusetts and attracts many sport-fishermen and whitewater enthusiasts. However, nonpoint source pollution has degraded segments of the mainstem and its major tributaries and poses a threat to other tributaries in the watershed. The Massachusetts Department of Environmental Protection (MA DEP) has identified several potential sources of nonpoint pollution in the Deerfield Watershed, including failing septic systems, stormwater runoff, road runoff from paved and unpaved roads (many major roadways in the watershed run alongside rivers and streams), and agricultural activities in close proximity to rivers and streams. Approximately twenty-five (25) river miles of the mainstem and segments of five (5) major tributaries have been assigned to Category 5 Waters, which are waters that require a Total Maximum Daily Load (TMDL) calculation.

In March 2004, the Franklin Regional Council of Governments (FRCOG) was awarded a 604(b) grant from the MA DEP to conduct an assessment of nonpoint pollution in the Deerfield River watershed. The Notice to Proceed was received in November 2004. This project includes conducting a water quality monitoring program for *E. coli* in three (3) subwatersheds (the South River, the North River and the Chickley River) where pathogens have been detected during previous sampling efforts undertaken by the MA DEP. The FRCOG will be partnering with staff and volunteers from the Deerfield River Watershed Association to conduct the water quality monitoring program. The project will employ the Colilert System developed by IDEXX laboratories, Inc. to assay *E. coli* bacteria. This commercially-available system uses hydrolyzable substrates for the detection of target bacteria. The enzyme substrate method has been selected because it was 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) and because it is now widely used for DEP/DWM waterbody assessments, TMDL determinations, and bacteria source tracking.

A. Problem Statement

The DEP monitoring performed in 1995 (DEP/Division of Watershed Management, unpublished water quality assessment report) identified a threat to recreation in the Deerfield River Watershed by bacterial pollution. Because the State DEP/Division of Watershed Management monitors the water quality in the watershed only once every five years, in accordance with the Massachusetts Watershed Approach five-year basin cycle of activities, the DRWA started monitoring fecal coliform bacteria at popular recreation sites to provide data in the intervening years and to ensure that those sites are safe to use by the general public. In 1999 DRWA monitored bacteria above and below waste water treatment plants (WWTP)

and at a few recreational sites. WWTPs did not seem to pollute the streams, but high counts of bacteria were recorded from at least one recreational site, exceeding the Massachusetts Surface Water Quality Standards Class B standards for primary contact recreation. This finding prompted a focused examination of bacteria levels in the popular swimming holes of the watershed in the summer of 2003, which suggested that bacteria levels were generally safe in the popular swimming holes of the watershed during summertime dry weather.

However, concerns of impairment of streams and rivers of the watershed persist. According to the Massachusetts Year 2002 Integrated List of Waters, five important tributaries of the Deerfield are assigned to Category 5 Waters, including the Green River (pathogens and metals), Davis Mine Brook (pH), the Chickley River (pathogens), the North River (pathogens, taste, odor and color) and the South River (pathogens). Pathogens are of particular concern because of the heavy recreational use the Deerfield River watershed receives.

The DRWA has monitored the water quality of the river mainstem and several major tributaries since 1990 to document the current quality of the watershed. The new partnership between the FRCOG and the DRWA will expand the baseline water quality monitoring capacity of the DRWA and supplement the ongoing efforts of the MA DEP to identify potential sources of nonpoint pollution in the targeted subwatersheds. DRWA will continue its annual springtime volunteer-based monitoring of pH and alkalinity throughout the watershed and will partner with FRCOG to perform the 2005-2006 604(b)-funded Deerfield River watershed *E. coli* sampling project. In this effort, DRWA will integrate its existing water quality monitoring program with the 604(b)-funded bacteria monitoring project. This QAPP is intended to provide quality assurance for this integrated program. The DRWA will be collecting temperature data in addition to pH and alkalinity in concert with the 604b project.

B. Intended Usage of Data

Data collected as part of this project will be shared with watershed stakeholders, including the MA DEP. One of the goals of this project is to supplement the MA DEP's database for the Deerfield River watershed with data that can support the agency's efforts to address some of the priority nonpoint pollution issues identified in the Deerfield River Watershed Team's FY 2004 Work Plan, the MA DEP's *Nonpoint Source Action Strategy for the Deerfield River Watershed* (2004), and the MA DEP's *Deerfield River Watershed 2000 Water Quality Assessment Report* (2004), and to support the MA DEP's development of TMDLs for targeted subwatersheds.

Another goal of this project is to support the ongoing outreach efforts and expand the water quality monitoring capacity of the Deerfield River Watershed Association.

Quality control-approved results will be promptly reported during the sampling season to the volunteers

and to the public through the DRWA's web site (www.deerfieldriver.org). Samples will be taken on Sunday, results read on Monday, and results posted on the web and emailed to volunteers on Wednesday of the same week. Colony counts (MPN) will be compared to the Massachusetts Surface Water Quality Standards for public bathing beaches where *E. coli* is the indicator of choice. The Massachusetts Surface Water Quality Standards for Class B waters are for fecal coliform bacteria. Only public bathing beaches as defined by the Massachusetts Department of Public Health (DPH) may use *E. coli* as the chosen indicator. The Massachusetts Surface Water Quality Standard for public bathing beaches where *E. coli* is the chosen indicator is no single *E. coli* sample shall exceed 235 *E. coli* per 100 ml and the geometric mean of the most recent five samples within the same season shall not exceed 126 *E. coli* bacteria per 100 ml. The FRCOG, with the assistance of the DRWA, will compare water quality data to existing water quality data previously collected in the sub-watersheds by DEP, land use data, and Stream Team Shoreline Survey data. A summary report will then be prepared and both the report and the analytical data will be presented to stakeholders and the information will be posted on the DRWA's web site.

6. Project/Task Description

A. General Overview of Project

The FRCOG and the DRWA will review existing water quality data and previous sample collection locations for the three (3) targeted sub-watersheds, discuss proposed sampling locations with stakeholders; and select up to six (6) sampling locations in each of the targeted sub-watersheds for a total of eighteen (18) "screening-level" sampling locations that will be used to identify river reaches with potential bacteria pollution problems. For example, six sampling locations will be selected along the length of the Chickley River from above West Hawley down to the confluence with the Deerfield River. Data will be used from each site to identify lengths of river with potential bacteria sources.

DRWA staff and volunteers, with the assistance of FRCOG staff, if needed, will conduct one (1) round of sampling for *E. coli* during three wet and three dry-weather events in targeted sub-watersheds at the selected screening-level sampling locations. Approximately six (6) samples will be collected between May and October for each selected sampling location in each sub-watershed for a total of up to 108 samples. Samples will be analyzed for the presence of *E. coli* using the Colilert system.

Sampling results will be analyzed and compared to results obtained during the Stream Team Shoreline surveys and data obtained from GIS mapping. Suspected bacteria sources will be identified and then the DRWA/FRCOG will conduct a targeted sampling round to bracket these potential sources. Up to twelve (12) sampling locations will be selected in each sub-watershed in 2006 for the second sampling round for a total of up to thirty-six (36) locations. The targeted sampling will be conducted for *E. coli* bacteria during both wet and dry conditions. Approximately six (6) samples will be collected between

May and October for each selected sampling location for a total of up to two hundred and sixteen (216) samples.

The Deerfield River Watershed Association will coordinate the project. Volunteers will be trained by the project's QC Officer and Project Coordinator to collect pH/alkalinity and bacteria samples as well as to take field temperature measurements and make weather and river use observations at 12 to 18 sites on the Deerfield River and tributaries in Massachusetts and Vermont. The pH/alkalinity samples will be collected at our traditional 12 sites in April only. The 2005 bacteria samples will be collected at eighteen (18) sites on six (6) occasions between May and October. Sampling will occur during three wet and three dry-weather events in 2005. Three scheduled sampling events will occur between June and August (one each month). Depending on the number of wet events that occur during scheduled sampling events, it will likely be necessary to send volunteers into the field on short notice to collect wet weather samples. We will attempt to take this approach beginning in May, for at least one wet-weather event, to ensure we achieve our objective of sampling during wet weather on three occasions.

To identify wet-weather events, the Program Coordinator will track weather and notify volunteers of an impending wet-weather event at least 24 hours in advance to determine whether sufficient volunteers will be available for sampling. Only large weather fronts forecast to produce significant and widespread precipitation will qualify as wet weather appropriate for sampling across the watershed. When a front moves into the watershed, volunteers who have installed a rain gage in their yard will call the Program Coordinator once 0.3-0.5 inches of rainfall has been measured in their rain gage. At that time, the Program Coordinator will call the remaining volunteers and give them the go-ahead to sample within the next couple of hours.

Air and water temperature will be measured for every collection. Safety will also determine whether volunteers collect samples during high flows. Precipitation will be recorded at the Greenfield Water Pollution Treatment Facility. Dry-weather sampling will occur only when the antecedent weather has lacked any measurable precipitation for 72 hours (less than 0.1 inch of rain).

Samples will be stored on ice at 4°C and transported to the DRWA Lab in Shelburne Falls for analysis of pH/alkalinity in April and for analysis of *E. coli* bacteria in May through October. All samples will be delivered to the laboratory within six hours of collection.

B. Analytical Parameters

Samples for the analysis of pH, alkalinity, and bacteria will be collected, water and air temperature will be field-measured, and accompanying visual observations of weather, river use, etc. will be recorded (see Field Data Sheet, Appendix D).

C. Sampling Sites

Sampling will occur in three subwatersheds: the North, South, and Chickley rivers. Six (6) locations will

be sampled within each of these subwatersheds during the 2005 screening-level assessment. These locations are listed in Appendix A and were selected from knowledge of potential problem areas based on existing data and through consultation with DWM staff. 2005 sampling will occur during three dry-weather and three wet-weather events for a total six sampling events at each site. Sampling will occur between May and October, 2005. Three planned sampling events will take place between June and August, the second weekend of each month. Three additional sampling efforts will also occur within the May-October sampling window to collect the remaining wet and dry-event samples.

North River sampling will occur in two locations on the mainstem of the North River – Sunburn Beach and at the Adamsville Road iron bridge – where bacteria sampling by DRWA has occurred in previous years. Two sample stations are located on the East Branch of the North River, while one sample station occurs on Foundry Brook, a tributary to the lower East Branch. Finally, one 2005 sample station is located in the West Branch of the North River near its confluence with the East Branch.

South River sampling will occur in the lower watershed, with the upriver-most site occurring less than one mile upriver of the town of Conway. Three sample stations occur in and around the town on Conway – two in the mainstem of the South River and one in Pumpkin Hollow Brook. Finally, two sample stations occur in the lower reaches of the mainstem South River, one located at the Reeds Bridge crossing and the other at the mouth of the South River, where DRWA bacteria sampling has occurred in previous years.

Chickley River sampling will occur throughout the mainstem of the Chickley River. Sites are relatively equidistant from one another, each spaced approximately 1-2 miles from its respective upstream and downstream sites. The upriver-most site occurs on Savoy Road near the Hawley/Florida town line. All other sampling sites occur along, or immediately off of, the length of Rte 8A that parallels the Chickley River.

2005 data will be used to identify potential bacteria source areas. Any 2005 sample sites that exceed either criterion described earlier for *E. coli* will be identified as having a potential upstream bacteria source. Targeted sampling will focus on reaches bracketed by 2005 sites that had higher *E. coli* readings at the downstream site than at the upstream site. A second year of sampling in 2006 will further bracket and track these sources. Up to 12 sampling locations will be selected in each subwatershed in 2006 and each sampled six times between May and October. A first sampling event will occur in May using the same sites as those used in 2005 to determine whether bacteria data show similar patterns to those in 2005. If these 2006 data show new bacteria sources, targeted sampling will be refined to include sampling from these newly-identified sources. Up to twelve (12) sampling locations will be selected in each sub-watershed in 2006 for the second sampling round for a total of up to thirty-six (36) locations.

D. Project Timetable

| Activity | Projected Start Date | Anticipated Date of Completion |
|---|---|---------------------------------------|
| Recruit participants | March 14, 2005 | April 24, 2005 |
| 2005 Volunteer bacteria testing training workshop | May 22, 2005 | May 22, 2005 |
| 2005 Sampling for chemical testing occurs (using previously-trained volunteers) | April 24, 2005 May 22 potential makeup | April 24, 2005 May 22, 2005 |
| 2005 Sampling for bacteria testing occurs (will begin only AFTER volunteer training) | May 2005 | October 2005 |
| Quality Control procedures conducted (includes field audits, collection of field duplicates and blanks, analyzing lab duplicates, blinds, blanks, and positive controls, and data review) | April-October 2005 | October 2005 |
| 2005 Data entry | April 2005 | October 2005 |
| Distribute 2005 results to participants and media | April 2005 | October 2005 |
| 2006 Volunteer training workshop | April 2006 | April 2006 |
| 2006 Sampling for chemical testing occurs | April 2006 May potential makeup | April 2006 May 2006 |
| 2006 Sampling for bacteria testing occurs | May 2006 | October 2005 |
| Quality Control procedures conducted | April 2006 | October 2006 |
| 2006 Data entry | April 2006 | October 2006 |
| Distribute 2006 results to participants and media | April 2006 | October 2006 |

| | | |
|-----------------------------|--------------|---------------|
| Write and Distribute Report | October 2006 | December 2006 |
|-----------------------------|--------------|---------------|

7. Measurement Quality Objectives

A. Data Precision, Accuracy, Measurement Range

| Matrix | Parameter | Measurement Range | Accuracy | Precision |
|-------------|----------------|-------------------|--|---|
| River Water | Temperature | 0-30°C | ∓1°C | ∓10% |
| River Water | <i>E. coli</i> | 0-10,000 MPN | Blanks and negatives show no colonies, positives show colonies | A precision criterion will be calculated and applied using Sec. 8.b of Standard Methods 9020 B (20 th ed.) |
| River Water | pH | 0-14 | ∓0.3 pH unit | ∓0.3 pH unit |
| River Water | Alkalinity | 0-100mg/l | ∓3mg/l | ∓10% |
| Air | Temperature | 0-40°C | ∓1°C | ∓1°C |

These objectives will be measured as follows:

Temperature: All thermometers will be calibrated against the Standard NIST thermometer kept at the Environmental Analysis Lab at UMass Amherst. Only thermometers that agree within 1°C of the NIST thermometer will be used. The thermometers will be used to take readings of a water bath at freezing and at room temperatures. Thermometers that do not meet the objective will not be used. Collectors will be trained by the DRWA QC Officer and the Project Coordinator on how to use the thermometer and how to read it at a training session before the project begins. Volunteers will be given written instructions to bring along each collection (see Appendix C, Sampling Instructions). In the field, a randomly chosen volunteer collector will be checked by the DRWA QC Officer or the Project Coordinator at least once each collection to assess that proper procedures are being followed, and the volunteer readings will be compared to the trainer's readings. If the volunteer's readings do not agree within 1°C of the trainer's reading, the volunteer's readings will be censored and the volunteer will be retrained at that time.

Bacteria: Two blank field samples using a purchased sterile solution poured in a sterilized bottle in the

field (the sterile solution is buffered dilution water from North Central Laboratories, Birnamwood, WI) and two blank lab samples will be run every collection by the lab. The trays should show no colonies. If they do, the equipment and methods will be examined by the Program Coordinator to determine the source of contamination, and the data will be censored if necessary.

For a positive check, a positive control sample will be collected during each summer sampling event from the Green River at Meade Street, a site known for its historical high counts of bacteria. We have used that site successfully for the past four years as a positive sample. One hundred milliliters of that sample will be run and positive results should be obtained. If the results are negative by comparison of the sample to the comparator, we will conclude that our procedure killed the bacteria and our results will be censored. The equipment and methods will be examined by the Program Coordinator to determine the problem.

Two collectors will take duplicate field samples during each collection (amounting to two field duplicates per eighteen field samples). We will rotate which collectors take a field duplicate to check on as many collectors as possible. All samples will immediately be placed on ice and held at 4°C for no longer than six (6) hours before delivery to the lab.

Multiple duplicates will be run during the first two sampling collections to calculate a criterion for assessing precision using the methods described in Section 8. b of Standard Method 9020 B (American Water Works Association 20th ed., 1998). Duplicate analyses will be run on 15 positive samples. Once fifteen duplicates have been run, the relative percent difference of Log₁₀-transformed data will be calculated for each pair of duplicates. From these RPDs, a precision criterion will be calculated according to Sec. 8.b of Standard Methods 9020 B. Two samples will be run in duplicate with each sample batch to provide lab duplicates. Precision of Colilert testing will be assessed by calculating the relative percent difference of Log₁₀-transformed *E. coli* results for each duplicate pair and comparing this value to the precision criterion. The RPD of any pair should not exceed the precision criterion established by the lab. If any duplicates fail to meet the quality objective established by the precision criterion, the data will be censored, and equipment and methods will be examined to determine where improvements can be made. If our duplicate numbers continue to look questionable, we will consult with MA DEP staff member, Richard Chase, and then take appropriate action, including first running additional lab duplicates and/or qualifying or censoring the entire sample batch.

pH and alkalinity: One field duplicate will be collected by a volunteer (amounting to one in twelve (12) samples having a duplicate sample collected). A lab duplicate will be performed for each parameter. One blind sample from the EAL will be analyzed before and after analysis of river samples. If the field replicates and lab duplicates do not meet the above data quality objectives for precision, or if the blind samples do not meet the requirements for accuracy, the data will be censored and the equipment and methods will be examined by the Program Coordinator to determine the problem. Resampling in May

may be undertaken if April data must be censored. If we resample, all sites will be sampled in May, but we may limit analysis to the faulty parameter (pH or alkalinity).

B. Data Representativeness

The data obtained from this project are representative of the specific areas selected for monitoring; volunteer training will emphasize taking representative samples. Results will not be extrapolated to other unmonitored portions of the Deerfield River.

C. Data Comparability

We will use standard equipment and methods. In April we will monitor the same ten sites that we have monitored for chemistry for the past 12 years (plus two sites in Readsboro, VT, added in 2000). Our site locations will be well documented and can be found in Appendix A.

D. Data Completeness

We expect to have one reading (in April) for each of our 12 sites for water temperature, pH, and alkalinity. We expect to have at least five (preferably six) readings for each of our 18 sites for bacteria to allow computation of geometric means for the season.

| Parameter | No. 2005 Valid Samples Anticipated | No. 2006 Valid Samples Anticipated |
|-------------------|--|---|
| Water Temperature | 102 | 192 |
| Bacteria | 108 (5 per site, or a total of 90 is acceptable) | 216 (5 per site, or a total of 180 is acceptable) |
| pH/Alkalinity | 12 | 12 |

These data will be provided to DEP/DWM and to the Massachusetts Department of Health who will also use the data for their own programmatic purposes and at their discretion.

8. Training Requirements and Certification

A. Training Logistical Arrangements

| Type of Volunteer Training | Frequency of Training/Certification |
|---|--|
| Group Training Session for Field Sampling: lecture and demonstration | Once a year, in April or May |

B. Description of Training and Trainer Qualifications

Volunteer monitors will be trained by the DRWA QC Officer and Project Coordinator in sample collection at the April/May training meeting. One training will be held each year. These are not optional and collectors not attending the session will not be used in that season. Returning volunteers will be required to come for a re-certification that day. Safety considerations in getting to their sites and in handling samples will be covered. Measurement of temperature and sample collection procedures for pH/alkalinity and bacteria will be demonstrated. Instruction sheets will be distributed to participants for future reference (see Appendix C).

Volunteer monitor performance will be evaluated through a field audit. As many of the volunteer collectors as possible will be accompanied by the DRWA QC Officer or the Project Coordinator once during the sampling season. The volunteer collector's techniques will be observed and corrected if necessary. The Project Coordinator will likely also be collecting samples during each sampling event, so only the Quality Control Officer will be accompanying volunteer monitors.

Some volunteers (who may or may not be sample collectors as well) will be trained by the Lab Director to assist her in laboratory analyses. This training will take place on the first day of analyses, before analyses are begun, and will cover sample handling, glassware washing, sterility considerations, preparation of Colilert Quanti-Trays for incubation, analyzing incubated samples, and record keeping.

The DRWA QC Officer, Marie-Françoise Walk, is a water quality professional employed by the University of Massachusetts Water Resources Research Center. The Project Coordinator, Marie Jose Iken, is a lab technician for the Greenfield Water Pollution Control Facility and was trained in sampling techniques by our QC Officer in 2002.

9. Documentation and Records

Field data sheets (See Appendix D) will be completed on-site at the time of sampling. Collectors will record the data and time of sample collection, the name and number of their site, weather and river use observations, air and water temperature, and their name. Data sheets will accompany the samples to the Drop-Off Site and/or to the Lab, where they will be collected by the Project Coordinator within six (6) hours of collection.

Labels on bottles will include the site name and identification number.

Chain-of-Custody Forms (See Appendix D) will be signed by collectors as they relinquish their samples to the Drop-Off Site and/or lab. Sample ID Number, Date, Sample Collection Time, Sample Drop-Off Time, Cooler Internal Temperature, and Signature of Sample Relinquisher will be filled on the Chain-of-Custody Form.

The Lab Director will use lab data sheets (see Appendix D), on which she will record the date and time of sample analysis. For bacteria analysis, Colilert media lot number, dilution, incubation start time and temperature, incubation end time and temperature, UV reader name, and UV fluorescence results will be recorded. Raw data from pH and alkalinity analyses will be recorded (pH units, digits to 4.5, digits to 4.2). These lab data sheets will be kept by the Lab Director/Project Coordinator, Marie Jose Iken.

The Project Coordinator will enter the raw data into a MS Excel spreadsheet fitted with formulas to compute results (for alkalinity and bacteria counts). When she is finished entering the data in electronic form, all data sheets will be given to the QC Officer for error checking. The QC Officer will also check the electronic data entry. The computer files will reside on the QC Officer's personal computer and will be backed up on a zip disk each time new data are entered. Finally all paper forms will be filed indefinitely in a filing cabinet at the lab in Shelburne Falls.

The Chain-of-Custody Form will follow the samples from the Drop-Off Site to the lab, where it will be signed by the Samples Relinquisher and Receiver. Chain-of-Custody Forms will be checked by the Project Coordinator and filed in the DRWA filing cabinet at the lab.

10. Sampling Process Design

A. Rationale for Selection of Sampling Sites

The sites selected for pH and alkalinity are historical monitoring locations selected in 1990. The Cold River was chosen to serve as a benchmark against which to compare other sites because it is a pristine tributary that runs through undeveloped land. 2005 bacteria sampling sites were selected to represent locations that bracket areas within each subwatershed that, based on observed land use practices or mapped land use types and discussions with DWM personnel, may contribute to the bacteria problems occurring in each of these three waterbodies. 2006 sample sites will be relocated after examination of the 2005 data and consultation with DWM for further bracketing and tracking of suspected sources. See Appendix A for list of 2005 site descriptions.

In April, samples will be collected between 7 and 10 A.M. Bacteria samples taken between May and October will be collected between 10 A.M. and 1 P.M. This timing matches actual recreational use of the river better than the earlier April times, and also allows for enough time for analysis in the afternoon. It is also more convenient for counting Quanti-Tray sample wells to derive MPN estimates the next day (in the afternoon when the Lab Director is available).

Select volunteers who live in close proximity to the North, South, and Chickley Rivers will be provided with a Tru-Chek Direct-Reading rain gage and asked to monitor precipitation in their gages daily and to closely monitor rainfall rates and amounts during specific rain events that are identified as potential wet-weather sampling events. Volunteers will be asked to install the rain gage on a 4-ft post in their yard where it will not stand under trees or other obstructions (or in the path of the lawn sprinkler). These volunteers will keep a record of rainfall amounts for the duration of the sampling season and will give this record to the Program Coordinator at the end of the season.

Rainfall will also be recorded at the Greenfield Water Pollution Control Facility for the preceding 24 hours (following an antecedent dry period of 72 hours). The Greenfield Water Pollution Control Facility is part of the National Oceanic and Administration National Weather Service, Weather Station Index # 19-3229-2 and furnished with the official observation equipment. The rain gage is an official non-recording rain gage read daily at 7:30 a.m. (no specific name or number to rain gage). This gage amplifies collection to increase the accuracy of measurement.

To identify wet-weather events, the Program Coordinator will track weather and notify volunteers of an impending wet-weather event at least 24 hours in advance to determine whether sufficient volunteers will be available for sampling. Only large weather fronts forecast to produce significant and widespread precipitation will qualify as wet weather appropriate for sampling across the watershed.

When a front moves into the watershed, volunteers who have installed a rain gage in their yard will call the Program Coordinator once 0.3-0.5 inches of rainfall has been measured in their rain gage. At that time, the Program Coordinator will call the remaining volunteers and give them the go-ahead to sample within the next couple of hours.

When sampling during wet-weather events, samplers will make a qualitative assessment of the water level at their sampling station by “reading” the water level at a point of reference such as a large rock or bridge abutment, and making a note of it on their field sheet. This point of reference will be selected prior to the first sampling event (wet or dry) and will be used to document the water level during each wet and dry-weather sampling event.

We will also document water levels during both wet and dry-weather sampling events with USGS gage station data for the North River (gage #01169000) and the Deerfield River in Charlemont (gage

#01168500). These supporting data will be included with our data and observations in our final report.

B. Sample Design Logistics (same logistics will follow in 2006)

| | Type of Sample/ Parameter | Number of Samples per collection | Sampling Frequency | Total Number of 2005 Samples (includes field QC samples) | Sampling Period |
|------------|--------------------------------------|---|---------------------------|--|---------------------------------|
| Biological | Bacteria | 20 (includes a field blank and a field duplicate) | 1X-2X/month | 120 | May - October 2005 |
| Physical | Air Temperature | 10/12 | once/twice a month | 120 | April 2005/ May-October2005 |
| | Water Temperature | 10/12 | once/twice a month | 120 | April 2005/ May-October 2005 |
| Chemical | pH/Alkalinity | 13 (includes a field duplicate) | once a year | 13 | April 2005 |

There will be nine sampling crews of one or two volunteers, each visiting two sites. The crews will first hang their thermometer in the shade on a tree alongside the stream to measure air temperature. While the thermometer is reaching equilibrium, the crews will take water samples for pH/alkalinity (in April) or bacteria (summer). They will place samples on ice at 4°C in the cooler. They will then read the thermometer for air temperature and then hold the thermometer in the water for two minutes to obtain a water temperature reading. Finally, they will fill out the rest of the field data sheet (see Appendix C).

11. Sampling Method Requirements

| Parameter | Sampling Equipment | Sampling Method* |
|-------------------|--|--|
| Air Temperature | Hand-held Alcohol Thermometer | MassWWP Standard Operating Procedures Rivers-1 for Temperature, Revision 0 |
| Water Temperature | Hand-held Alcohol Thermometer | MassWWP Standard Operating Procedures Rivers-1 for Temperature, Revision 0 |
| Bacteria | New disposable Sterilized Plastic Sample Bottle (120ml) (no thiosulfate) | MassWWP Standard Operating Procedures River- 3 for Bacteria, Revision 0 |
| pH and Alkalinity | Plastic Sample (HDPE) Bottle (500ml) | MassWWP Standard Operating Procedures Rivers-4 for pH and Alkalinity, Revision 0 |

* All MassWWP Protocols are DEP-approved and can be found at <http://www.umass.edu/tei/mwwp/protocols.html>

12. Sample Handling and Custody Procedures

Samples will be collected and labeled by the volunteer collector between 7A.M. and 10 A.M. on sampling day in April, and between 10 A.M. and 1 P.M. on sampling days in the summer (May through October).

pH and alkalinity samples will be collected and labeled by the volunteer collector between 7A.M and 10 A.M. The samples will be placed inside two Ziploc bags; the first bag (“zipped” closed before placement in the second bag) will contain only the sample bottle, the second bag will contain the first bag with the sample bottle and ice. This double-bagged sample will be placed inside a cooler, and immediately brought by the collector with the data sheets to the DRWA lab. All pH/alkalinity samples will be delivered to the DRWA lab within six (6) hours of collection. The DRWA lab is in the basement of Water Quality Committee member Ted Merrill’s house at 30 High Street in Shelburne Falls, MA. Samples must arrive at this location by 11 A.M. The date and time of arrival to the lab will be recorded and signed by the collector on the Chain of Custody Form and by the QC Officer, Ted

Merrill (receivers), or Project Coordinator/Lab Director. The samples will be analyzed by the Project Coordinator/Lab Director, or by trained volunteers under the Lab Director's supervision within two hours of receipt of samples. The Chain of Custody Form will be checked by the Lab Director and later by the QC Officer. After analysis, samples will be taken by the Project Coordinator/Lab Director to the Greenfield Water Pollution Control Facility for proper disposal. Samples will be discarded after analysis.

E. coli samples will be collected and labeled by the volunteer collector between 10 A.M and 1 P.M. The samples will be kept inside a 1-gallon Ziploc bag, inside a cooler with filled with ice and immediately brought by the collector with the data sheets to the DRWA lab. All *E. coli* samples will be delivered to the DRWA lab within six (6) hours of collection. Samples must arrive at this location by 2 P.M. in the summer. The date and time of arrival to the lab will be recorded and signed by the collector on the Chain of Custody Form and by the QC Officer, Ted Merrill (receivers), or Project Coordinator/Lab Director. The samples will be analyzed by the Project Coordinator/Lab Director, or by trained volunteers under the Lab Director's supervision, within two hours of receipt of samples. The Chain of Custody Form will be checked by the Lab Director and later by the QC Officer. After analysis, samples will be taken by the Project Coordinator/Lab Director to the Greenfield Water Pollution Control Facility for proper disposal. After analysis, samples, used trays, and reagents will be taken by the Project Coordinator/Lab Director to the Greenfield Water Pollution Control Facility for proper sterilization via autoclaving and disposal.

13. Analytical Methods Requirements

All analytical determinations will be performed at the DRWA lab in Shelburne Falls by the Lab Director, Marie Jose Iken. One or two volunteers will be recruited to assist the Director. Volunteers will be trained and supervised by the Director. In the past, our volunteers have held B.S. and even Ph.D. degrees in microbiology and we hope to enlist their help again this year. We built the lab in 2000 in a ground-level basement at the house of Water Quality Committee member Ted Merrill and have had great success at that location.

| Indicator | Method Number | Source | Reporting Units | Modifications or Options |
|------------------|--|------------------|------------------------|---------------------------------|
| pH | Standard Operating Procedures Rivers-4 for pH and Alkalinity, Revision 0 | MassWWP | pH units | n/a |
| Alkalinity | Standard Operating Procedures Rivers-4 for pH and Alkalinity, Revision 0 | MassWWP | mg/l | n/a |
| <i>E. coli</i> | 9223(B) for Enzyme Substrate Test | Standard Methods | MPN/100ml | n/a |

All bacteria samples will be analyzed for *E. coli* using the Colilert enzyme substrate test, as described in Standard Methods 9223B (APHA 1998; Appendix E). We will use a Colilert incubator for *E. coli* Colilert Quanti-Trays. The Colilert incubator, Quanti-trays, and associated supplies will be obtained from IDEXX Laboratories, Inc.

14. Quality Control Requirements

Quality control procedures will be performed for each sample collection batch and will consist of

- (1) 2 field duplicates,
- (2) 1 field blank,
- (3) 1 lab duplicate,

- (4) 1 negative lab control (lab blank),
 - (5) 1 positive lab control, and
 - (6) 1 lab blind,
- as well as a check of collectors by either trainer.

A. Volunteers Check - Field Audit

The QC Officer, the Project Coordinator, or both will accompany a crew of volunteers (each) to their site each collection. The trainers will observe the volunteers' performance and check their temperature measurements and field data sheets for accuracy.

B. Field QC Checks

Two Field Duplicates will be taken each sampling day. The lab will analyze both samples. Duplicates will be collected for pH/alkalinity and for bacteria.

One Field Blank will be taken by two volunteers at one site each per sampling day for bacteria. We will rotate the field duplicates and blank samples collection responsibility among the volunteers to increase the number of collectors checked.

C. Laboratory QC Checks

Two Lab Duplicates will be analyzed each sampling day. The lab will split two previously randomly chosen samples and analyze both subsamples. Alkalinity, pH, and bacteria lab duplicates will be run. Blind QCs: A blind sample will be obtained from the UMass Environmental Analysis Lab (EAL) for the analysis of pH, alkalinity. This sample will be analyzed first and last. Results will be checked with EAL after analyses to verify that they are acceptable. The lab will run lab blanks, positive sample, and lab duplicates for bacteria each collection (see section 7).

D. Data Analysis QC Checks

River data and QC data will be reviewed by the QC Officer as soon as they come in from the lab after each collection. QC data will be compared to the quality objectives (e.g. blanks should have MPN of zero, bacteria sample duplicates should meet DQOs stated earlier, positives should have positive MPN, etc). River data will be reviewed as well to ensure that no field or lab contamination has occurred (data will be compared to those of other sites and of previous collections).

Bacteria, pH, and alkalinity computations will be checked and validated. If discrepancies are found, the Lab Director will be called to resolve the discrepancy and corrections will be made by the QC Officer.

Field data sheets will be also reviewed the day of collection by the Project Coordinator and later by the QC Officer to ensure that samples were taken at the right times and that all of the required information has been filled out. The Project Coordinator or collectors will be called if omissions or errors are detected. If needed, corrections will be made (signed and dated) on the field sheet by the QC Officer.

15. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

| Equipment Type | Inspection Frequency | Type of Inspection |
|--|--|---|
| Autoclave (at the Greenfield Water Pollution Control Facility) | Monthly Weekly | Visually Spore check is run with a batch to ensure the autoclave is reaching proper temperature and pressure |
| IDEXX Quanti-Tray Sealer | Prior to each sampling | Visual inspection, clean, and maintain according to manufacturer's recommendations. |
| IDEXX Incubator | Prior to each sampling | Check temperature with max/min electronic thermometer (traceable to NIST) |
| Beckman phi-400 pH Meter | Prior to April Sampling and day of analysis | Calibration and running a QC sample from EAL. Check that electrode is clean and free of buffer solution. |
| Pocket Thermometers (Hach product 2676300) | 1. Twice a year by QC Officer (before and after sampling season) 2. Prior to each sampling by collectors | 1. Calibration 2. Visually check for separations in column |

16. Instrument Calibration and Frequency

| Equipment Type | Calibration Frequency | Standard or Calibration Instrument Used |
|----------------|--|--|
| pH Meter | Every analysis day | 4.01 and 7.0 pH buffers (2-point)* |
| Thermometers | Twice a year: Before and after sampling season | NIST Thermometer at EAL, putting thermometers in ice water and tepid water |

* Reports will include a reference to our two-point calibration procedure to allow other users of the data to determine whether to censor any pH values greater than 7. We will also note that pH values greater than 7 may not be as accurate as those between 4 and 7.

17. Inspection/Acceptance Requirements for Supplies

Sample bottles will be provided by DRWA. pH/Alkalinity bottles will be cleaned by the Lab Director or a lab volunteer between collections and stored in a closed cabinet. We will use disposable IDEXX sample bottles for bacteria sampling. IDEXX sample bottles will be used only once before sterilization and proper disposal.

- For pH and alkalinity, sample bottles must be clean, large enough to hold 200ml of sample and unopened until sample collection. Dirty lab ware will first be visually inspected, scrubbed with a brush if residue remains, rinsed three times with tap water, rinsed three times with distilled water, filled with distilled water for 24 hours, and then air dried.
- For bacteria, sample bottles must be sterile, large enough to hold at least 120-ml of sample and be unopened until sample collection. Disposable IDEXX 120-ml sample vessels will be used. They will not contain thiosulfate.
- Rubber Inserts for the Quanti-Trays must be autoclaved or cleaned with isopropyl alcohol or bleach prior to each use.
- Thermometers must be clean, free of column separation, and previously calibrated.

The Project Coordinator will inspect sample bottles before distributing them to collectors. She will reject bottles that are cracked or misshapen.

Volunteer collectors will inspect their thermometers for column separation prior to each sampling event. The program does not own enough replacement thermometers to provide each crew with a spare. Therefore, if a thermometer breaks or is lost at the sampling site, no temperature reading will be made that day. Temperature is no a critical parameter for this study and the potential omission of this

measurement will not compromise the study. The volunteer collector should alert the Project Coordinator upon relinquishing his/her samples. The broken or lost thermometer will be replaced by the next sample collection.

Chemicals (sulfuric acid cartridges and bacteria growing media) will be checked for expiration dates before the program begins in April to ensure that they will be usable throughout the sampling season.

18. Data Acquisition Requirements

Geographical information from USGS topographic maps at 1:25,000 scale for the Deerfield River region was used to locate sampling sites. Rainfall records will be obtained from the Greenfield Water Pollution Control Facility for local 24-hour amounts. These rainfall data will be used for an approximate correlation between precipitation amounts for the 72 hours preceding sampling and bacteria counts. Because rainfall can be quite variable from location to location in the summer, these rainfall data will be used as an approximation only and need not be of strict accuracy.

19. Data Management

Field data sheets will be inspected for omissions and errors by the Project Coordinator or the QC Officer before the collectors leave the drop off location. Field data sheets and Chain-of-Custody Forms will also be inspected by the QC Officer as soon as they are received, and the collectors will be contacted if any problem is suspected. If either person discovers that samples did not arrive at the lab at 4°C (as determined by a temperature reading taken in the cooler at the time of sample drop-off and as documented on the Chain-of-Custody Form), the samples will be discarded.

Lab data sheets will be checked by the Lab Director before she enters data in an MS Excel spreadsheet. All data sheets will be forwarded to the QC Officer who will check them and will also proof the electronic data entry.

Descriptive statistics (means, medians, quartiles) and graphical analyses will be performed on the data to uncover any outliers or errors. Any questionable data points will be flagged and rechecked. The Project Coordinator and QC Officer will work together to determine that the data meet the project requirements. Any data not meeting the DQOs will be noted. A review of the procedures will be made to determine where problems arose. Steps will be then taken to correct the problem.

A table presenting the latest quality-controlled results will be posted on the DRWA web site after each collection and will be accompanied by a brief interpretation of these results, comparing the results to

state water quality standards, with some indication of the rainfall prior to sampling.

A final report summarizing the survey's results will be written after the conclusion of each sampling season (2005 and 2006). These reports will be distributed to volunteers, local, regional, and state officials as well as other concerned parties and will detail the program's goals, methods, results, data interpretation, and recommendations. DEP will receive electronic and hard-copies of the report which will include all raw data and QC results in tabular form.

The data will be stored in the QC Officer's personal computer and backed up on a separate zip disk. Paper forms will be stored indefinitely in a DRWA file cabinet at the DRWA lab.

20. Assessment and Response Actions

As described above, review of field data sheets, lab data sheets, river data and QC data will occur immediately after each collection.

When an error is discovered, it will be corrected if possible. If no correction is possible, or if quality control objectives are not met, the data will be discarded. No re-sampling will occur, except for the April collection if resources allow.

Assessment of this water quality monitoring program will be the responsibility of the Program Coordinator, assisted by the QC Officer. They will train new volunteers and certify returning ones by observing them performing measurements and taking samples.

Field audits will consist of accompanying volunteer collectors to the field during the project. Any volunteer not performing to our specifications will be immediately retrained. Specifications include going to the correct sampling site, reading the thermometer accurately, and following proper procedures for sampling, as well as adequate sample storage (i.e., the volunteer has a frozen koolit as well as an ice-filled bag in the cooler). Any identified problems or shortcomings will be rectified promptly. If it is determined that identified problems may invalidate data, then those specific data will be discarded. The Program Coordinator and QC Officer will document problems identified and corrective actions taken during the field audits and will include these findings in the annual report.

If necessary, the spring collection may be repeated in May. Summer collections will not be repeated beyond the six collection dates.

21. Reports

Data that has passed preliminary QC analysis will be posted on the DRWA Web site and shared with the local media. A caveat will accompany these data explaining that they are preliminary and that a final data set and report will be available at the end of the season.

A final report will be written by the Project Coordinator and QC Officer and filed with DRWA documents as well as sent to any funders, local Boards, DEP, and other interested parties. The final report will include the table and graphs that were developed for the web site and media, and it will describe the program's goals, methods, quality control results, data interpretation, and recommendations.

22. Data Review, Validation, and Verification

Data will be reviewed for consistency and errors initially by the QC Officer, and then by the Technical Advisory Committee. This review includes a confirmation of the preliminary quality control results (field sheets and QC results on the lab sheets). It also includes a check of the season's overall results, looking in particular for outliers or extreme data always associated with a particular crew. The reviewers will determine what steps should be taken to rectify any problems encountered. For example, if errors are discovered that escaped detection at the preliminary QC analysis step, and these errors in some way alter the data that had been distributed, a correction will be posted on the web site and the corrected data with an explanation will be sent to the media.

23. Validation and Verification Methods

The following validation procedures will be established throughout the project: Equipment will be calibrated at the start of the season and checked before each collection; field blanks, field replicates, and blind samples will be submitted to the laboratory, which will also analyze lab duplicates, blanks, and positives; volunteers will be evaluated during the project; chain of custody will be maintained; field sheets and data entry will be checked by the QC Officer; descriptive statistics and graphs will be produced; and review by the technical advisory committee will occur at the end of the sampling season.

24. Reconciliation with DQOs

Whenever feasible, calculations and determinations for precision, completeness and accuracy will be made. Any corrective action will be implemented, noted and initialized by the QC Officer. If data quality

indicators do not meet the project=s specifications, then data may be discarded. Investigation of problems will take place and corrections will be documented. If equipment failure is found to be the cause, calibration and maintenance techniques will be reassessed. Any limitations on the data will be noted.

25. References

American Water Works Association 20th ed., 1998.

Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Water Pollution Control Federation, Washington, DC.

Massachusetts Department of Environmental Protection - 1995

Draft Deerfield River Water Quality Assessment Report (unpublished)

Massachusetts Department of Environmental Protection - 2004

Standard Operating Procedure. Analytical Quantification of Escherichia Coli and Enterococci Bacteria in Ambient Surface Waters Using Enzyme Substrate Test (Standard Methods 9223B). MA DEP, Worcester, MA.

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Deerfield River Watershed 2000 Water Quality Assessment Report.
MA DEP, Worcester, MA.

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Massachusetts Surface Water Quality Standards
(<http://www.state.ma.us/dep/bwp/iww/files/314cmr4.htm>)

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Massachusetts Year 2002 Integrated List of Waters
<http://www.state.ma.us/dep/brp/wm/files/303dpt2.pdf>

Massachusetts Water Watch Partnership, 2001

River Sampling Protocols. Rev. 0

R-1: Temperature <http://www.umass.edu/tei/mwwp/acrobat/R1temp.pdf>

R-2: Dissolved Oxygen <http://www.umass.edu/tei/mwwp/acrobat/R2DO.pdf>

R-3: Bacteria <http://www.umass.edu/tei/mwwp/acrobat/R3bacteria.pdf>

R-4: pH and Alkalinity <http://www.umass.edu/tei/mwwp/acrobat/R4pHalk.pdf>

Blaisdell House, UMass Amherst MA 01003

Appendix A: Sites

Deerfield River Monitoring Project Comprehensive Site List

Sites for 2005/2006 Baseline Water Chemistry

| Site Number | Site Name | Town |
|-------------|---|----------------|
| COR-010* | Cold River | Charlemont, MA |
| SOR | South River | Conway, MA |
| NOR-010 | North River, above Wastewater Treatment Plant | Colrain, MA |
| NOR-015 | North River, below Wastewater Treatment Plant | Colrain, MA |
| DER-025* | Deerfield River, Zoar Gap | Charlemont, MA |
| DER-021 | Deerfield River, below Charlemont WWTP | Charlemont, MA |
| DER-020 | Deerfield River, Old Willow | Charlemont, MA |
| DER-016* | Deerfield River, Gardner Falls (below WWTP) | Buckland, MA |
| DER-015* | Deerfield River, Stillwater | Deerfield, MA |
| DER-010* | Deerfield River, Rtes 5&10 bridge | Greenfield, MA |
| DER-100 | Main Branch Deerfield River | Readsboro, VT |
| WBD-050 | West Branch of Deerfield River | Readsboro, VT |

Sites for 2005 Bacteria Testing

| Site Number | Site Name | Town |
|-------------|----------------|----------------|
| NOR-010* | North River | Colrain, MA |
| NOR-002* | North River | Colrain, MA |
| NOR-003 | North River | Colrain, MA |
| NOR-004 | Foundry Brook | Colrain, MA |
| NOR-005 | North River | Colrain, MA |
| NOR-006 | North River | Colrain, MA |
| SOR-001* | South River | Conway, MA |
| SOR-002* | South River | Conway, MA |
| SOR-003* | South River | Conway, MA |
| SOR-004* | South River | Conway, MA |
| SOR-005* | South River | Conway, MA |
| SOR-006* | South River | Conway, MA |
| CHR-001* | Chickley River | Charlemont, MA |
| CHR-002* | Chickley River | Hawley, MA |
| CHR-003* | Chickley River | Hawley, MA |
| CHR-004* | Chickley River | Hawley, MA |
| CHR-005* | Chickley River | Hawley, MA |
| CHR-006* | Chickley River | Hawley, MA |

*These sites are located within the Category 5 of the Massachusetts Year 2002 Integrated List of Waters.

Appendix A: Sites

Sites for Baseline Water Chemistry

SITE LOCATION SHEET

Site Name: Cold River

Site Number: COR-010

Town: Charlemont

Nearest major highway: Rte 2

Road names and/or numbers connecting major highway to the site access road:

Coming from Shelburne on Rte 2, follow road through Charlemont Center, toward Mohawk State Forest. There is a pullout on the right hand side before the State Forest.

Specific directions from access road (named above) to exact location of sampling site:

Park in pullout.

Walk over guard rail, there is a trail that veers to the right, down to the river.

Follow trail. You'll see old pipes standing.

Take sample at bottom of trail.

SITE LOCATION SHEET

Site Name: Deerfield River -5&10 Bridge

Site Number: DER-010

Town: Deerfield

Nearest major highway: Rtes 5&10

Road names and/or numbers connecting major highway to the site access road:

From Deerfield, follow Rtes 5&10 north to Greenfield.

Just before bridge over Deerfield River, park on River Rd. on right.

Specific directions from access road (named above) to exact location of sampling site:

Cross River Rd., go over guard rail, walk down to river on worn path.

Walk under bridge, keep walking about 100 yards.

Take sample by large flat rocks.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: Deerfield River - Stillwater

Site Number: DER-015

Town: Deerfield

Nearest major highway: Rte 116

Road names and/or numbers connecting major highway to the site access road:

From Conway, travel south on Rte 116.

Near Mill River@turn left onto Lee Rd.

Then turn left onto West Road.

Stop and park at intersection with Upper Rd (just before bridge above Deerfield River)

Specific directions from access road (named above) to exact location of sampling site:

Follow path from parking area to river, where you will take sample.

SITE LOCATION SHEET

Site Name: Deerfield River, Below Shelburne WWTP: Gardner Falls

Site Number: DER-016

Town: Buckland

Nearest major highway: Rte 2

Road names and/or numbers connecting major highway to the site access road:

From Rte 2 in Shelburne, take left at Sweetheart Restaurant (S. Maple St).

Cross iron bridge and take left onto Conway Rd.

Take road on left to Gardner Falls hydroelectric station

Specific directions from access road (named above) to exact location of sampling site:

Take sample in flowing water below the dam.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: Deerfield River, Old Willow

Site Number: DER-020

Town: Charlemont

Nearest major highway: Rte 2

Road names and/or numbers connecting major highway to the site access road:

Travel west on Rte 2 from Shelburne.

Pass Stillwater Restaurant. There is a large pullout on the left. Go to the next pullout 100 yards farther, directly across the road from a red house. Park.

Specific directions from access road (named above) to exact location of sampling site:

Walk to river.

Take sample from rocks at upstream end or area.

SITE LOCATION SHEET

Site Name: Deerfield River, Below Charlemont WWTP

Site Number: DER-021

Town: Charlemont

Nearest major highway: Rte 2

Road names and/or numbers connecting major highway to the site access road:

In Charlemont center, take the driveway to the waste water treatment plant.

Park at end of road.

Specific directions from access road (named above) to exact location of sampling site:

Walk around left of garage., through a field to woods on other side of field.

About 30 feet into the woods, turn right toward river bank.

Sample at large boulder.

Note: Call landowner ahead of time for permission to walk on his land.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: North River, Above BBA (ex-Veratec)

Site Number: NOR-010

Town: Colrain

Nearest major highway: Rte 112

Road names and/or numbers connecting major highway to the site access road:

From Rte 2 in Shelburne, take Rte. 112 N to Colrain (about 3-4 miles)
Pass BBA Nonwoven factory on left.
Take a left on to Adamsville Road.
Cross the steel bridge.

Specific directions from access road (named above) to exact location of sampling site:

Park past bridge.
Downstream of bridge there is a trail. Walk down path to river. (beware of POISON IVY!)
Take sample at beach with large rocks. Big rock in middle of river.

SITE LOCATION SHEET

Site Name: North River, Below BBA (ex Veratec)

Site Number: NOR-015

Town: Colrain

Nearest major highway: Rte 112

Road names and/or numbers connecting major highway to the site access road:

From Rte 2 in Shelburne, take Rte. 112 N to Colrain (about 3-4 miles)
Just before BBA Nonwoven factory and steel bridge, turn right down dirt road (private driveway for 3 families).
At end of driveway (Crossman residence), park.

Specific directions from access road (named above) to exact location of sampling site:

Take private path to river for sampling

Appendix A: Sites

SITE LOCATION SHEET

Site Name: West Branch Deerfield River

Site Number: WER-050

Town: Readsboro

Nearest major highway: Rte 100

Road names and/or numbers connecting major highway to the site access road:

From center of Readsboro, travel north on Rte 100. Pass Fuel Oil on right and sawmill on left. The sample site is just upstream of the footbridge crossing the river on the left of the road.

Specific directions from access road (named above) to exact location of sampling site:

Walk down to footbridge. Sample site is just upstream, on east bank.

SITE LOCATION SHEET

Site Name: Main Branch Deerfield River

Site Number: DER-100

Town: Readsboro

Nearest major highway: Rte 100

Road names and/or numbers connecting major highway to the site access road:

From center of Readsboro, travel south on Rte 100. Park where road crosses river.

Specific directions from access road (named above) to exact location of sampling site:

Walk downstream on the west bank. Sample site is half-way between Rte 100 and confluence with West Branch.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: South River

Site Number: SOR

Town: Conway

Nearest major highway: Rte 116

Road names and/or numbers connecting major highway to the site access road:

From Rte 116 in Conway, take Shelburne Falls Rd.

Take a right onto Bardswell Ferry Rd.

Take a right on Reeds Bridge Rd.

Specific directions from access road (named above) to exact location of sampling site:

Take sample at bridge on Reeds Bridge Rd. (take sample upstream of bridge)

Appendix A: Sites

2005 Bacteria Testing Sites

SITE LOCATION SHEET

Site Name: North River, Above BBA (ex-Veratec)

Site Number: NOR-010

Town: Colrain

Nearest major highway: Rte 112

Road names and/or numbers connecting major highway to the site access road:

From Rte 2 in Shelburne, take Rte. 112 N to Colrain (about 3-4 miles)
Pass BBA Nonwoven factory on left.
Take a left on to Adamsville Road.
Cross the steel bridge.

Specific directions from access road (named above) to exact location of sampling site:

Park past bridge.
Downstream of bridge there is a trail. Walk down path to river. (beware of POISON IVY!)
Take sample at beach with large rocks. Big rock in middle of river.

SITE LOCATION SHEET

Site Name: Sunburn Beach

Site Number: NOR-002

Town: Colrain

Nearest major highway: Rte 2

Road names and/or numbers connecting major highway to the site access road:

From west of Shelburne Falls: Turn north off route 2 at the Big Indian Shop onto North River Road and go 1.0 miles to the iron bridge over the North River.

From Shelburne Falls: Follow signs to route 112 north (Main Street becomes 112 at the edge of town) and

go 1.2 miles from the start of route 112 north to the left turn over the iron bridge.

Specific directions from access road (named above) to exact location of sampling site:

Park at the bridge and follow the path down to the Deerfield River and turn left. Sample the North River before it empties into the Deerfield. The North River flows under the iron bridge.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: North River

Site Number: NOR-003

Town: Colrain

Nearest major highway: Rte 112

Road names and/or numbers connecting major highway to the site access road:

From west of Shelburne Falls: Turn north off route 2 at the Big Indian Shop onto North River Road and go 1.0 miles to the iron bridge over the North River. Turn left onto route 112 north and follow for approximately 2.5 miles to Adamsville Road in Griswoldville. Turn left onto Adamsville Road and follow for 0.7 miles to the bridge crossing over the North River. Turn right after crossing bridge into the cemetery driveway and park on your right next to the river.

From Shelburne Falls: Follow signs to route 112 north (Main Street becomes 112 at the edge of town) and go approximately 3.5 miles from the start of route 112 north to Adamsville Road at the north end of the Fiberweb company property. Turn left onto Adamsville Road and follow for 0.7 miles to the bridge crossing over the North River. Turn right after crossing bridge into the cemetery driveway and park on your right next to the river.

Specific directions from access road (named above) to exact location of sampling site:

Park by the river and collect sample from the downstream side of the bridge.

SITE LOCATION SHEET

Site Name: Foundry Brook

Site Number: NOR-004

Town: Colrain

Nearest major highway: Rte 112

Road names and/or numbers connecting major highway to the site access road:

From west of Shelburne Falls: Turn north off route 2 at the Big Indian Shop onto North River Road and go 1.0 miles to the iron bridge over the North River. Turn left onto route 112 north and follow for approximately 4.5 miles to Foundry Village Road. Turn left onto Foundry Village Road and follow for approximately 0.5 mile to Foundry Village.

From Shelburne Falls: Follow signs to route 112 north (Main Street becomes 112 at the edge of town) and go approximately 5.5 miles from the start of route 112 north to Foundry Village Road. Turn left onto Foundry Village Road and follow for approximately 0.5 mile to Foundry Village.

Specific directions from access road (named above) to exact location of sampling site:

Park on left side of the bridge crossing Foundry Brook in the center of the village. Walk to stream from the downstream side of the bridge.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: North River

Site Number: NOR-005

Town: Colrain

Nearest major highway: Rte 112

Road names and/or numbers connecting major highway to the site access road:

Follow Rte. 112 out of Colrain. Turn left into Colrain Central School immediately crossing first bridge outside of town (immediately outside of center of town).

Specific directions from access road (named above) to exact location of sampling site:

Pull into lower parking lot immediately adjacent to the North River. Collect samples at this location on the downriver side of the river.

SITE LOCATION SHEET

Site Name: North River

Site Number: NOR-006

Town: Colrain

Nearest major highway: Rte 112

Road names and/or numbers connecting major highway to the site access road:

Follow Rte 112 north out of Colrain for approximately 4 miles to iron bridge.

Specific directions from access road (named above) to exact location of sampling site:

Cross over bridge and park on right side. Collect sample from downriver side of bridge.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: South River

Site Number: SOR-001

Town: Conway

Nearest major highway: Rte 116

Road names and/or numbers connecting major highway to the site access road:

From Rte 116 in Conway, take Shelburne Falls Rd.
Take a right onto Bardswell Ferry Rd.
Take a right onto Station Road.
Follow Station Road for approximately 1.0 mile to the terminus.

Specific directions from access road (named above) to exact location of sampling site:

Follow trail down to confluence of South River and Deerfield River. Collect sample from the South River about 50-100 m upstream of confluence.

SITE LOCATION SHEET

Site Name: South River

Site Number: SOR-002 (Previously SOR)

Town: Conway

Nearest major highway: Rte 116

Road names and/or numbers connecting major highway to the site access road:

From Rte 116 in Conway, take Shelburne Falls Rd.
Take a right onto Bardswell Ferry Rd.
Take a right on Reeds Bridge Rd.

Specific directions from access road (named above) to exact location of sampling site:

Take sample at bridge on Reeds Bridge Rd. (take sample upstream of bridge)

Appendix A: Sites

SITE LOCATION SHEET

Site Name: Pumpkin Hollow Brook

Site Number: SOR-003

Town: Conway

Nearest major highway: Rte 116

Road names and/or numbers connecting major highway to the site access road:

Locate the Rte 116 bridge over the South River in the center of Conway (yellow house on east side of bridge).

Specific directions from access road (named above) to exact location of sampling site:

Park on the east side of the bridge between bridge and yellow house. Walk along bridge down to stream bank. Walk upstream for ~30 m to confluence of Pumpkin Hollow Brook with the South River (facing upriver, Pumpkin Hollow Brook is on the left). Collect sample SOR-003 from Pumpkin Hollow Brook.

SITE LOCATION SHEET

Site Name: South River

Site Number: SOR-004

Town: Conway

Nearest major highway: Rte 116

Road names and/or numbers connecting major highway to the site access road:

Locate Rte 116 bridge over South River in the center of Conway (yellow house on east side of bridge).

Specific directions from access road (named above) to exact location of sampling site:

Park on the east side of the bridge between bridge and yellow house. Walk along bridge down to stream bank. Walk upstream for ~30 m to confluence of Pumpkin Hollow Brook with the South River (facing upriver, the South River is on the right). Collect sample SOR-004 from the South River about 5-10 m upstream of the confluence with Pumpkin Hollow Brook.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: South River

Site Number: SOR-005

Town: Conway

Nearest major highway: Rte 116

Road names and/or numbers connecting major highway to the site access road:

From Rte 116 in Conway, turn south onto Maple Street. Park on wide shoulder on the right side immediately following the intersection of Rte 116 and Maple.

Specific directions from access road (named above) to exact location of sampling site:

Walk the embankment (be careful, it's fairly steep) down to the river and sample on the upriver side of the Rte 116 bridge.

SITE LOCATION SHEET

Site Name: South River

Site Number: SOR-006

Town: Conway

Nearest major highway: Rte 116

Road names and/or numbers connecting major highway to the site access road:

From Conway, drive approximately 0.5 mile west on Rte. 116 to the first bridge crossing over the South River.

Specific directions from access road (named above) to exact location of sampling site:

Park on the west side of the bridge, on the left (facing west). Walk down to the stream along the guard rail and bridge and sample on the downstream side of bridge.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: Chickley River

Site Number: CHR-001

Town: Charlemont

Nearest major highway: Rte 2

Road names and/or numbers connecting major highway to the site access road:

Turn south onto Rte 8A in Charlemont. Follow 8A south for approximately 1.0 mile (RR tracks will be on your right). Turn right onto Tower Road at the first southward (left) bend in 8A. Follow Tower Road for approximately 200 m to the bridge crossing the Chickley River.

Specific directions from access road (named above) to exact location of sampling site:

Park on the left (facing west) on the west side of the bridge and walk down to the river next to the bridge. Collect the sample from the upriver side of the bridge.

SITE LOCATION SHEET

Site Name: Chickley River

Site Number: CHR-002

Town: Hawley

Nearest major highway: Rte 2/Rte 8A (site along Rte 8A)

Road names and/or numbers connecting major highway to the site access road:

Turn south onto Rte 8A from Rte 2 in Charlemont. Follow Rte 8 for approximately 2 miles to the first bridge crossing over the South River.

Specific directions from access road (named above) to exact location of sampling site:

Park on the left (facing south) on the north side of the bridge and walk down to the river. Sample on the upriver side of the bridge.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: Chickley River

Site Number: CHR-003

Town: Hawley

Nearest major highway: Rte 2/Rte 8A.

Road names and/or numbers connecting major highway to the site access road:

Turn south onto Rte 8A from Rte 2 in Charlemont. Follow Rte 8 for approximately 3.5 miles to Puddin Hollow Road. Turn left onto Puddin Hollow Road. Cross the bridge over the Chickley River (bridge being rebuilt in winter 2004/2005) and make another left onto Middle Road. Follow Middle Road for approximately 70 yds and park on the left side of the road along narrow forested strip through which the South River can be seen.

Specific directions from access road (named above) to exact location of sampling site:

Walk 30 yards through the forested strip to the river and collect sample immediately upstream of the confluence with Mill Brook.

SITE LOCATION SHEET

Site Name: Chickley River

Site Number: CHR-004

Town: Hawley

Nearest major highway: Rt. 8A

Road names and/or numbers connecting major highway to the site access road:

Turn south onto Rte 8A from Rte 2 in Charlemont. Follow Rte 8A for approximately 5.5 miles to Forge Hill Road. Cross bridge crossing the Chickley River as soon as soon as turn onto Forge Hill is made and park on the right on the far side of the bridge.

Specific directions from access road (named above) to exact location of sampling site:

Walk down to river on the upriver side of the bridge and collect sample.

Appendix A: Sites

SITE LOCATION SHEET

Site Name: Chickley River

Site Number: CHR-005

Town: Hawley

Nearest major highway: Rte 8A

Road names and/or numbers connecting major highway to the site access road:

Turn south onto Rte 8A from Rte 2 in Charlemont. Follow Rte 8A for approximately 7.0 miles to the small town of West Hawley. The Chickley River makes an abrupt turn to the west and crosses under Rte 8A in West Hawley

Specific directions from access road (named above) to exact location of sampling site:

Park on the left side (facing south) before crossing the bridge and walk down to the stream on the downstream side of the bridge.

SITE LOCATION SHEET

Site Name: Chickley River

Site Number: CHR-006

Town: Hawley

Nearest major highway: Turn south onto Rte 8A from Rte 2 in Charlemont. Follow Rte 8A for approximately 7.0 miles to the small town of West Hawley. The Chickley River makes an abrupt turn to the west and crosses under Rte 8A in West Hawley. Turn right onto Savoy Road (marked by a small wooden street sign) just past the bridge and follow for approximately 1 mile to the second bridge spanning the Chickley River on this road.

Road names and/or numbers connecting major highway to the site access road:

Park by bridge and collect sample from the upstream side.

Specific directions from access road (named above) to exact location of sampling site:

Appendix B: Job Descriptions

Deerfield River Monitoring Program JOB DESCRIPTION LABORATORY DIRECTOR

Laboratory Director is responsible for:

- Maintaining lab and equipment in clean and proper working order
- Maintaining a safe working environment in the lab
- Training and supervising DRWA volunteers in the lab
- Signing Chain-of-Custody Forms as they are brought in the lab
- Analyzing samples for pH, Alkalinity and using Standard Methods 4500B, 2320B, Analysis date will be April 24, 2005
- Analyzing samples for E. coli bacteria using Standard Methods 9223 (B) within 8 hours of sample collection (samples will arrive at lab within 6 hours of collection).
- 18-22 hours after the beginning of incubation, remove trays from incubators and read number colonies on each. Write results on lab sheet.
- For all determinations, run quality control samples as prescribed by project
- Completing lab data sheets for each analysis day
- Sending Chain-of-Custody Forms and lab data sheets to Project Coordinator as soon as possible after completion of each analysis day
- Contacting QC Officer as soon as possible if he/she must discontinue their participation in the project before the end of sampling season

Appendix B: Job Descriptions

Deerfield River Monitoring Program

JOB DESCRIPTION

Project Coordinator

Week before sampling:

- email all the volunteers to make sure they are available and all set for the next collection
- if they don't reply in a couple of days, call them on the phone
- make sure the dirty labware has been sterilized by Greenfield WWTP and someone is bringing it back to our lab (it can be brought by the person bringing the Greenfield samples to Shelburne on Sunday, or we can ask Bob Walker to get everything on his way to SF as he is coming from Montague)
- make sure someone will take the positive sample at the Green River at Mead Street.
- make sure someone will take a field blank (done with 2 bottles of buffer solution)
- make sure someone will take a field duplicate (rotate the field blank and field duplicate among volunteers)
- print or copy 1 lab sheet and enough field sheets for all volunteers

Day of sampling:

- Go out with a sampling volunteer for field audit
- Arrive at lab before 2pm to set up
- Check field sheets to make sure they are complete and error-free. (Call volunteers if you have questions)
- Check Chain-of-Custody Forms to make sure all samples are accounted for and that the holding times were respected
- Lab bench must be cleaned with disinfectant (in silver spray bottle)
- Fill out lab notebook with required information in anticipation of sample delivery.
- Samples should be kept cool until you are ready to start analyses
- Turn on the Quanti-Tray sealer to allow preheating (usually takes about 10 minutes)
- Prepare the counter work area with the necessary dilution bottles, reagents, trays, pipettes, etc.
 - Make sure to leave sufficient space to prepare the samples for incubation.
- Check incubator(s) to ensure they're turned on (that's something Ted does 24 hours ahead)
- Set on low bench (or table) behind lab bench: Sample bottles, in the same order that they appear on the lab sheet)
- Mark the lab notebook with the date, sample ID, 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. Identify the dilution scheme for sample at this point.
- Dilute samples, as needed and pour them into new, sterile 120-ml vessels up the 100-ml line (see

- DWM SOPs for additional steps required for dilution of “hot” samples.
- Add entire contents of a Colilert reagent packet into each 100-ml sample. Cap and invert the bottle >25 times until all reagent is dissolved.
- Dispense each mixed sample into a Quanti-Tray and place each Quanti-Tray into the rubber tray carrier of the Quanti-Tray Sealer.
- Seal each Quanti-Tray by moving the rubber tray into the sealer.
- Prepare the quality control samples using DWM SOPs.
- Incubate all prepared Colilert Quanti-Trays in the incubator(s) at $35\pm 0.5^{\circ}\text{C}$ for 18-22 hours. Record the incubation start time in the notebook.

When all done with samples:

- Empty and rinse sample bottles
- Put all the dirty labware and sample bottles back in blue tub
- Thoroughly clean the work area. Place all “bio-waste” items (i.e. disposable items that been in contact with sample water, such as pipettes, sample bottles, dilution bottles, etc.) in plastic bio-waste bags.

Bring the tub and bio-waste bags to the Greenfield Water Pollution Control Facility.

Monday:

- After 18-22 hours, remove the Colilert trays from the incubator and record the time in the notebook.
- Examine each Quanti-Tray under a hand-held 365 nm UV light. Compare the level of fluorescence in each well to the comparator, count the number of fluorescent wells, and record in the lab notebook.
- Write results on lab sheet
- Enter and calculate results in Excel spreadsheet
- Check quality control (blanks are zero, positive has colonies, duplicates are reasonably close). If all's well, send the results to the volunteers and Marie-Francoise. At some point the QC officer will check the data entry.
- Place used Colilert trays in “bio-waste” bags, tie securely, and temporarily store in the lab in a designated location.
- Gather the field sheets, Chain-of-Custody Forms, and lab sheets and file in cabinet

Between Samplings:

- Make sure that clean bottles, field sheets and Chain-of-Custody Forms are available at the drop-off point (can coordinate with MF and Ted)

Appendix C: Field Sampling Instructions (adapted from MassWWP DEP-approved SOPs) Deerfield River Monitoring Program

Field Sampling Instructions - April

You should have:

- Directions to sample site
- Cooler and Frozen Koolit
- Plastic sample bottle
- Data Sheet
- First aid kit and Pair of latex gloves
- Thermometer

Temperature:

Air: hang thermometer in shade. Wait 2 minutes or more, then read temperature to nearest degree and record on data sheet

Water: place thermometer in a shady area of the river (attach to rock or hold on to string). Read temperature after 2 minutes or more and record on data sheet.

Be sure to record thermometer number on data sheet.

pH and alkalinity sample

1. Use the 500 ml plastic sample bottle.
2. Carefully wade into the stream, avoid stirring up bottom sediments and walking upstream. Stand so that you are facing upstream. Note: if stream is not wadeable, do not sample.
3. Remove the cap of the sample bottle. Submerge the bottle and fill partway. Cap loosely and shake to rinse. Empty bottle downstream and repeat process twice.
4. To fill sample, plunge bottle mouth first into the water to mid-arm depth, turn upside right, let it fill and cap under water.
5. Be sure sample has the proper site code label, place in a Ziploc bag, put it in the cooler with ice or the Koolit.

Fill in all the information on the data sheet: your name, date and time, weather and activity observations.

~ Keep all rubbish (empty pillow containers, etc) in your cooler ~

~ Use latex gloves if you suspect the water to be contaminated ~

Appendix C: Field Sampling Instructions (adapted from MassWWP DEP-approved SOPs)

Deerfield River Monitoring Program

Instructions for Field Sampling - Bacteria samples

You should have:

- Cooler
- Two new 1-gallon Ziploc bags (one empty, the other half-filled with ice)
- Thermometer
- Sample Bottle
- Data Sheet
- Directions to Sample Site
- Pair of Latex Gloves
- First Aid Kit
- If sampling more than once today, rinse water and towel

Temperature:

1. Hang thermometer in the shade. Wait 2 minutes or more, then read temperature to nearest degree and write on data sheet.
2. Place thermometer in a shady area of the river (attach to rock or hold on to the string). Read temperature after 2 minutes or more and write on data sheet. Record thermometer number.

Weather and Use:

Observe the weather, the current use of the river at this time, and the physical characteristics of the water, and fill out the data sheet

E. coli Bacteria Sample:

1. Use only sterile bottles obtained from DRWA. Be sure sample bottle is labeled with proper with sample code. Record site code on data sheet.
2. Wash hands before starting sample run - if you sample in an area that may have high fecal levels rinse hands before handling the next sample bottle. Be careful not to touch your hands to yourself before you have cleaned them in order to avoid coming into contact with pathogens. If you are sampling from waters known to be contaminated with sewage, wear latex gloves to protect yourself.
3. Wade in the water. Note: if stream is not wadeable, do not sample. Always sample upstream of your body and point the bottle opening upstream. Keep the bottle closed until just ready to sample. Do not rinse the bottle. Remove cap and hold it to the side. Hold the bottle near its base in the hand and plunge it, neck downward, below the surface. Turn bottle until neck points slightly upward and mouth is directed toward the current. If there is no current, create a current artificially by pushing bottle forward horizontally in a direction away from the hand. Be sure not to collect any sediment you may have suspended by walking on the streambed. Also avoid collecting any water from the surface layer of the water as this is uncharacteristic of the water flowing through. Replace cap leaving 1/2" of air space in bottle. It is important to leave the air space so that the sample will constantly mix while transported. 4. Place sample bottle in the empty Ziploc bag and "zip" it closed. Place this Ziploc

back containing the sample into the Ziploc bag ½ filled with ice, zip closed and place in the cooler.

Appendix D: Forms

Deerfield River Monitoring Program

FIELD DATA SHEET

SITE NAME: _____ SITE NUMBER: _____

DATE: _____ TIME: _____

VOLUNTEERS: _____

WEATHER OBSERVATIONS (check appropriate boxes):

Weather now: Clear Partly Cloudy Overcast Cloudy Drizzle Raining Other:

Air Temperature: _____°C Time: _____

Weather past three (3) days: _____

If it has rained in past three days, estimate amount of precipitation: _____ inches

Was the rain light, moderate, or heavy?

WATER OBSERVATIONS (check appropriate boxes):

Water level (on reference object compared to low-flow water level):

Water color: clear cloudy muddy green brown tea colored iridescent
other: _____

Water odor: none rotten egg gasoline sewage detergent fishy
other: _____

Water Temperature: _____°C Thermometer Number: _____

Any floating debris? (describe): _____

RIVER USE OBSERVATIONS (check appropriate boxes):

swimming wading boating fishing picnic hanging out other: _____

Any wildlife? Describe: _____

SAMPLES TAKEN (check appropriate boxes):

bacteria – time of sample collection: _____ pH/alkalinity – time of sample collection: _____

Comments: _____

Appendix D: Forms

Deerfield River Monitoring Program CHAIN OF CUSTODY FORM

Fill in the Date and Time, and Sign in the Released/Received Columns. Please record temperature inside cooler at the time the samples are released.*

| SAMPLE ID | RELEASED BY COLLECTOR: | RECEIVED BY LAB ATTENDENT: | RELEASED BY LAB ATTENDENT: | RECEIVED BY ANALYST: |
|-----------|------------------------|----------------------------|----------------------------|----------------------|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

*** Temperature inside the cooler should be measured by placing the thermometer inside the bag with the sample upon arrival at the laboratory, resealing and re-enclosing in the Ziploc bag with ice, closing the cooler for two minutes, and then taking a reading.**

Appendix D: Forms. Lab Data Sheet

LAB ANALYSTS: _____

DATE: _____

| Sample ID | Begin Time | pH | Digits to 4.5 | Digits to 4.2 | Alkalinity (mg/l) |
|-----------|------------|----|---------------|---------------|-------------------|
| April QC | | | | | |
| COR-010 | | | | | |
| DER-010 | | | | | |
| DER-015 | | | | | |
| DER-016 | | | | | |
| DER-020 | | | | | |
| DER-021 | | | | | |
| DER-025 | | | | | |
| DER-100 | | | | | |
| NOR-010 | | | | | |
| NOR-015 | | | | | |
| SOR | | | | | |
| WBD-050 | | | | | |
| Field Rep | | | | | |
| Lab Dup | | | | | |
| April QC | | | | | |

| | | | | |
|--|--|--|--|--|
| | | | | |
| | | | | |

* The start date and start time will typically be the same as the previous end date and end time.

Appendix D: Forms. DRWA Colilert E. coli Analysis Lab Sheet

| LAB NAME: | | | DATE: | | | | | | |
|---------------|---------------------|------------------|----------|----------------------------------|---------------------------------|----------------|--------------------------|--------------------------|-------------|
| LAB ANALYSTS: | | | | | | | | | |
| Site | Analysis start time | Media lot number | Dilution | Incubation Start Time & Temp (C) | Incubation Start End & Temp (C) | UV Reader Name | # of large fluores wells | # of small fluores wells | E. coli MPN |
| Lab Blank | | | | | | | | | |
| NOR-001 | | | | | | | | | |
| NOR-002 | | | | | | | | | |
| NOR-003 | | | | | | | | | |
| NOR-004 | | | | | | | | | |
| NOR-005 | | | | | | | | | |
| NOR-006 | | | | | | | | | |
| SOR-001 | | | | | | | | | |
| SOR-002 | | | | | | | | | |
| SOR-003 | | | | | | | | | |
| SOR-004 | | | | | | | | | |
| SOR-005 | | | | | | | | | |
| SOR-006 | | | | | | | | | |
| CHR-001 | | | | | | | | | |
| CHR-002 | | | | | | | | | |
| CHR-003 | | | | | | | | | |
| CHR-004 | | | | | | | | | |
| CHR-005 | | | | | | | | | |
| CHR-006 | | | | | | | | | |
| Lab Blind | | | | | | | | | |
| FD: | | | | | | | | | |
| FD: | | | | | | | | | |
| FD: | | | | | | | | | |
| LD: | | | | | | | | | |
| LD: | | | | | | | | | |
| LD: | | | | | | | | | |
| Lab Positive | | | | | | | | | |
| Field Blank | | | | | | | | | |

FD = field duplicate, LD = lab duplicate

Appendix E: DRWA Colilert SOP

Deerfield River Watershed Association

STANDARD OPERATING PROCEDURE

FOR

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

Prepared By:

(Michael B. Cole and Marie Jose Iken)

Monitoring Coordinator:

(Michael Cole)

QA/QC Analyst:

(Marie-Francoise Walk)

Project/Laboratory Coordinator

(Marie Jose Iken)

May 20, 2005

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1.0 SCOPE & APPLICATION

This SOP describes the detection and enumeration of total coliform, *Escherichia coli* (*E-coli*) 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” method is employed to detect and enumerate both total coliform and *E-coli* bacteria. This defined enzyme substrate method was 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 DRWA (and its volunteers) and analyzed by DRWA staff at the DRWA laboratory in Shelburne Falls, MA. The main target analyte for DRWA bacteria assessments is *E. coli*.

2.0 SUMMARY OF LABORATORY METHODS

Because pathogenic (disease-causing) organisms are difficult to isolate and identify, fecal coliform bacteria are used to indicate the potential presence of pathogens in water. Coliform 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

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 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 method detection limit (MDL) for total coliform and *E. coli* using Colilert 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 $35.0^{\circ} \pm 0.5^{\circ}\text{C}$ 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.

3.0 INTERFERENCES

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

Colilert:

3.1 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.

3.2 *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.

3.3 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*.

3.4 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.

3.5 Incubation beyond 28 hours may yield a false positive Colilert result, due to cessation of suppression of non-coliform heterotrophic bacteria.

3.6 Presence of free chlorine in the sample may result in a transient blue color upon addition of the Colilert reagent. To avoid this, bacteria samples collected in waters suspected of containing chlorine should be taken using sample bottles containing sodium thiosulfate.

4.0 LAB SAFETY

4.1 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.

- 4.2 The sealer device is a burn hazard if not properly used and maintained. See 11.1.
- 4.3 Material Safety Data Sheets (MSDS) for all chemicals used in this SOP shall be stored in the laboratory
- 4.4 Adherence to the waste storage and disposal procedures in Section 14 shall be maintained at all times.

5.0 LABORATORY EQUIPMENT AND SUPPLIES

The following lab equipment and supplies are needed to employ the multi-well tray Colilert method.

Colilert and Colilert-18:

- 5.1 50% Bleach or 70% Alcohol and paper towels
- 5.2 Disposable plastic gloves and “hot” gloves (incubator and sealer)
- 5.3 Lab notebook
- 5.4 Lab fridge and storage areas for reagents and supplies
- 5.5 Model 2X Quanti-tray sealer
- 5.6 Quanti-tray 2000 97-well sample trays [**Store in cabinet**]
- 5.7 Quanti-tray 2000 tray rubber inserts (minimum 2)
- 5.8 Colilert reagent [**Store in dark at 2-30 deg. C; use within 12 months of manufacture**]
- 5.9 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.
- 5.10 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**]
- 5.11 Sterile pipettes
- 5.12 Sterile water

- 5.13 Positive (*E. coli*, *Klebsiella pneumoniae*) and negative (*Pseudomonas*, other) control cultures (for QC) in Quanti-cult QC kit **[Store at 2-8 deg. C until use; use within 18 months of manufacture]**
- 5.14 Two-shelf, bench-top, mechanical-convection incubator (5-65 deg. C; +/- 1.5 deg. C uniformity; +/- 0.5 deg. C stability) with thermometer.
- 5.15 NIST-certified (or traceable) thermometer
- 5.16 365 nm UV lamp (6 watt)
- 5.17 UV viewing cabinet
- 5.18 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]**
- 5.19 MPN tables
- 5.20 Three-clock timer

6.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1 Field Equipment and Supplies

- Cooler
- Two new 1-gallon Ziploc bags (one empty, the other half-filled with ice)
- Thermometer
- IDEXX 120-mL Sample Bottle
- Data Sheet
- Directions to Sample Site
- Pair of Latex Gloves
- First Aid Kit
- If sampling more than once today, rinse water and towel

- 6.2 Upon arrival to the sample site, hang the thermometer in the shade. Wait 2 minutes or more, then read temperature to nearest degree and write on data sheet. Place thermometer in a shady area of the river (attach to rock or hold on to the string). Read temperature after 2 minutes or more and write on data sheet. Record thermometer number. Observe the weather, the current use of the river at this time, and the physical characteristics of the water, and fill out the data sheet

6.3 Collecting the E. coli Bacteria Sample

Use only sterile IDEXX bottles obtained from DRWA. Be sure sample bottle is labeled with proper with sample code. Record site code on data sheet. Wash hands before starting sample run - if you sample in an area that may have high fecal levels rinse hands before handling the next sample bottle. Be careful not to touch your hands to yourself before you have cleaned them in order to avoid coming into contact with pathogens. If you are sampling from waters known to be contaminated with sewage, wear latex gloves to protect yourself.

Wade in the water. Note: if stream is not wadeable, do not sample. Always sample upstream of your body and point the bottle opening upstream. Keep the bottle closed until just ready to sample. Do not rinse the bottle. Remove cap and hold it to the side. Hold the bottle near its base in the hand and plunge it, neck downward, below the surface. Turn bottle until neck points slightly upward and mouth is directed toward the

current. If there is no current, create a current artificially by pushing bottle forward horizontally in a direction away from the hand. Be sure not to collect any sediment you may have suspended by walking on the streambed. Also avoid collecting any water from the surface layer of the water as this is uncharacteristic of the water flowing through. Replace cap leaving 1/2" of air space in bottle. It is important to leave the air space so that the sample will constantly mix while transported. 4. Place sample bottle in the empty Ziploc bag and "zip" it closed. Place this Ziploc bag containing the sample into the Ziploc bag ½ filled with ice, zip closed and place in the cooler.

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.

7.0 LABORATORY QUALITY CONTROL

Colilert:

7.1 Media Lot-Specific Quality Control: DRWA's main intent in running the following QC samples is to verify that the Colilert media does not provide false positives or false negatives for *E.coli*. Each lot of media is tested using *Quanti-cult* (see Appendix D) cultures as follows:

- Blank: sterile buffer dilution water
- Negative Culture Control: sterile buffer dilution water inoculated with non-fluorescent *Pseudomonas* sp. (i.e., total coliform & *E. coli* negative).
- Positive Culture Controls: sterile buffer dilution water inoculated with *E. coli* (i.e., total coliform & *E. coli* positive) and sterile buffer dilution water inoculated with *Klebsiella* (i.e., total coliform positive & *E. coli*, fluorescence, negative).
-

7.2 Sample Batch-Specific Quality Control Samples: With each new batch of samples run, DRWA will perform the following QC sample analysis:

- 2 Field Duplicates
- 1 field blank
- 1 Lab blank
- 1 Positive Control
- 1 Lab Duplicate

7.3 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.

7.4 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.

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

8.0 CALIBRATION AND STANDARDIZATION

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

8.2 Incubator: The incubator has an internal temperature monitoring device and maintains a temperature of $35^{\circ} \pm 0.5^{\circ}\text{C}$. 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 should be known to ensure that the specified incubation period occurs. When in use, incubator temperature will be recorded at least twice per day with readings separated by at least 4 hours.

9.0 DETAILED LAB PROCEDURE (assumes reagent lot has been tested and passed; see 7.1)

Colilert:

- 9.1 Coordinate and plan analytical work with DRWA volunteer field staff regarding the quantity and timing of sample delivery. For each DRWA survey, one primary Colilert analyst and one backup analyst will be pre-identified and pre-scheduled to run the survey samples at the DRWA laboratory in Shelburne Falls. If the primary analyst is not available for any reason, the backup analyst must be available to run them.
- 9.2 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.
- 9.3 Prepare all work areas by cleaning with lab disinfectant.
- 9.4 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.
- 9.5 Check incubator to ensure it is ON, maintaining the proper temperature and has sufficient space for sample trays when they are ready.
- 9.6 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.
- 9.7 Prepare counter work area with the necessary dilution bottles, reagents, trays, pipettes, etc., leaving sufficient space to prepare samples for incubation.
- 9.8 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.

- 9.9 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.
- 9.10 Use a Sharpie pen to mark all sample bottles with "HI" suspected of having high counts.
- 9.11 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.
- 9.12 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 DRWA QC Analyst.
- 9.13 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 and deliberate so that each sample is put into its corresponding labeled tray.
- 9.14 **NO DILUTION SAMPLES:** If no dilutions are to be performed on the sample, mix sample (as above). If over the 100-ml 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. 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 volumes errors are minimized (<2.5 %), this level of measurement accuracy and precision is currently deemed acceptable.)
- 9.15 **10 ML DILUTION SAMPLES:** (*for known or suspected "HOT" samples to be quantified at a level > 2420 org./100 ml.*). 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 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 of using pre-filled dilution bottles containing 90 mls. of phosphate buffer is under investigation).
- 9.16 **100 ML DILUTION 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 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.
- 9.17 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 of the reagent is dissolved. Let any foam settle.

- 9.18 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. Allow any foam to settle.
- 9.19 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.
- 9.20 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.)
- 9.21 Prepare **LAB QC SAMPLE(S)** as follows: 1) **Negative Control.** Prepare a lab blank by pouring 100 mls of sterile water into an IDEXX vessel and then adding reagent (as above). Cap/tray/seal/incubate.
- 9.22 Prepare **LAB BLANK** as follows (minimum one per batch): Pour 100 mls. of buffer dilution water into an IDEXX vessel, add reagent, cap and mix, pour into tray, seal and incubate.
- 9.23 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.
- 9.24 Incubate all prepared Colilert Quanti-Trays in the incubator at **35 ±0.5 °C** for 24 - 28 hours. Avoid any opening and closing the incubator during use in order to maintain stable proper temperature.
- 9.25 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. Tie the bag(s) when 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.
- 9.26 After 24 hours, remove the Colilert trays from the incubator. Record the time in the lab notebook. 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.
- 9.27 To determine the *E. coli* result, expose each tray to 365 nm UV light by placing them one-by-one into the UV light viewing cabinet. Count and record the number of fluorescent wells, large and small respectively, in the lab notebook. Use comparator to verify fluorescence. Use the MPN table (Appendix a) to record the confirmed E-coli value as MPN/100 mL sample in the lab notebook.
- 9.28 If the sample color is questionable in comparison to the color comparator after 24 hours of incubation, incubate the sample tray for an additional 4 hours (total of 28 hours for Colilert), and recheck 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, 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.

- 9.29 Colilert results are definitive at 24-28. Any positives for both total coliform and *E.coli* observed before the minimum time and negatives observed after the maximum time are also valid.
- 9.30 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.
- 9.31 Place used Colilert trays in “bio-waste” bags, tie securely and temporarily store in the lab in the designated, secured location.

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

Table 1: Summary of General Dilution 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 |

** These procedures assume that the IDEXX vessel 100 ml line can be used as an accurate (+/- 2.5%) measure.*

10.0 PREVENTATIVE MAINTENANCE

- 10.1 Sealer: Trained personnel (only) must inspect, clean and maintain the sealer according to the manufacturer's recommendations (Appendix C).
- 11.2 Rubber Inserts: Autoclave or clean with isopropyl alcohol or bleach.

11.0 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.

Data Validation: DRWA's QA/QC Analyst shall perform a comprehensive data validation in relation to data quality objectives (DQOs). The reviewer shall discuss any problems, concerns and issues with the lab analyst as needed, and if all data appear OK, approve the MPN results.

Table 2: Colilert reactions

| Reaction | Result |
|--|---|
| Yellow | Total Coliform positive |
| No color or indeterminant | Negative (censored) |
| Fluorescent (yellow) | <i>E. coli</i> positive |
| Yellow color intensity at or near the comparator color | Inconclusive; re-incubate an additional 4 hours |

Table 4: 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 5: QC Validation Criteria

| Quality Control | Frequency | Acceptance Criteria | Corrective Action |
|--|--------------|--|---|
| Positive Culture Control (<i>E. coli</i>) | 5% | Positive | Qualify or censor data |
| Negative Control (sterile water) | 5% | Negative | Qualify or censor data |
| 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 M qualifiers (exceeded holding time or method of preservation not followed) |

12.0 METHOD PERFORMANCE

12.1 The method detection limit for Colilert has been determined to be a MPN of 1 colony-forming unit (CFU) per sample volume or dilution tested. The DRWA 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.

13.0 POLLUTION PREVENTION

13.1 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.

14.0 WASTE MANAGEMENT

15.1 All materials that have come in contact with samples (trays, bottles, pipettes, etc.) shall be double-bagged as “bio-waste” and temporarily stored in 32 gallon, plastic cans in a designated, secured location at the DRWA laboratory, prior to transport to the Greenfield Water Pollution Control Facility for proper disposal (autoclave sterilization, plastics recycling and solid waste disposal).

15.0 REFERENCES

- 15.1 *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
- 16.7

17.0 APPENDICES

- Appendix A: MPN Tables for Colilert (by reference)
- Appendix B: MSDS Sheets (by reference; 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

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

