BUREAU OF WATER

South Carolina Department of Health and Environmental Control

Synopsis: Development and Adoption of the *Escherichia coli* Freshwater Water Quality Standard

Technical Report No. 015-2020

dhec

S.C. Department of Health and Environmental Control

Synopsis: Development and Adoption of the *Escherichia coli* Freshwater Water Quality Standard

Technical Report No. 015-2020

Prepared By:
David Chestnut and Bryan Rabon
SCDHEC – Bureau of Water, Aquatic Science Programs

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Introduction

This document is a synopsis of the activities undertaken that led the Department (DHEC) to the adoption of *Escherichia coli* (*E. coli*) as the new freshwater fecal pathogen indicator in *Regulation 61-68 Water Classifications and Standards*, replacing fecal coliform bacteria. This report is primarily a collection of the documentation presented to the US Environmental Protection Agency (EPA) Region 4 to support the change to R.61-68, with additional original material to connect the various attachments.

Background

Historically, DHEC had used fecal coliform bacteria as the bacterial indicator for the protection of all waters for recreational use relative to the presence of fecal material from warm-blooded animals. In the 1986, EPA criteria document *EPA440/5-84-002 Ambient Water Quality Criteria for Bacteria – 1986,* EPA documented that other bacterial indicators were more closely correlated with the occurrence of illness at freshwater lake beaches due to fecal matter and were, therefore, preferable over fecal coliform bacteria. For freshwaters, EPA recommended either *E. coli* or *Enterococcus* species and published criteria values for each.

While none of the indicator bacteria may be directly responsible for illness, they serve as indicators that other disease-causing organisms (pathogens) may be present. In almost all cases of water-borne illnesses, the pathogens come from inadequately treated waste of humans or other warm-blooded animals. *Enterococcus* and *E. coli* are more specific to sewage from fecal sources than the more general fecal coliform bacteria group.

In the mid to late 2000s, a discussion began between DHEC and a group of NPDES permittees about changing the bacteria indicator in R.61-68 and NPDES permits from fecal coliform bacteria to either *Enterococcus* or *E. coli*.

The Clean Water Act, as amended by the Beaches Environmental Assessment and Coastal Health (BEACH) Act in 2000, required the EPA to conduct studies associated with pathogens and human health and to publish new or revised Water Quality Criteria recommendations for the pathogens and pathogen indicators based on those studies (EPA 2012).

So, in the late 2000s, EPA began an effort to collect new data and update the recreational water quality criteria. In 2007, EPA Office of Water, Office or Research and Development, published a *Criteria Development Plan & Schedule for Recreational Water Quality Criteria* (EPA, 2007, 823-R-08-003) to guide this process. As part of this plan, the *Analysis and Synthesis of Data and Peer Review of Results and Analyses* was scheduled for January 2011-March 2011.

During this same timeframe, an EPA Workgroup was formed that consisted of staff-level representatives from throughout EPA, including representatives from offices that manage various Clean Water Act Programs such as the NPDES Permitting Program, the TMDL Program, and the Beach Monitoring and Advisory Program; the Office of Research and Development; the Office of Enforcement and Compliance Assurance; and, the Office of General Counsel.

Mr. Joel Hansel, EPA Region 4's Standards Coordinator, and EPA standards coordinator for South Carolina, was serving on the national EPA Workgroup. DHEC maintained close communication with Mr. Hansel to stay informed of the thinking and direction of the EPA Workgroup.

With all of the discussion of potential changes to criteria and indicator recommendations, DHEC decided it would be beneficial to collect the Department's own data on the all three: fecal coliform bacteria, *E. coli*, and *Enterococcus*. Attachment 1, Evaluation of Alternative Freshwater Pathogen Indicators, is the original QAPP for this effort. The actual raw data are contained in *Synopsis: Development and adoption of the Escherichia coli Freshwater Water Quality Standard, Volume II – Raw Data*. The raw data are also available electronically from the Water Quality Portal, https://www.waterqualitydata.us/ under Organization ID 21SC60WQ_WQX. The goal of this investigative effort was to determine whether the use of either *Enterococcus* or *E. coli* was reasonable and thereby develop meaningful and realistic protection for primary contact recreational uses of freshwaters of the State. Primary contact recreation typically includes activities where immersion and ingestion are likely and there is a high degree of bodily contact with the water, such as swimming, bathing, surfing, water skiing, tubing, skin diving, water play by children, or similar water-contact activities.

During the planning process, the most current bacteria water quality was the EPA *Ambient Water Quality Criteria for Bacteria – 1986*. In this criteria document, there were multiple Single Sample Maximum (SSM) Allowable Density values for *E. coli* and enterococci based on different confidence limits from the EPA datasets. These different values were equated to different intensities of use for full body contact recreation. So at this stage of the project process, DHEC anticipated the possible need for multiple SSM values. This is reflected in some of the language of the QAPP (Attachment 1).

When the final 2012 Recreational Water Quality Criteria were released, the acceptance of the multiple SSM values had been scrapped. Mr. Hansel informed DHEC that any standards change based on that concept would be denied. There was also no support from the stakeholder group for multiple values. So although such ideas may be referred to in some of the attachments, they were not pursed to the end of the project's final submission of proposed standards changes to EPA.

Project Description

DHEC undertook a statewide effort to collect weekly data for a comparison of all three indicators, fecal coliform bacteria, *Enterococcus*, and *E. coli*, in freshwater locations across South Carolina.

The data collected were compared to the published criteria developed by EPA and were used to examine reasonable criteria for South Carolina reservoirs and flowing freshwater resources.

Input from the DHEC Regional monitoring staff was used to help identify potential sampling sites from the routine ambient monitoring network for inclusion in this effort. Considerations for selecting existing sites included a representation of:

- Different ecoregions
- All freshwater waterbody types, i.e. streams and lakes
- A wide range of stream sizes, multiple stream orders
- Different stream types, e.g. blackwater, swamps, mountain streams, etc.
- Sites on §303(d) list for fecal coliform bacteria, extreme and borderline

- Sites that currently meet fecal standards, extreme and borderline
- Neighborhoods on septic tanks
- Suburban sewered
- Urban runoff with no NPDES discharge
- Rural agricultural settings around pastureland or livestock operations
- Wooded, primarily undeveloped with little potential for human input

This resulted in an initial list of 74 locations (list of sites Attachments 1 and 3, map of sites Attachments 1 and 4).

All sites selected were to be sampled weekly for one year beginning January 1, 2009;these samples analyzed for all three parameters: fecal coliform bacteria using Membrane Filter, Enterococci by Enterolert, and *E. coli* by Colilert (Quantitray). However, by the third week of sampling it was necessary to drop one of the monitoring sites, SV-291, Clarks Hill Reservoir at US 378, due to dangerous conditions to staff associated with very heavy traffic on the bridge. This left 73 locations for the remainder of the study period.

As data began coming in it, became apparent that the *E. coli* results were producing many values in the range of 300 to 800 Most Probably Number (MPN) of Colony Forming Units (CFU) per 100 ml, where the Quantitray most probable number (MPN) table has less discriminatory power, with several values reported as >2419.6 CFU/100 ml The distribution of possible values (concentrations) from the Quantitray MPN table shows much better resolution between values within a range near the lower values and a wider spread of numbers towards the higher end of the possible range. IDEXX, the manufacturer of the Quantitray, recommends dilution to get within the MPN range needed with the least dilution necessary being preferable.

With a 1 to 4 dilution [e.g., 25 milliliters (ml) sample, 75 ml deionized (DI) water] much better resolution was achieved in the range of values important for picking a standard within EPA-acceptable ranges. While this did somewhat reduce resolution for very low values (i.e., <40 CFU/100 ml) this range was less than any of the possible criteria for *E. coli*. An addendum was made to the original QAPP (Attachment 2) to incorporate the noted 1 to 4 dilution factor for all samples collected beginning the week of June 15, 2019.

In order to explore relationships between the different indicator concentrations, it was essential to have quantifiable values for the indicators being compared. Values reported as estimated or greater than reporting limits were not of use in assessing the statistical relationships between indicators. Considering the impact and importance of setting a new statewide pathogen standard to be protective of human health that will impact a wide range of Bureau of Water (BOW) activities and the regulated community, it was imperative that there be adequate resolution within the results to evaluate the different available criteria options.

Results and Data Evaluation

Weekly sampling for three pathogen indicators: fecal coliform bacteria, *Escherichia coli*, and *Enterococcus*, was conducted at 73 locations during 2009. From January 5, 2009 through December 30, 2009, there were a total of 10, 922 analyses conducted of which: 3,717 were for fecal coliform bacteria; 3,602 for *Escherichia coli*; and, 3,603 for *Enterococcus*.

Statistical analyses of the resulting data (Attachment 5) were performed using R (2009, R Development Core Team, http://www.R-project.org). The statistical analyses excluded censored data as discussed below.

For microbial analyses, dilution of the sample is often necessary to obtain concentrations within a quantifiable range. With different dilution factors, this can result in a variety of different *Less Than* or *Greater Than*, or *Estimated* values when the resulting value is not within the quantifiable range. Censored data are those where an individual number is not known, but it is known that the value is less than or greater than a threshold value or the value is estimated where a precise value could not be measured but only estimated as a possible value (*Less Than, Greater Than*, or *Estimated*).

Correlation or regression of data where censored data are present can alter the variation from what would have occurred in nature and introduce error in the estimates of the relationships between the variables being compared. Therefore, all of the analytical analyses presented and discussed below are based on only the uncensored data with all values reported as *Greater Than* , *Less Than* , or *Estimated* excluded.

Bacteria commonly reproduce by asexual cell division called *binary fission*, whereby a single bacterial cell divides into two identical cells. Under favorable conditions, this results in logarithmic population growth, a very rapid form of growth where the population initially doubles, then quadruples, then grows to 8 times the original number, then 16 times, 32 times, etc. Arithmetic evaluations of such populations are often improved by transforming the raw data to logarithmic values prior to statistical analyses. Therefore, all of the analyses were conducted using both raw values and log base 10 transformed data.

Correlations were evaluated using Pearson's Product-Moment Correlations for fecal coliform bacteria vs. *Escherichia coli*, and fecal coliform bacteria vs. *Enterococcus* (Table 1).

To examine relationships between the different indicators tested, regressions were performed on the same data sets. Because the measures of all the indicators compared have associated measurement errors, simple linear regression is not suitable. A more appropriate regression method in such situations is the orthogonal least squares regression. To illustrate this difference, in Figures 1-4 a simple linear regression line is also included as the red dashed line.

The correlation analyses for this study (Table 1) indicated that of the evaluated alternative pathogen indicators, *E. coli* was most closely correlated with the historic fecal coliform bacteria indicator. This was also supported by the regression analyses. Figures 1 and 2 illustrate better relationships between the current fecal coliform indicator and *E. coli* than between fecal coliform and *Enterococcus* (Figures. 3 and 4).

A series of stakeholder meetings were held throughout the data evaluation and proposed standard Single Sample Maximum/Statistical Threshold Value development (May 26, 2011; 1une 30, 2011; July 21, 2011).

Table 1. Pearson Product-Moment Correlation Results, Uncensored Data Only

Comparison	Correlation Coefficient	Lower 95th Percent Confidence Interval	Upper 95th Percent Confidence Interval
Fecal coliform vs. *E. coli*	0.8102	0.7967	0.8230
Log10 Fecal coliform vs. Log10 *E. coli*	0.8765	0.8673	0.8851
Fecal coliform vs. *Enterococcus*	0.3826	0.3488	0.4154
Log10 Fecal coliform vs. Log10 *Enterococcus*	0.6930	0.6722	0.7128

Discussion

EPA's 2012 Recreational Water Quality Criteria states Scientific advancements in microbiological, statistical, and epidemiological methods have demonstrated that culturable enterococci and E. coli are better indicators of fecal contamination than the previously used general indicators, total coliforms and fecal coliforms. Fecal contamination in recreational waters is associated with an increased risk of gastrointestinal (GI) illness and less often identified respiratory illness. As such, fecal contamination and its indicators are considered "pathogen indicators," as defined by §502(23) of the CWA.

EPA also stated that *E. coli* is the most fecal-specific of the coliform indicators.

The 2012, EPA criteria redefined what constituted an *illness* from the definitions previously used. The new definition used a more comprehensive definition of GI illness, referred to as NEEAR-GI (NGI). Because NGI is broader than HCGI (i.e., NGI includes diarrhea without the requirement of fever), more illness cases were reported and associated with aquatic recreation in the NEEAR study using the NGI definition of illness, at the same level of water quality observed using the previous illness definition (i.e., HCGI).

EPA's 1986 criteria recommendations correspond to a level of water quality that is associated with an estimated illness rate expressed in terms of the number of highly credible gastrointestinal illnesses (HCGI) per 1,000 primary contact recreators. EPA's National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) study used a more comprehensive definition of GI illness, referred to as NEEAR-GI (NGI). Because NGI is broader than the older definition of highly credible gastrointestinal illness HCGI (i.e., NGI includes diarrhea without the requirement of fever), more illness cases were reported and associated with aquatic recreation in the NEEAR study using the NGI definition of illness, at the same level of water quality observed using the previous illness definition (i.e., HCGI).

The new criteria have two components, a geometric mean (GM) and statistical threshold value (STV). The STV approximates the 90th percentile of the water quality distribution.

Figure 1. Fecal Coliform vs. E. Coli
Updated Analysis 6-28-2011

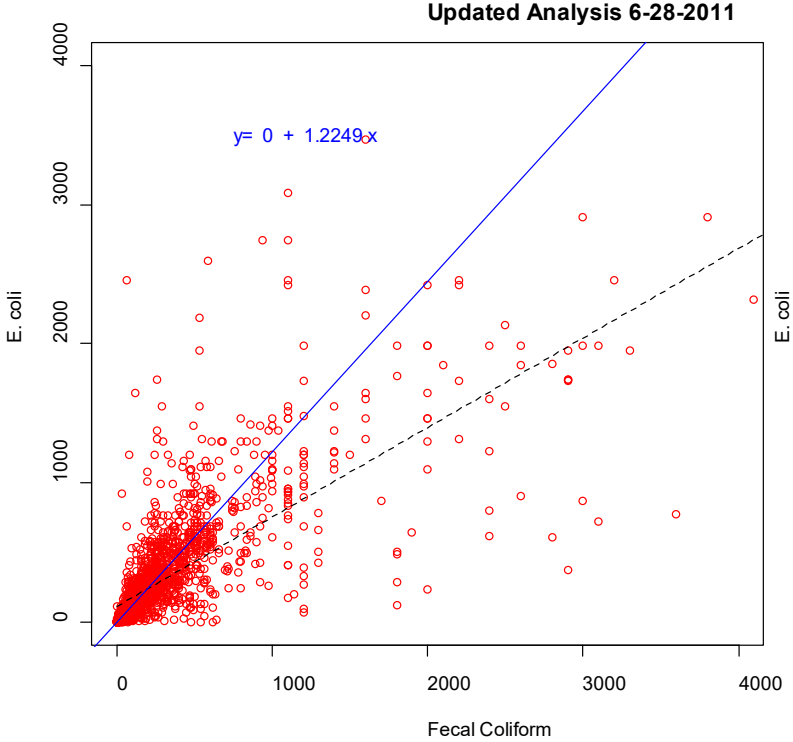

Figure 2. Log10 Fecal Coliform vs.
Updated Analysis 6-28-2011

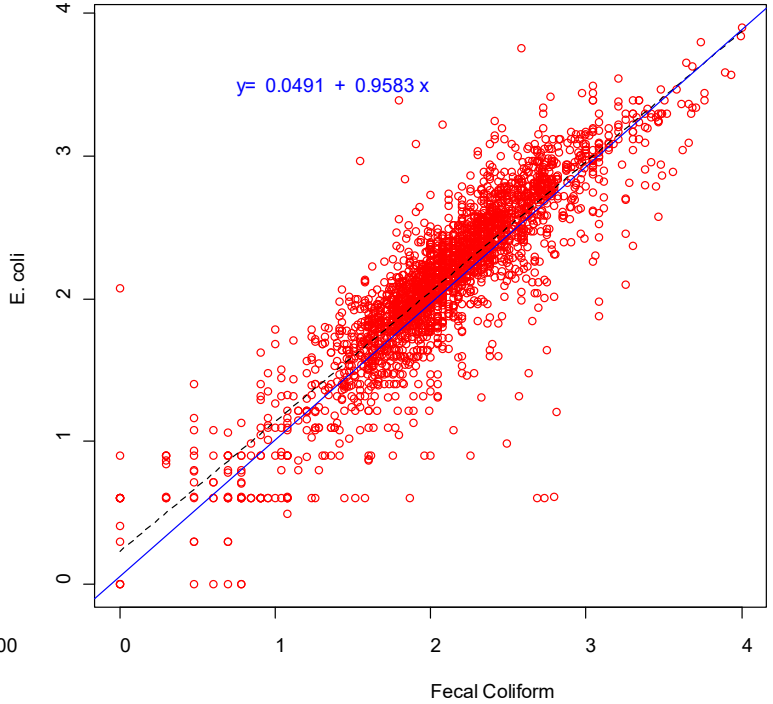

Figure 3. Fecal Coliform vs Enteroc

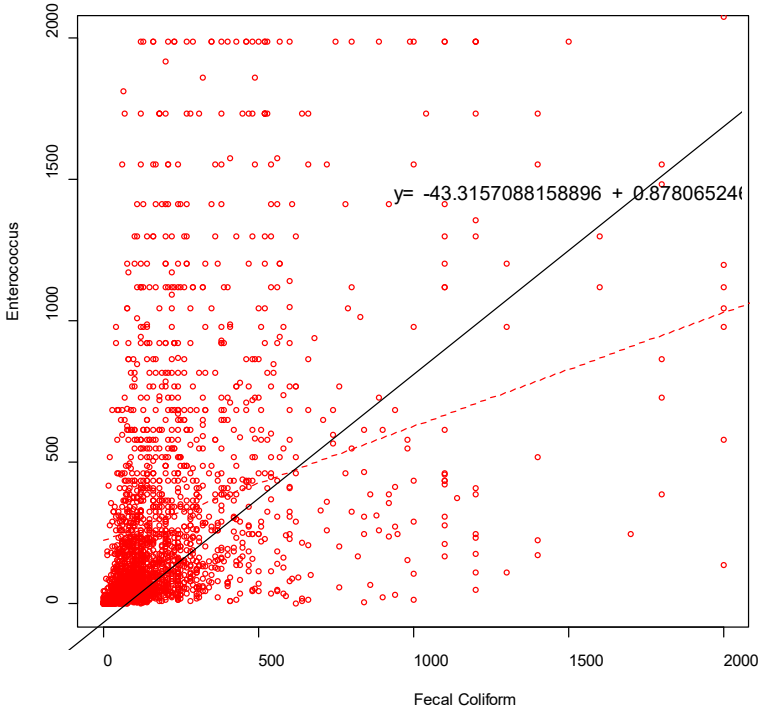

Figure 4. Log10 Fecal Coliform vs.

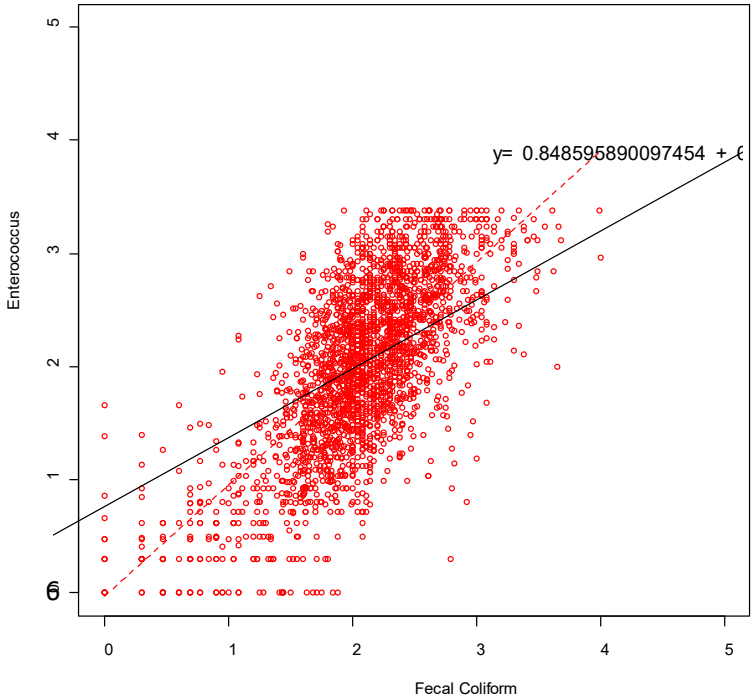

Table 2. Recommended 2012 RWQC

Criteria Elements	Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators			Estimated Illness Rate (NGI): 32 per 1,000 primary contact recreators	
	Magnitude			Magnitude	
Indicator	GM (cfu/100 mL)[a]	STV (cfu/100 mL)[a]		GM (cfu/100 mL)[a]	STV (cfu/100 mL)[a]
Enterococci – marine and fresh	35	130	OR	30	110
OR					
E. coli – fresh	126	410		100	320
Duration and Frequency: The waterbody GM should not be greater than the selected GM magnitude in any 30-day interval. There should not be greater than a ten percent excursion frequency of the selected STV magnitude in the same 30-day interval.					

[a]EPA recommends using EPA Method 1600 (U.S. EPA, 2002a) to measure culturable enterococci, or another equivalent method that measures culturable enterococci and using EPA Method 1603 (U.S. EPA, 2002b) to measure culturable E. coli, or any other equivalent method that measures culturable E. coli; cfu = colony forming units

Derivation of a Single Sample Maximum Allowable Density/Statistical Threshold Value

The R script used is part of Attachment 5.

An *E. coli* concentration equivalent to a fecal coliform bacteria density of 400 per 100 ml (the current maximum for fecal coliform bacteria not to be exceeded by more than 10% of the total samples during any 30 day period) was calculated using the regression formula from Figure 2 as below.

$Log10(Y)=0.0491+0.9583Log10(x)$
$Log10(E.\ coli)=0.0491+0.9583Log10(Fecal\ Coliform)$
$Log10(E.\ coli)=0.0491+0.9583Log10(400)$
$Log10(E.\ coli) =2.5426$
$E.\ coli =10^{2.5426}$
$E.\ coli =348.8$
E. coli 349= Fecal Coliform 400

The single sample maximum allowable *E. coli* density of 349 per 100 ml falls between the 2012 Statistical Threshold Values for freshwater *E. coli* (Table 2). This value had a great deal of consensus support from the stakeholder community that participated in the discussions leading to the pathogen indicator change.

EPA Submittal

Proposed revisions to South Carolina Regulation 61-68, Water Classifications and Standards, and Regulation 61-69, Classified Waters, were submitted for the EPA review by letter dated July 2, 2012 from Mr. W. Marshall Taylor, Jr., General Counsel for the South Carolina DHEC to Ms. Gwendolyn Keyes Fleming, Regional Administrator of the EPA's Region 4 Office. The State's request for review included a certification by the DHEC's General Counsel that the revisions were duly adopted pursuant to State law.

These revisions were duly promulgated by the Department's Board and became effective for purposes of State law upon publication in the State Register on June 22, 2012. These revisions included the removal of the fecal coliform indicator and adoption of the *E. coli* indicator for recreational uses in Freshwaters of the State and other editorial revisions.

Attachments 1-5 were included in the submission to EPA's Region 4 Office, along with the raw data contained in Synopsis: Development and adoption of the Escherichia coli Freshwater Water Quality Standard, Volume II – Raw Data.

DHEC received approval of the removal of the fecal coliform indicator and adoption of the *E. coli* indicator for recreational uses in Freshwaters of the State in a letter dated February 28, 2013, from Mr. Jim Giattina, then EPA Region 4 Director of the Water Protection Division, to then SCDHEC Bureau of Water Chief Mr. David Wilson (Attachment 6).

Citations

EPA. 1986. Ambient Water Quality Criteria for Bacteria – 1986. EPA440/5-84-4402.

EPA. 2012. Recreational Water Quality Criteria. Office of Water 820-F-12-058.

Attachment 1. Evaluation of Alternative Freshwater Pathogen Indicators QAPP

A. Project Management

A1. Title Page

Evaluation of Alternative Freshwater Pathogen Indicators

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Prepared By:

David Chestnut
Bureau of Water
South Carolina Department of Health & Environmental Control

December 2008

Lead Organization: South Carolina Department of Health & Environmental Control
2600 Bull Street
Columbia, SC 29201

Project Manager: _____ Date: 12/29/08
David Chestnut, Senior Scientist, Bureau of Water

Project Director: _____ Date: 12-29-08
David Baize, Assistant Bureau Chief, Bureau of Water

SCDHEC QA Officer: _____ Date: 12/18/2008
Nydia Burdick, Manager, Office of Quality Assurance

ARESD Director: _____ Date: 12/18/2008
Sandra Flemming

State Microbiologist: _____ Date: 12/22/2008
Karen Suber, Manager, Microbiology Section, ARESD

A2. Table of Contents

A3. Distribution List

Name	Region/Office	Phone	Fax	E-mail
David Baize	Bureau of Water	803-898-4272	803-898-3795	BAIZEDG@dhec.sc.gov
Nydia Burdick	Office of Quality Assurance, Columbia	803-896-0862	803-896-0980	BURDICNF@dhec.sc.gov
Karen Suber	Microbiology Section ARESD, Columbia	803-896-0834	803-896-0868	SUBERKE@dhec.sc.gov
Susan Turner	EQC Region 2 Director, Greenville	864-241-1090	864-241-1092	TURNERSG@dhec.sc.gov
Tamika Watt	Region 2 Lab Manager, Greenville	864-241-1090	864-241-1092	WATTTR@dhec.sc.gov
Harry Mathis	EQC Region 3 Director, Columbia	803-896-0620	803-896-0617	MATHISHL@dhec.sc.gov
Jimmy Owens	EQC Region 4 Director, Florence	843-661-4825	843-661-4858	OWENSJE@dhec.sc.gov
Leigh Plummer	Region 4 Lab Manager, Florence	843-661-4825	843-661-4858	PLUMMELW@dhec.sc.gov
Jennifer Hughes	EQC Region 5 Director, Aiken	803-641-7670	803-641-7675	HUGHESJR@dhec.sc.gov
Tabitha Corley	Region 5 Lab Manager, Aiken	803-641-7670	803-641-7675	CORLEYTG@dhec.sc.gov
Christine Sanford-Coker	EQC Region 7 Director, Charleston	843-953-0150	843-953-0151	SANFORCC@dhec.sc.gov
Sharon Gilbert	Region 7 Lab Manager, Charleston	843-953-0150	843-953-0151	GILBERSM@dhec.sc.gov
Russell Berry	EQC Region 8 Director, Beaufort	843-846-1030	843-846-0604	BERRYRE@dhec.sc.gov
Penny Cornett	Region 8 Lab Manager, Beaufort	843-846-1030	843-846-0604	CORNETPA@dhec.sc.gov

A4. Project/Task Organization

David Chestnut is the Project Manager and the Data Validator. Mr. Chestnut will also develop and maintain the QAPP.

Bill McDermott is the Data Verifier for this project.

Nydia Burdick will review and approve the QAPP.

The Analytical and Radiological Environmental Services Division (ARESD) Microbiology Section and the EQC Regional Laboratories of Regions 2, 4, 5, 7, and 8 will be responsible for the analyses of all samples.

The Regional Directors for Regions 2, 3, 4, 5, 7, and 8 will be responsible for overseeing the coordination of sample collection with Regional staff.

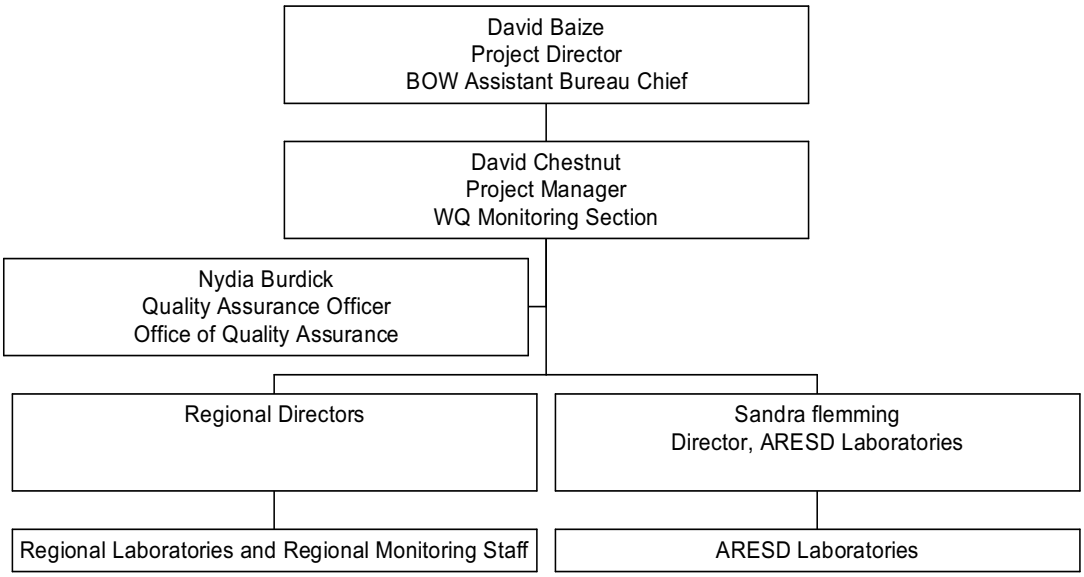


Figure 1. Project Organization Chart

A5. Problem Definition/Background

For more than twenty-five years, DHEC has monitored the quality of our surface waters. Presently in freshwater DHEC uses fecal coliform as the bacterial indicator of recreational water quality, and all waters of the state must meet swimming standards.

Changes in science and technology now enable us to consider the use of other indicators to ensure that we are suitably protecting the citizens of South Carolina. Based on the occurrence of gastroenteritis (upset stomach, nausea, diarrhea), in 1986 the US Environmental Protection Agency documented that other bacterial indicators were more closely correlated with the occurrence of illness at freshwater lake beaches during the summer and were therefore preferable over fecal coliform bacteria.

For freshwaters EPA is recommending either *Escherichia coli* (*E. coli*) or *Enterococcus*, and published criteria values for each based on the summer lake data. While none of the indicator bacteria may be directly responsible for illness, they serve as indicators that other disease-causing organisms (pathogens) may be present.

In almost all cases of water-borne illnesses the pathogens come from inadequately treated waste of humans or other warm-blooded animals. *Enterococcus* and *E. coli* are more specific to sewage and fecal sources than the more general fecal coliform bacteria group.

EPA is strongly encouraging states to replace fecal coliform bacteria in their standards with one of these alternatives.

The South Carolina Department of Health and Environmental Control is beginning an evaluation of our freshwater water quality standards, classifications, and uses as they relate to recreation. The goal of this investigative effort is to determine whether the use of either *Enterococcus* or *E. coli* is reasonable and thereby develop meaningful and realistic protection for recreational uses of freshwaters of the State. Recreational uses include activities with frequent full body immersion (swimming, water skiing, other whole body water-contact sports) and those with a low chance of total body immersion or ingestion of water (wading, boating, fishing). This project will also determine if the different criteria values incorporated in the EPA criteria based on the frequency of use for full body contact recreation are reasonable for application in South Carolina freshwaters.

Changing the bacteria indicator would require changes in State Standards, and possibly water classifications, and in addition to changing what is sampled for monitoring activities, would also have impacts on NPDES permitting, stormwater permitting, water quality assessment, and the §303(d) list of impaired waters.

A6. Project/Task Description

DHEC is undertaking a statewide effort to collect weekly data for a comparison of all three indicators, fecal coliform bacteria, *Enterococcus*, and *E. coli*, in freshwater locations across South Carolina.

The data collected will be compared to the published criteria developed from EPA's freshwater lake data and will be used to examine reasonable criteria for South Carolina reservoirs and flowing freshwater resources.

The intent of this change is to identify the most heavily used swimming areas in our state, focus our limited resources on providing the greatest level of protection for these areas, and to determine reasonable protection for areas less frequently used for swimming.

Input from the Regional monitoring staff was used to help identify potential sampling sites from the routine ambient monitoring network for inclusion in this effort. Considerations for selecting existing sites included a representation of:

- Different ecoregions
- All freshwater waterbody types, i.e. streams and lakes
- A wide range of stream sizes, multiple stream orders
- Different stream types, e.g. blackwater, swamps, mountain streams, etc.
- Sites on §303(d) list for fecals, extreme and borderline
- Sites that currently meet fecal standards, extreme and borderline
- Neighborhoods on septic tanks

- Suburban sewered
- Urban runoff with no NPDES discharge
- Rural agricultural settings around pastureland or livestock operations
- Wooded, primarily undeveloped with little potential for human input

All sites selected will be sampled weekly for one year and these sample analyzed for all three parameters: fecal coliform bacteria using Membrane Filter, Enterococci by Enterolert, and *E. Coli* by Colilert (Quantitray). The list of the sites selected for weekly sampling is provided in Appendix A, and a map of the statewide distribution of these sites is included in Appendix B.

The following table lists main Project activities and predicted milestone dates.

Activity	Name/Group	Anticipated Start Date	Anticipated Completion Date	Comments
Sampling	Regions 2, 3, 4, 5, 7, and 8 staff	1/1/2009	12/31/2009	Weekly sampling at selected sites
Data Verification	ARESD and Regions 2, 3, 4, 5, 7, and 8 laboratory staff	As samples analyses are completed	1/15/2010	
Data Validation	Bill McDermott	As data verification is complete	3/1/2010	
Final Database	Type to be determined in the near future.	As data is acquired, once the database is set up.	3/15/2010	

A7. Data Quality Objectives (DQOs) and Data Quality Indicators (DQIs)

Method	DQI	QC or other Activity used to determine Performance	Measurement Performance
Membrane Filtration	Sensitivity	MDL	1 CFU/100 ml
Membrane Filtration	Accuracy	PT Samples	Pass
Membrane Filtration	Accuracy	Count verification by a second analyst	Counts within 10%
Membrane Filtration	Accuracy	Number of Colonies	Adhering to the 20-60 range of colonies when counting.
Enterolert	Sensitivity	MDL	1 CFU/100 ml
Enterolert	Accuracy	PT Samples	Pass
Enterolert	Accuracy	Positive/Negative /Sterility Controls	Media works properly.*
Colilert(Quantitray)	Sensitivity	MDL	1 CFU/100 ml
Colilert(Quantitray)	Accuracy	PT Samples	Pass
Colilert(Quantitray)	Accuracy	Positive/Negative Controls	Media works properly*.

QC Criteria

* Positive controls for Colilert change the media color to yellow and fluoresce under UV light. Positive controls for Enterolert fluoresce under UV light. Negative controls do no fluoresce, but for Colilert some negative controls will change the media to yellow but do no fluoresce.

DQO Process

1. The USEPA has published new criteria for alternative pathogen indicators, *Escherichia coli* and *Enterococcus*. EPA recommends replacing fecal coliform bacteria criteria with either of these two pathogen indicators. These indicators are more strongly correlated with the occurrence of illness related to the ingestion of water during recreational activities than fecal coliform bacteria. However, the State of SC has not evaluated these indicators in the waters of SC.

2. The new EPA criteria for these two indicators allows some flexibility in selecting specific values based on the frequency of use of a waterbody for full body contact recreation and/or different acceptable illness rates. This study is of the investigative type which will provide data that can be compared with fecal coliform bacteria results and evaluate both of the alternative indicators with the possibility of adopting one as a new State water quality standard.

3. Samples will be collected weekly for one year in 500 ml bottles at the sites selected and split for analysis of fecal coliform bacteria, *E. coli*, and *Enterococcus*. This will allow evaluation of seasonal variability at individual sites and across the state.

4. The selected sites are listed in Appendix A and mapped in Appendix B. Samples will be collected weekly at each site by staff from the appropriate Region or Central Office Water Quality Monitoring or Aquatic Biology Sections staff. It is intended that sampling will begin in January, 2009 and continue throughout a full year.

5. Since this is an investigative study to determine whether it is appropriate or reasonable to change State Standards, a decision rule is not applicable. How the data will be interpreted and the final decision on which criteria to use and what, if any, changes to the State standards will be proposed will be determined with discussion with the USEPA Region 4 staff. Consideration may be given to waterbody size, season, ecoregion, landscape setting, frequency of use as a swimming resource, etc.

6. Specifying the limits on decision error is not applicable since this is an investigative type study. In addition microbiological samples do not lend themselves to precision measurements because of the tendency for bacterial colonies to clump and thus not be evenly distributed throughout the sample. The study does include Membrane Filtration for fecal coliform bacteria which can be directly compared to historical data. Accuracy can be assured by staff following the same methods to collect and analyze the samples. Moreover, the inclusion of a large number of sites of various types will also help to ensure the data as a whole are unbiased and representative of the State of SC various waterbodies.

7. The number and distribution of sites included in this study took into consideration which Regional laboratories could participate, distance from the laboratory and holding times, predominant land use surrounding the sites, stream size, bridge access to lake sites, suggestions from Regional monitoring staff, and input from Central Office staff. Weekly sampling frequency for one year should allow for seasonal differences to be examined, as well as a variety of flow and meteorological conditions.

At the time of sample collection, monitoring personnel will make on-site observations for evidence of recent rainfall and include codes on the laboratory analysis request sheets (see Section B9).

Completeness as a data quality indicator is important; however, samples will not be recollected or replaced. It is believe that the number of samples will more than be enough to make comparisons and determine what indicator should be used.

A8. Training and Certification

All monitoring will be conducted by DHEC staff and will require no special training. All sample collection activities will follow the Quality Management Plan For the South Carolina Department Of Health and Environmental Control (2008) and EQC Environmental Investigations Standard Operating Procedures and Quality Assurance Manual (SCDHEC, 2006).

Analyses will be conducted by the EQC regional labs and ARESD which will be certified for all methods.

A9. Documentation and Records

Documentation and records protocols for the collected samples and field observations will follow the EISOP (EQC Environmental Investigations Standard Operating Procedures and Quality Assurance Manual (SCDHEC, 2006)) and the requirements as given in the ARESD Microbiology Laboratory Manual. Data will be kept in LIMs, handled and backed up as per ARESD SOPs.

There will be no report per se. Data will be transferred electronically from the laboratories to the Program. Initially data will be transferred following established ARESD and BOW procedures. The goal is to move toward downloading the data in electronic format directly from LIMs.

Mr. Chestnut will be responsible for distributing the QAPP. These will go out in courier mail to those listed in the Distribution List.

If a weekly sample is not collected at any site the reason should be documented in the Field Logbook and David Chestnut, Bill McDermott, or Bryan Rabon, of the Water Quality Monitoring Section in the Bureau of Water should be notified.

B. Measurement/Data Acquisition

B1. Sampling Process/Experimental Design

Study sites will be collected at least once each week for one year beginning in January 2009. All samples will be collected by Regional staff or Central Office Water Quality Monitoring or Aquatic Biology Sections staff. The exact days on which the samples are collected is not critical and is left to Regional Laboratories to determine the day of the week which will work best for them.

The selected sites are listed in Appendix A and mapped in Appendix B, including the Regional office responsible for sampling each site. All but two of the locations have been sampled by the Regional staff in the past as part of the Routine Ambient Surface Water Monitoring Program. This allows data collected as part of the study to be compared to historical data to assure that results from the study are similar to historical observations.

Sites were selected to represent different ecoregions of the state, different stream and lake sizes in different ecoregions, and different predominant land use characteristics. Regional staff should document the recreational use (if any) for the area in which the site is located.

While the intent of this study is to collect 52 samples a year from each site, it is recognized that there will be some loss of data. Due to limited resources samples will not be recollected. Moreover, the study has enough sites of various types with which to draw conclusion even if some data are lost.

As previously discussed, variability is inherent in enumerating bacteria. Other sources of variability include rainfall. It is well known that a rain event will increase the number of bacteria detected. Lack of rain, such as in a drought, will also affect this study and may make it impossible to collect samples. If a site becomes dry or otherwise inaccessible David Chestnut, Bill McDermott, or Bryan Rabon, of the Water Quality Monitoring Section in the Bureau of Water should be notified by e-mail.

B2. Sampling Methods

All monitoring will be conducted by DHEC staff following the EQC Environmental Investigations Standard Operating Procedures and Quality Assurance Manual (SCDHEC, 2006), see Appendix C.

Samples will be collected as grab samples. All three analyses, fecal coliform bacteria, *Enterococcus*, and *E. coli*, must come from the same bottle. Fisher 500 mL bottles (fisher # 02-911-934) and autoclavable caps (53mm-400 caps--fisher # 13-757-164) will be used for this study.

If problems occur in the field, Mr. Chestnut or Mr. McDermott should be contacted.

B3. Sample Handling and Custody

Sample handling and custody will follow EQC Environmental Investigations Standard Operating Procedures and Quality Assurance Manual (SCDHEC, 2006). See Appendices C and E. For this study all analyses will have travel times of 6 hours (Appendix D) and laboratory set up time of 2 hours.

B4. Analytical Methods

General laboratory methods will follow Laboratory Procedures Manual for Environmental Microbiology-- Analytical Services (SCDHEC, 1998). Specifically, fecal coliform bacteria will follow Standard Methods 9222D – Membrane Filter Procedure, *Enterococcus* will follow the Idexx Enterolert procedures, and *E. coli* will use Standard Methods 9223A,B - Enzyme Substrate Coliform Test with a holding time of 6 hours to match the other methods (Appendix D). Quality Control measures and Method Performance requirements are outlined in the tables below and fully discussed in the Laboratory SOPs. If the required method performance and QC is not met, the appropriate Lab Manager will be responsible for corrective action. Because the samples are for bacterial analysis, QC and method performance failures will result in the invalidation of the sample results. This will be documented in the Lab Micro Logbook and in the LIMs report.

Laboratory analyses, verification by the lab and data entry into LIMS should be no more than 1 week after collection

B5. Quality Control Requirements

QC Requirements for this study include the following:

Item	Data Quality Indicator (DQI)	Frequency
Sterility check on glass sample bottles	Accuracy/Bias	Per autoclaved batch
Sterility check on disposable plastic Idexx sample bottles	Accuracy/Bias	Per manufacturer's lot
Pre and post sterility checks on filter funnels	Accuracy/Bias	Each funnel is checked before and after a filtration series
Positive and Negative controls on Enterolert or Colilert	Accuracy/Bias	Each lot
Sterility and Positive Controls on Enterolert or Colilert	Accuracy/Bias	Weekly
Duplicated Counts for Membrane Filter Analysis	Accuracy/Bias	!0% of all samples

Table 1 QC, DQIs and Frequency

Item	Requirement	Resolution of Deficiency
Sterility check on glass sample bottles	Randomly chosen bottle shows no growth with non-specific broth.	Entire batch is re-autoclaved.
Sterility check on disposable Idexx sample bottles	Randomly chosen bottle from each manufacturer's lot shows no growth with non-specific broth.	Manufacturer is called-lot is replaced.
Pre and post sterility checks on filter funnels	Filters from the sterility check show no growth.	The samples associated with the sterility checks that grow organisms are invalidated.
Positive and Negative controls on Enterolert or Colilert	Positive controls (E.Coli for Colilert or Enterococcus for Enterolert)show fluorescence. Negative controls do not.	Replace lot.
Sterility and Positive Controls on Enterolert or Colilert	Sterility controls show no growth, no fluorescence. Positive controls (E.Coli for Colilert or Enterococcus for Enterolert) show fluorescence.	Invalidate samples and/or don't analyze samples until problem is determined.
Duplicated Counts for Membrane Filter Analysis	Analysts must be within 10% of each other.*	Each analyst will re-count.

Table 2 QC Requirements

For more information see Laboratory Procedures Manual for Environmental Microbiology-- Analytical Services (SCDHEC, 1998).

B6. Instrument/Equipment Testing, Inspection, and Maintenance

Equipment	Type of Testing	Frequency	Supplies Needed	Person Responsible	Resolution of Deficiency
Autoclave	Check with bacillus spores to determine if autoclave kills them.	Monthly	Spore ampule or strip	Microbiologist	Call for service; invalidate media
Incubators	Check temperature to determine that the incubator is staying at the required temperature.	Twice daily, at least 4 hours apart	Thermometer	Microbiologist	Readjust, determine if incubator has been opened, call for service-may invalidate sample results.
Quantitray Sealer	Check for leaks	Monthly	Bromcresol purple or other dark dye	Microbiologist	Call for service
Filter	Inspections for	With every	N/A	Microbiologist	Replace

Equipment	Type of Testing	Frequency	Supplies Needed	Person Responsible	Resolution of Deficiency
Funnels for MF	scratches	use			

Table 3. Equipment testing

For more information see Laboratory Procedures Manual for Environmental Microbiology-- Analytical Services (SCDHEC, 1998).

B7. Instrument Calibration and Frequency

Equipment	Calibration Procedure	Frequency	Acceptance Criteria	Person Responsible	Resolution of Deficiency
Balance	Check weights on the balance	Series of 5 weights monthly, plus a check with a single weight when used.	Within 10% of the true weight	Lab Manager or Microbiologist	Re-zero and try again; call for service
Lab Thermometers	Check against NIST traceable	Once per year	Within 1 degree of NIST-correction factor is noted and used	Lab Manager or Microbiologist	Replace
Field Thermometers and Temperature Probes	Check against NIST traceable	Quarterly	Within 1 degree of NIST-correction factor is noted and used	Sample Collector	Replace
Filter Funnels	Pour 100 ml water into the funnel and inscribe a line where the meniscus falls on the funnel	When Received	The Volume must be accurate for the analysis.	Microbiologist	N/A
Graduated Cylinders	Check volume using balance	When received	The Volume must be accurate for the analysis.	Microbiologist	N/A

Table 4. Calibration Criteria

For more information see Laboratory Procedures Manual for Environmental Microbiology-- Analytical Services (SCDHEC, 1998).

B8. Inspection/Acceptance Requirements for Supplies and Consumables

Item	Vendor	Acceptance Criteria	Handling/Storage	Person responsible for inspection
Nitrile or other non-latex disposable gloves	Any Vendor	No holes, non-latex	Room Temperature	Lab Manager
Sample bottles	Fisher	500 ml with autoclavable lids.	Room temperature, sealed with cap	Microbiologist
Sample Bottles for mixing samples with Colilert or Enterolert media	Idexx	120 ml, Sterile with cap.	Room temperature, sealed with cap	Microbiologist
Media for Membrane Filtration	Any Vendor	Made according to SM 20th Ed.	Stored in cool dry place	Microbiologist
Filters for MF	Any Vendor	47 mm cellulose ester, white, 45µm pore size, grid marked	Store at room temperature	Lab Manager
Petri dishes for MF	Any Vendor	Sterile, fits 47 mm filter	Store at room temperature	Microbiologist
Quantitrays	Idexx	Sterile, 2000 series	Store at room temperature	Microbiologist
Media for Colilert and Enterolert	Idexx	Sterile, works properly with negative and positive controls	Stored in cool dry place	Microbiologist

Table 5. Inspection of Supplies

For more information see Laboratory Procedures Manual for Environmental Microbiology-- Analytical Services (SCDHEC, 1998).

B9. Data Acquisition Requirements (Non-Direct Measurements)

Historical data that may be used for comparison to data generated through this study will be data stored in EPA's STORET database. This can be directly compared to the Fecal Coliform analysis by Membrane Filtration in this study since the same methodology is being used for collection and analysis. In addition many of the samples will come from the same sites as the historical data.

Information related to recent rainfall will be of interest as it may relate to extremes in analytical results.

At the time of sample collection, monitoring personnel will make on-site observations for evidence of recent rainfall, e.g. high water level, high turbidity, knowledge of recent local weather patterns, wetness of surrounding area, etc.

The following Weather Codes should be used in the Weather field, parameter 00041, on the field data sheet, DHEC form 2186:

22 - Indicates that it is raining at the time of sample collection

24 - Indicates that it has rained in the past 24 hours

26 - Indicates that it has rained longer than 24 hours ago, but within the last 48 hours

The other weather codes (01 fair, 00 clear and 02 for cloudy) should be used as needed when it has not rained within the last 48 hours.

This documentation is needed in order for the Laboratory Microbiologist to determine the correct dilution of the samples.

Any other observations (dead animals in the creek, etc.) should be noted in the comments section of the data sheet and brought to the attention of the Laboratory Manager.

Some rainfall web resources are available that may be useful, although the lag time for updates is such that information for the last 24 hour period may not be available before sample collection takes place. Field observations need to be made at the time of sample collection to the best of the sampling personnel's judgment to aid the microbiologist's choice of dilutions for the analyses. Verification of rainfall and more detail on rainfall amounts should be made from available resources after returning to the office.

Some web resources include:

The State Climatology Office:
http://www.dnr.sc.gov/climate/sco/Observations/hyd_data.php

The Weather Underground, www.wunderground.com has a good network of smaller weather stations. A list of all the South Carolina stations can be found at:
http://www.wunderground.com/weatherstation/ListStations.asp?selectedState=SC&selectedCountry=United+States

The accuracy of these weather information sources will vary. However, the information will only be used to determine if there was enough rain to increase bacterial counts.

Another potential DHEC source includes the continuous air quality monitoring sites, most of which have rain gauges in place. Scott Reynolds with the Division of Air Quality Analysis can supply a list of available sites.

An Excel spreadsheet will be provided to each Regional Monitoring Supervisor containing a list of the monitoring sites for which their Region is responsible. This spreadsheet should be kept up to date each week with documentation of sample collection date and any more detailed rainfall information that can be obtained, including actual rainfall amounts at nearest rain gage in tenths of inches and when it occurred, e.g. last 24 hours, last 48 hours, etc.

If a site is missed one week, include the reason why in the spreadsheet.

Initially this spreadsheet should be e-mailed each week to Bryan Rabon and cc'ed to David Chestnut. This process will be re-evaluated after the first few weeks to determine if the frequency can be reduced.

B10. Data Management

Data will be managed for this study the same way as routine samples. The sample collector will follow the chain of custody procedure as outlined in Appendix E. All documentation concerning the sample will be logged in the field logbook and on the chain of custody. The samples will be brought to the regional lab within the travel holding time of 6 hours.

Regional lab staff will enter the sample information into the Micro logbook and assign the sample a regional number. The samples will be inoculated onto media as outlined in the Microbiology SOPs. One part of the chain of custody record will be sent via courier to ARESD. The sample will be entered into LIMs and receive a LIMs number that is associated with the regional number already assigned. When the sample results are obtained, the microbiologist enters the data directly into the logbook or workbook associated with the analyses. QC information is entered into both the Microbiology Logbook as well as the QC logbook. Once results have been obtained, the data is reviewed by a data verifier (a second analyst or the Lab Manager) for accuracy. The results are entered into LIMs and this transcription is also verified for accuracy.

The LIMs system has been in use a number of years as has STORET. Both have been found to be acceptable and accurate. Nevertheless it is intended that the Data Verifier will review the electronic data against hard copies.

The LIMs database is backed up nightly and monthly. Each Friday's backup is stored off site. Currently, the LIMs data is on a single server so there has been no need, thus far, to archive the data.

Discussions are underway with Central Laboratory Data Management Personnel to allow for direct downloading of results from LIMs as soon as the data is verified and released by the lab. This will be done by querying LIMs using the list of weekly pathogen station numbers.

The parameter codes for the analyses are:

31616 - Fecal Coliform Bacteria by Standard Methods 9222D – Membrane Filter Procedure
50589 - *Enterococcus* by Idexx Enterolert
31633 - *E. coli* by Standard Methods 9223A,B - Enzyme Substrate Coliform Test

The charge code for this study is PIS (Pathogen Indicator Study).

If one of the study sites is collected by personnel as part of the routine monthly ambient stream runs, the WPC code will be used since this data will be used for the regular stream data as well as the pathogen indicator study. **SAMPLING PERSONNEL MUST BE SURE TO INCLUDE THE PARAMTER CODES FOR *ENTEROCOCCUS* (50589) AND *E. COLI* (31633) AND BE SURE THOSE ANALYSES ARE CHECKED FOR THE APPROPRIATE MONITORING SITE.**

C. Assessment and Oversight

C1. Assessment and Response Actions

The following table lists the type and frequency of assessment activities that are conducted on EQC Internal Laboratories as well as the individuals responsible for the audit and corrective action.

Assessment External or Internal	Frequency	Organization Responsible	Individual Receives Report & Notification of Deficiencies	Time-frame of Notification	Individual that Implements Corrective Actions?	Corrective Action Effectiveness Documented where?	Individuals Receiving Corrective Action Response
Lab Cert-TSA/ Internal Regional Labs only	Every 2 years*	Office of Quality Assurance (OQA)	Regional Director, Lab Manager, BES ABCs	Within 1 month of the Audit	Lab Manager	Response to the Audit	OQA
PT/external Regional and ARESD	Annually, Varies	A2LA certified proficiency provider	Lab Manager	Roughly 1 month after the study closes	Lab Manager	Memo to OQA	OQA
On-Site TSA/ Internal ARESD	As requested, done per method	OQA	Lab Manager and ARESD Director	1 month	Lab Manager	Memo	OQA and ARESD Director
TSA – External	Every 3 years, January 2009	EPA	EQC Deputy Commissioner	Varies	Lab Managers	Memo	EPA, BES ABCs, and OQA

Assessment External or Internal	Frequency	Organization Responsible	Individual Receives Report & Notification of Deficiencies	Time-frame of Notification	Individual that Implements Corrective Actions?	Corrective Action Effectiveness Documented where?	Individuals Receiving Corrective Action Response
Data Review	As requested	OQA or Project Manager	Project Manager (if not doing the audit) Project Director and Lab Managers	Varies	Lab Managers	Memo	OQA and ARESD Director

*Approximate dates that the Regional Labs will be audited by OQA: Region 2 2009, Region 3 2010, Region 4 2009, Region 5 2009, Region 7 2010, and Region 8 2009.

C2. Reports to Management

For this project there are no formal QA Status reports, however, the Lab Manager is expected to contact the Project Manager or Bill McDermott via e-mail or phone should a situation arise that would cause a change in the quality of the data. This includes information gleaned from the field documentation as well as problems in the laboratory.

D. Data Validation and Usability

D1. Data Review, Verification and Validation

When a particular sample is out of holding time or fails any portion of the laboratory QC procedures the data are flagged in the LIM system according to Section IV-G of the Procedures and Quality Control Manual for Chemistry Laboratories--Analytical Services (SCDHEC, 2005). These results will not be reported or used for this study.

D2. Validation and Verification Methods

As described in data management, the data results are verified as well as the transcription into LIMS. Any mistakes are corrected before entry to LIMs. Any transcription errors in LIMs are also corrected.

The Project Verifier then compares hard copy results with the data in LIMs. This is a second check for transcription errors. This check will also include a check to make sure that all samples are accounted for – whether they have been invalidated or data has been received.

The Project Validator will examine the data as a whole. This examination will include a search for anomalies in the data, unexpected trends/bias, and comparison with historical data as appropriate. Should questions arise about the data, the Validator may end up contacting the laboratory and possibly the sample collector to determine if

there are reasons for the anomaly. The Project Validator is responsible for communicating any concerns or questions about the data to the committee in charge of determining what indicator will be used in South Carolina.

In addition to the process identified in Section D1, the Water Quality Monitoring Section will perform a minimum 10 percent review of all data to ensure quality assurance of the data.

D3. Reconciliation with User Requirements

Any errors detected by the Project Verifier will be handled through established DHEC procedures and corrected.

Problems with trends and bias in the data will be examined by the Project Validator and the uncertainties and limitations associated with parts of the data will be communicated to the Committee.

Appendix A

Evaluation of Alternative Freshwater Pathogen Indicators

Sampling Locations

DHEC Regional Office – Office responsible for sample collection
Approx Stream Order – Approximate stream order at sampling location
Recreational Use Support
    Meets – supports recreational use standards for last assessment
    Impaired – does not meet recreational use standards for last assessment
    Unassessed – not included for last assessment
Reason if specified – rationale for site inclusion, if available
Majority Land Use within 1/2 mile radius – predominant class of land use within a one-half mile radius of sampling location

DHEC Regional Office	Station	Approx Stream Order	Recreational Use Support	Location Description	Reason, if specified	Majority Land Use within 1/2 mile radius	Level 3 Ecoregion	Latitude	Longitude
Aiken	SV-324	2	Meets	TIMS BR AT SRP ROAD C	SRS	Developed, Medium Intensity	SOUTHEASTERN PLAINS	33.28712897950	-81.69740720610
Aiken	E-107	3	Meets	DEAN SWAMP CK AT SC 4	Region interest	Developed, Low Intensity	SOUTHEASTERN PLAINS	33.51246447210	-81.31836508450
Aiken	SV-069	3	Meets	SAND RVR AT OLD US 1 1.2 MI SE WARRENVILLE	Urban, Domestic Animals, Wildlife	Developed, High Intensity	SOUTHEASTERN PLAINS	33.55443360580	-81.78865322640
Aiken	SV-353	3	Impaired	BEAVERDAM CREEK AT FOREST SERVICE ROAD 621 OFF S-19-68	Wildlife	Scrub/Shrub	PIEDMONT	33.79981006960	-82.12331455920
Aiken	E-007	4	Meets	N FORK EDISTO RVR AT US 601 AT ORANGEBURG	Urban	Scrub/Shrub	SOUTHEASTERN PLAINS	33.48280107880	-80.87396804290
Aiken	E-059	4	Impaired	FOUR HOLE SWP AT S-38-50 5.2 MI SE OF CAMERON	Region interest	Grassland/Herbaceous	SOUTHEASTERN PLAINS	33.49044104860	-80.67995112650
Aiken	SV-325	4	Impaired	UPPER THREE RUNS AT SRP ROAD A	SRS	Pasture/Hay	SOUTHEASTERN PLAINS	33.23902931890	-81.74369715960
Aiken	SV-072	5	Meets	HORSE CK AT S-02-145	WWTP, Septic	Developed, High Intensity	SOUTHEASTERN PLAINS	33.48552047510	-81.89616937840
Aiken	SV-366	8	Meets	SAVANNAH RVR OFF JACKSON LANDING OFF END OF S-02-299	Urban	Developed, Open Space	SOUTHEASTERN PLAINS	33.27807900320	-81.84447143220
Aiken	SV-291	Lake	Meets	CLARKS HILL RESERVOIR AT US 378 7 MI SW MCCORMICK	Lake / Bridge	Mixed Forest	PIEDMONT	33.85816623450	-82.39021441110
Beaufort	RS-08260	1	Unassessed	UNNAMED TRIB TO LITTLE SALKEHATCHIE RIVER AT CULVERT ON SC 362 JUST NORTH OF S-15-465	First order stream		MIDDLE ATLANTIC COASTAL PLAIN	33.05908176070	-80.87976759890
Beaufort	CSTL-071	3	Impaired	HORSESHOE CREEK AT SC 64	Region interest	Developed, Low Intensity	MIDDLE ATLANTIC COASTAL PLAIN	32.81282517070	-80.53199215160
Beaufort	CSTL-121	3	Impaired	COOSAWHATCHIE RIVER AT SC 363	Region interest	Mixed Forest	MIDDLE ATLANTIC COASTAL PLAIN	32.85329363360	-81.16110067330
Beaufort	CSTL-122	3	Meets	CYPRESS CREEK AT S-27-108	Region interest	Developed, Low Intensity	MIDDLE ATLANTIC COASTAL PLAIN	32.69581194400	-80.98920127970
Beaufort	CSTL-109	4	Meets	COOSAWHATCHIE RVR AT S-25-27 2.5 MI SW CUMMINGS	Region interest	Emergent Herbaceous Wetland	MIDDLE ATLANTIC COASTAL PLAIN	32.76125649590	-81.02003482000
Beaufort	RS-08076	4	Unassessed	BUCKHEAD CREEK AT US 21	Region interest		MIDDLE ATLANTIC COASTAL PLAIN	33.01826926940	-80.81174363470
Beaufort	CSTL-075	Lake	Meets	LAKE WARREN, BLACK CK ARM, AT S-25-41 5 MI SW OF HAMPTON	Minor Lake	Pasture/Hay	MIDDLE ATLANTIC COASTAL PLAIN	32.82681229170	-81.18030838490
Catawba	CW-088	1	Impaired	GRASSY RUN BR AT SC 72 1.6 MI NE CHESTER	Urban	Cultivated Crops	PIEDMONT	34.71771468050	-81.19228425150

DHEC Regional Office	Station	Approx Stream Order	Recreational Use Support	Location Description	Reason, if specified	Majority Land Use within 1/2 mile radius	Level 3 Ecoregion	Latitude	Longitude
Catawba	CW-036	4	Impaired	SUGAR CREEK AT S-46-36	Urban, NC	Developed, Low Intensity	PIEDMONT	34.95077641670	-80.86868032940
Catawba	CW-016	7	Meets	CATAWBA RVR AT SC 9 AT FT LAWN	Major river below NC	Scrub/Shrub	PIEDMONT	34.70832342570	-80.86756144250
WQM & ABS	C-076	3	Unassessed	CEDAR CK CANOE ACCESS OFF S-40-1288 (SO CEDAR CK RD)	Background/National Park		SOUTHEASTERN PLAINS	33.81840198270	-80.78798276390
WQM & ABS	C-077	3	Unassessed	CEDAR CK - BRIDGE B	Background/National Park		SOUTHEASTERN PLAINS	33.81988312540	-80.82308357150
Cent Mid	B-123	1	Impaired	WINNSBORO BR AT US 321-AB WINNSBORO MILLS OUTFALL	Urban, Golf Course	Woody Wetlands	PIEDMONT	34.37128783600	-81.08935624380
Cent Mid	C-021	1	Impaired	MILL CK AT SC 262	Suburban	Pasture/Hay	SOUTHEASTERN PLAINS	33.98014695560	-80.91460740420
Cent Mid	B-280	2	Impaired	SMITH BR AT N MAIN ST (US 21) IN COLA	Urban, no NPDES	Pasture/Hay	SOUTHEASTERN PLAINS	34.02723011690	-81.04197905580
Cent Mid	S-287	2	Impaired	RAWLS CREEK AT S-32-107	Suburban	Scrub/Shrub	PIEDMONT	34.05384144410	-81.18636514470
Cent Mid	S-306	3	Impaired	HOLLOW CK AT S-32-54	Livestock	Barren Land	PIEDMONT	33.99208232800	-81.46496652760
Cent Mid	C-001	4	Impaired	GILLS CK AT BRDG ON US 76 (GARNERS FERRY ROAD)	Urban	Scrub/Shrub	SOUTHEASTERN PLAINS	33.98965502440	-80.97411392710
Cent Mid	S-298	5	Meets	SALUDA RVR AT USGS GAGING STATION, 1/2 MI BELOW I-20	Urban runoff, rec use area	Scrub/Shrub	PIEDMONT	34.01385513060	-81.08780920450
Cent Mid	C-068	Lake	Meets	FOREST LAKE AT DAM	Lake/ Suburban	Mixed Forest	SOUTHEASTERN PLAINS	34.02199837450	-80.96255680580
Cent Mid	CW-208	Lake	Meets	LK WATEREE AT S-20-101 11 MI ENE WINNSBORO	Major Lake	Developed, High Intensity	PIEDMONT	34.42192264290	-80.86743212470
Cent Mid	S-213	Lake	Meets	LAKE MURRAY AT S-36-15	Major Lake	Scrub/Shrub	PIEDMONT	34.12514632320	-81.43367351170
Charleston	CSTL-043	2	Impaired	SAWMILL BR AT SC 78 E OF SUMMERVILLE	Suburban	Deciduous Forest	MIDDLE ATLANTIC COASTAL PLAIN	33.02228925090	-80.16348542910
Charleston	RS-01056	2	Impaired	CEDAR CREEK AT CNTY RD 857 HAMPTON PLANTATION STATE PARK	Region interest		MIDDLE ATLANTIC COASTAL PLAIN	33.19401262670	-79.45328603470
Charleston	RS-02461	2	Impaired	WADBOO SWAMP AT S-08-447 THIRD BRIDGE FROM WEST	Wildlife	Developed, Open Space	MIDDLE ATLANTIC COASTAL PLAIN	33.30186924210	-79.91208159830
Charleston	RS-05572	2	Impaired	GUM BRANCH AT S-18-167 4.9 MI SE OF ST GEORGE	Domestic Animals	Open Water	MIDDLE ATLANTIC COASTAL PLAIN	33.13206998170	-80.52191265790
Charleston	CSTL-063	3	Meets	WASSAMASSAW SWP AT US 176	Wildlife	Cultivated Crops	MIDDLE ATLANTIC COASTAL PLAIN	33.15092640890	-80.17538101080
Charleston	CSTL-113	3	Meets	WADBOO SWP AT SC 402	Wildlife	Developed, Medium Intensity	MIDDLE ATLANTIC COASTAL PLAIN	33.19531049050	-79.95155117230

DHEC Regional Office	Station	Approx Stream Order	Recreational Use Support	Location Description	Reason, if specified	Majority Land Use within 1/2 mile radius	Level 3 Ecoregion	Latitude	Longitude
Charleston	E-016	3	Meets	POLK SWP AT UNIMP RD S-18-180 2 MI S OF ST GEORGE	Domestic Animals	Mixed Forest	MIDDLE ATLANTIC COASTAL PLAIN	33.15478934790	-80.58505492930
Charleston	E-032	3	Impaired	INDIAN FIELD SWAMP AT S-18-19	Domestic Animals	Grassland/Herbaceous	MIDDLE ATLANTIC COASTAL PLAIN	33.09142760530	-80.51275626720
Charleston	E-109	3	Impaired	POLK SWAMP AT S-18-19	Domestic Animals	Open Water	MIDDLE ATLANTIC COASTAL PLAIN	33.08921301340	-80.52144934860
Charleston	RS-02467	3	Impaired	ECHAW CK AT PITCH LANDING FRANCIS MARION NATL FOREST	Wildlife	Pasture/Hay	MIDDLE ATLANTIC COASTAL PLAIN	33.24736710690	-79.57740951040
Charleston	RS-03333	3	Impaired	CANE GULLEY BRANCH AT S-08-97 6.1 MI NE OF MONCKS CORNER	Wildlife	Grassland/Herbaceous	MIDDLE ATLANTIC COASTAL PLAIN	33.21918222580	-79.91319486020
Charleston	CSTL-112	4	Meets	WAMBAW CK AT EXTENSION OF S-10-857 (BRIDGE NEAR BOAT LANDING)	Wildlife	Developed, Open Space	MIDDLE ATLANTIC COASTAL PLAIN	33.20833204390	-79.46903540410
Florence	PD-065	1	Impaired	GULLEY BR AT S-21-13, TIMROD PARK	Urban	Mixed Forest	SOUTHEASTERN PLAINS	34.18339141950	-79.76975666380
Florence	PD-258	1	Impaired	SNAKE BR AT RR AVE IN HARTSVILLE	Urban	Emergent Herbaceous Wetland	SOUTHEASTERN PLAINS	34.37370233310	-80.06077039840
Florence	PD-167	3	Impaired	WILLOW CREEK AT S-21-57	Urban - Looks Ag to us	Developed, Low Intensity	SOUTHEASTERN PLAINS	34.11052697700	-79.59418241890
Florence	PD-230	3	Impaired	MIDDLE SWP AT SC 51 3.5 MI SSE OF FLORENCE	Urban, Swamp	Developed, High Intensity	SOUTHEASTERN PLAINS	34.15195293970	-79.73473349950
Florence	PD-256	3	Impaired	JEFFRIES CK AT S-21-112 4.8 MI W OF FLORENCE	AG	Emergent Herbaceous Wetland	SOUTHEASTERN PLAINS	34.18613706210	-79.85134708150
Florence	PD-346	3	Impaired	CAMP BRANCH AT S-21-278	Urban	Open Water	MIDDLE ATLANTIC COASTAL PLAIN	33.88170522410	-79.76394561040
Florence	PD-353	3	Meets	BLACK RIVER AT S-43-57	AG	Mixed Forest	SOUTHEASTERN PLAINS	33.95034608990	-80.17971577290
Florence	PD-035	4	Meets	JEFFERIES CK AT SC 327 AT CLAUSSEN	Urban		SOUTHEASTERN PLAINS	34.13580514330	-79.63162556920
Florence	PD-367	4	Meets	THREE CREEKS AT SC 38, S OF BLENHEIM	AG	Emergent Herbaceous Wetland	SOUTHEASTERN PLAINS	34.50019416950	-79.64991137960
Florence	PD-027	5	Meets	BLACK CK AT S-16-35 5.5 MI SE DARLINGTON	AG	Grassland/Herbaceous	SOUTHEASTERN PLAINS	34.27074444190	-79.78645668570
Florence	PD-041	5	Meets	LYNCHES RVR AT US 52 NEAR EFFINGHAM	WWTP, AG	Developed, High Intensity	MIDDLE ATLANTIC COASTAL PLAIN	34.05149422450	-79.75360879280
Florence	PD-042	6	Meets	LITTLE PEE DEE RVR AT US 501, GALIVANT'S FERRY	WWTP, AG	Deciduous Forest	MIDDLE ATLANTIC COASTAL PLAIN	34.05705586880	-79.24781846830
Florence	PD-337	7	Meets	GREAT PEE DEE RVR AT US 301/76	WWTP, AG	Emergent Herbaceous Wetland	SOUTHEASTERN PLAINS	34.20357138570	-79.54927014080

DHEC Regional Office	Station	Approx Stream Order	Recreational Use Support	Location Description	Reason, if specified	Majority Land Use within 1/2 mile radius	Level 3 Ecoregion	Latitude	Longitude
Florence	CL-077	Lake	Meets	LAKE ASHWOOD, FOREBAY MOVED TO CATWALK NEAR DAM	Lake / AG	Mixed Forest	SOUTHEASTERN PLAINS	34.09966293210	-80.31662730190
Florence	PD-081	Lake	Meets	PRESTWOOD LK AT US 15	Lake / Urban		SOUTHEASTERN PLAINS	34.38633662720	-80.07679206260
Greenville	BE-035	2	Impaired	BRUSHY CK AT HOWELL RD (S-23-273/335) APPROX 5 MI NE OF GREENVILLE (BIO B-798)	Urban	Mixed Forest	PIEDMONT	34.87876816270	-82.33074176680
Greenville	RS-02330	2	Meets	ADAMS CK AT UNPVD RD FROM SC 8 AND END OF S-39-34	Rural	Mixed Forest	PIEDMONT	34.98722804850	-82.65660682860
Greenville	SV-341	2	Impaired	LITTLE EASTATOE CREEK AT S-39-49	Blue Ridge ecoregion	Scrub/Shrub	BLUE RIDGE	34.94919541370	-82.83309687680
Greenville	B-246	3	Impaired	BEAVERDAM CK AT S-30-97, 7 MI NE OF GRAY COURT	Septic, Livestock, SPST	Developed, Medium Intensity	PIEDMONT	34.64623594580	-81.99552644730
Greenville	B-317	3	Impaired	MUSH CK AT SC 253 BL TIGERVILLE	SPST	Open Water	PIEDMONT	35.05487919140	-82.36721541670
Greenville	S-319	3	Impaired	REEDY RVR AT RIVERS ST, DOWNTOWN GREENVILLE	Urban, Local Interest	Deciduous Forest	PIEDMONT	34.84489991260	-82.40167687050
Greenville	SV-230	3	Meets	EASTATOE CREEK AT S-39-143	Reference	Scrub/Shrub	BLUE RIDGE	34.95812944850	-82.85319035840
Greenville	SV-342	3	Impaired	CANE CREEK AT S-37-133	Local Interest, upper Piedmont ecoregion	Mixed Forest	PIEDMONT	34.76653493770	-83.02571045320
Greenville	SV-343	3	Impaired	LITTLE CANE CREEK AT S-37-133	Local Interest, upper Piedmont ecoregion	Mixed Forest	PIEDMONT	34.76926882230	-83.01150169190
Greenville	BL-001	4	Impaired	LAWSONS FORK CK AT S-42-108	Local Interest	Developed, Low Intensity	PIEDMONT	34.94370037520	-81.78854862910
Greenville	S-004	4	Impaired	N SALUDA RVR AT BRDG AB JCT WITH SALUDA RVR E OF SC 186	SPST, Local Interest	Scrub/Shrub	PIEDMONT	34.97890320770	-82.52201756330
Greenville	B-339	Lake	Meets	LAKE BOWEN 0.3 MI W OF SC 9	Lake/ Bridge	Mixed Forest	PIEDMONT	35.11285121980	-82.04553096510
Greenville	RL-02307	Lake	Meets	LAKE OOLENOY SAMPLED FROM S SIDE OF SC 11 BRIDGE	Minor Lake	Scrub/Shrub	PIEDMONT	35.02157963190	-82.69494984370
Greenville	RL-04461	Lake	Meets	LAKE BLALOCK AT US 221	Minor Lake	Barren Land	PIEDMONT	35.06693019040	-81.87737030480
Greenville	SV-288	Lake	Meets	LK HARTWELL, SENECA RVR ARM AT USACE BUOY BTWN MRKRS S-28A & S-29	Major Lake	Mixed Forest	PIEDMONT	34.52647023750	-82.81533736630

Appendix B. Weekly Monitoring Site Locations

APPENDIX C.
EXCERPTS FROM PERTINENT SECTIONS OF
EQC ENVIRONMENTAL INVESTIGATIONS STANDARD OPERATING PROCEDURES AND QUALITY ASSURANCE MANUAL
(SCDHEC, 2006)

AMBIENT MONITORING

1. Preparation and General Considerations

Proper planning for sampling is essential to ensure that the facility is sampled correctly. Some important considerations are as follows:

- The necessary equipment should be inspected for cleanliness and made sure that the said equipment is in proper working order before leaving the Region office.
- Consider weather forecast and possible affects on sampling.
- Each parameter to be sampled, should be appropriately collected in the proper container, properly preserved, and the sample chain-of-custody maintained.
- When collecting samples or installing sampling equipment, field investigators always wear a new pair of the appropriate protective gloves (disposable latex gloves, rubber gloves, etc.) to prevent contamination of the sample and reduce exposure.
- At no time shall sampling equipment other than DHEC's be used for sample collection.

2. Ambient Monitoring (Surface Water) Sampling Site Selection:

Streams and lakes are monitored routinely at specified locations. Care must be taken to locate the exact location using landmarks such as marker buoys or bridge mid-points. In streams, the samples must be taken from an area that is well mixed and where the stream is deep enough to submerge sampling equipment. Unless predetermined stations have been established then the location is to be recorded as percent from right bank.

The following factors should be considered in the selection of surface water sampling locations:

- Study objectives;
- Water use;
- Point source discharges;
- Nonpoint source discharges;
- Tributary locations;
- Changes in stream characteristics;
- Type of stream bed;
- Depth of stream;
- Turbulence;
- Presence of structures (weirs, dams, etc.);

- Accessibility; and
- Tidal effect (estuarine).

Before any sampling is conducted, an initial reconnaissance should be made to locate suitable sampling locations. Bridges and piers are normally good choices as sites since they provide ready access and permit water sampling at any point across the width of the water body. However, these structures may alter the nature of water flow and thus influence sediment deposition or scouring. Additionally, bridges and piers are not always located in desirable locations with reference to waste sources, tributaries, etc. Wading for water samples in lakes, ponds, and slow-moving rivers and streams must be done with caution since bottom deposits are easily disturbed, thereby resulting in increased sediments in the overlying water column. On the other hand, Wadeable areas may be best for sediment sampling. In slow-moving or deep water, a boat is usually required for sampling. Sampling station locations can be chosen without regard to other means of access if the stream is navigable by boat, especially in estuarine systems where boats frequently provide the only access to critical sampling locations.

Water environments are commonly separated into two types:

- Flowing water, including estuarine environments, rivers, creeks, and small to intermittent streams; and

- Water that is contained, with restricted flow including lakes, ponds, and manmade impoundments.

3. Ambient Water Quality Monitoring Locations:

A network of ambient monitoring stations has been established throughout South Carolina to assess water quality trends across the State. Specific sampling stations, their locations, and parametric coverage at those stations are presented annually in the State of South Carolina Monitoring Strategy.

In addition to ambient water quality monitoring, the guidelines and methodologies presented in this manual are followed for intensive water quality surveys, lake studies, and any other special water quality studies conducted by DHEC.

In order for the ambient stations to be monitored effectively, proper planning and organization is necessary throughout the sampling program. Guidelines presented here will not only assist monitoring personnel in meeting their responsibilities but also will provide consistency in sampling procedures throughout the State.

4. Sample Types

Sample types include split, duplicate, blank, grab, and composite. For this project only grab samples will be collected, however all parameters will be analyzed from the same bottle.

Grab Samples

A grab sample is a discrete sample collected at a specific point and at a particular instance in time.

Grab sampling is conducted when:

- the water stream is not continuous (e.g., batch discharges or intermittent flow);

- the characteristics of the water stream are known to be constant or nearly so;

- the sample is to be analyzed for parameters whose characteristics are likely to change significantly with time, i.e., dissolved gases, bacteria, etc.;

- the sample is to be collected for analysis of a parameter such as oil and grease where the compositing process could significantly affect the observed concentration relative to the true concentration; and,

Except where otherwise specified (Special Sample Collection Procedures), grab samples may be collected by submersing the sample container in the water. When possible, the sample should be collected directly into the appropriate sample container. The container should not be overfilled if preservatives are present in the container. If the material to be sampled cannot be physically reached, an intermediate collection device may be used. When the sample container must be lowered into the stream, either because of safety or impracticality care must be taken to avoid contamination. The most desirable sampling location is the area of greatest mixing. Quiescent areas should be avoided. The sample container should be plunged into the water using a swooping motion with the mouth facing upstream

When analyzing grab samples in the field, holding times, usually fifteen (15) minutes, must be adhered to. The time the sample is collected and the time the sample is actually analyzed must be noted in the logbook and field lab form. Although one sample maybe collected for use in several analyses, if all tests cannot be completed within the holding time allowed, then another sample must be collected and times documented separately.

5. Bacterial Sample Collection

5.1 Preparation and Sample Collection:

When preparing for stream or lake monitoring sample collection, the collector should organize a list of those stations to be sampled and the parameters required for each station. All necessary sample containers should be obtained with care taken to select the proper containers for the parameters required. If pre-labeled, the containers can be segregated, by station, and placed in the sampling vehicle so they will be convenient to the collector during the sample collection. Sample containers as well as sampling equipment should be secured and controlled access maintained to prevent contamination or tampering.

It is recommended that extra containers be kept in the vehicle in case of accidental loss or breakage of the prepared containers. A quantity of ice sufficient to keep the samples at or below the required 10°C should be in each cooler used to transport the samples back to the laboratory. Bacteria samples should be preserved in a separate cooler from containers for other parameters (i.e., Little Oscar or other small cooler) to prevent contact and/or contamination through submergence of the sample bottle in water. All nutrients, metals, mercury, chlorides, and bacteria sample bottles will be placed in Whirl-Pak bags or zip lock plastic bags at the time of collection. The bags should be securely sealed, and the sample will then be preserved in ice at 6°C. Bags will be reused and discarded only when they can no longer be securely sealed or become punctured. No sample bottle should ever be allowed to become submerged in water within a cooler.

When sampling any stream station, the sample should be collected from an area in the stream that is well mixed and where the stream is deep enough to submerge sampling equipment. Unless predetermined stations have been established then the location is to be recorded as percent from right bank. For sampling purposes, the right bank is observed when facing upstream at the sampling site.

Bacteriological, organic, and oil and grease samples must be collected from the stream directly into their respective containers. These containers are specially prepared and the investigator must be careful not to contaminate the sample bottle by touching the inside of the container or the inside of the lid of the container. If the stream cannot be physically reached by the sampling personnel, the container may be attached to a pole, chain, rope, or string and then lowered into the stream.

Manual sampling is normally used for collecting grab stream samples and/or for immediate in-situ field analyses. The best method to manually collect a sample is to use the actual sample container which will be used to transport the sample to the laboratory; this eliminates the possibility of contaminating the sample with an intermediate collection container. In general, samples are manually collected by first selecting a location in the stream that is well mixed and then dipping the container in the water so the mouth of the container faces upstream. The container should not be overfilled if preservatives are present in the container.

If the stream cannot be physically reached by the sampling personnel, the container may be attached to a pole, chain, rope, or string and then lowered into the stream. Samples are collected manually by tipping the collection container in the stream to a depth of 0.3 meters. **Samples for oil and grease, bacteria, phenols, volatile organic compounds, and sulfides analyses must always be collected directly into the sample container.**

5.2 Bacteriological Collection Procedure

Samples for bacteriological analyses must always be collected directly into the glass or plastic sample container prepared by a DHEC Laboratory. The sample container should be kept unopened until it is to be filled. When the cap is removed, care should be taken not to contaminate the cap or the inside of the bottle. The sample is collected directly into the container provided and the mouth of the bottle should be directed against the current. It is recommended that extra sample containers be kept in the vehicle in case of accidental loss, contamination or breakage of the prepared containers. The bacteriological sample will be collected directly from the water body. The inspector must be careful not to contaminate the sample bottle by touching the inside of the container or exposing the inside of the lid to any foreign agent (i.e. hand, etc.).See Section 4.2 for special procedures for splitting bacteria samples. See **Appendix B** for preservation procedures and holding times.

Samples for fecal coliform, Enterococci and E. Coli bacteriological testing must be taken from a single 500 ml sterilized DHEC sample container only. Proper procedures are:

1. Collect the sample using a sterile 500 ml bottle.
2. Shake the sample thoroughly by inverting back and forth at least 25 times to insure complete mixing of the contents.
3. Follow routine procedures for preserving and transporting sample back to the lab.

6. Sample Identification, Control, and Documentation

The success of any environmental monitoring program depends to a great degree on the capability to provide valid data and to be able to systematically demonstrate the validity of the data. It is essential that laboratories involved in the collection of primary evidence provide written procedures to be followed whenever evidence samples are collected, transferred, stored, analyzed, or destroyed. These procedures must provide for an accurate written record which systematically traces the possession of the sample within the laboratory organization from receipt of the sample to release of the data. The Chain of Custody procedures that will be discussed must be fully employed to fulfill the legal requirements of the South Carolina Department of Health and Environmental Control.

All sample identification, chain-of-custody records, receipt for sample forms, calibration records, analytical records, and field records should be recorded with waterproof, non-erasable ink. If errors are made in any of these documents, corrections should be made by crossing a single line through the error and entering the correct information. Correction fluid must not be used. All corrections should be initialed and dated. If possible, all corrections should be made by the individual making the error.

If information is entered onto logbooks and sample tags or sample containers using stick-on labels, the labels should not be capable of being removed without leaving obvious indications of the attempt. Labels should never be placed over previously recorded information. Corrections to information recorded on stick-on labels should be made as stated above.

The method of sample identification used depends on the type of sample collected. Samples collected for specific field analyses or measurement data are recorded directly in bound field logbooks or recorded directly on the Chain-of-Custody Record, with identifying information, while in the custody of the samplers. Examples include pH, temperature, conductivity, dissolved oxygen, and residual chlorine. Samples collected for laboratory analyses are identified by using standard sample labels which are attached to the sample containers. In some cases, particularly with biological samples, the sample labels may have to be included with or wrapped around the samples. The following information shall be included on the sample label using waterproof, non-erasable ink:

- field identification or sample station number;
- sample identification number;
- preservatives used; and
- the general types of analyses to be performed (tape on some containers).

Additional information about the sample should be recorded in a bound field logbook. The following information shall be included in the bound field logbook using waterproof, non-erasable ink:

- sample identification number;
- date and time of sample collection (compositing period if sample is a composite);
- designation of the sample as a grab
- type of sample (ambient or stream) or program area when applicable;
- brief description of sampling location, if pertinent;
- signature of the sample collector;
- if applicable--field parameter (pH, dissolved oxygen, residual chlorine, temperature, conductivity, turbidity) analytical results; and
- relevant comments including weather information (e.g. readily detectable or identifiable odor, color, toxic properties, sheen, etc).

Appendix D Preservation and Holding Times

Parameter(s)	Bottle Label	Number, Size, and Type of Containers	Preservation and Temperature	Maximum Holding Time
E. Coli by Colilert/Quantitray	None	1 – 500 ml, sterile plastic or amber glass	Cool $\leq 10^{o}C$	6 hours transport time
Enterococci-Enterolert			Cool, $\leq 10°C$	6 hours transport time
Fecal Coliform –MPN, MFC			Cool, $\leq 10°C$	6 hours transport time

APPENDIX E.
EXCERPTS FROM PERTINENT SECTIONS OF
EQC ENVIRONMENTAL INVESTIGATIONS STANDARD OPERATING PROCEDURES AND QUALITY ASSURANCE MANUAL
(SCDHEC, 2006)

SAMPLE CONTROL, FIELD RECORDS, AND DOCUMENT CONTROL

1. Introduction

All sample identification, chain-of-custody records, receipt for sample forms, calibration records, analytical records, and field records should be recorded with indelible ink. If errors are made in any of these documents, corrections should be made by crossing a single line through the error, entering the correct information and initialing and dating the correction. Correction fluid must not be used. If possible, all corrections should be made by the individual making the error.

If information is entered onto logbooks and sample tags or sample containers using stick-on labels, the labels should not be capable of being removed without leaving obvious indications of the attempt. Labels should never be placed over previously recorded information. Corrections to information recorded on stick-on labels should be made as stated above.

2. Sample and Evidence Identification

2.1 Sample Identification:

The method of sample identification used depends on the type of sample collected. Samples collected for specific field analyses or measurement data are recorded directly in bound field logbooks or recorded directly on the Chain-of-Custody Record, with identifying information, while in the custody of the samplers. Examples include pH, temperature, conductivity, dissolved oxygen, residual chlorine, sample filters, or grab samples. Samples collected for laboratory analyses are identified by using standard sample labels which are attached to the sample containers. In some cases, particularly with biological samples, the sample labels may have to be included with or wrapped around the samples. At minimum, the following information shall be included on the sample label using indelible ink:

- field identification or sample station number
- sample identification number and possibly the program area
- preservatives used
- the analyses to be performed (identified using tape on some sample types)
- Additional information about the sample should be recorded in a bound field logbook. The following information shall be included in the bound field logbook using indelible ink:
- sample identification number;

- date and time of sample collection (compositing period-- both end and beginning times if the sample is a composite);
- designation of the sample as a grab or composite;
- type of sample (drinking water, wastewater, soil, etc.) or program area;
- brief description of sampling location;
- simple site sketches/mapping of sample locations- (see Section 13 for GPS information)
- signature of the sample collector
- field parameter (pH, dissolved oxygen, residual chlorine, temperature, conductivity, turbidity) analytical results
- relevant comments (e.g. readily detectable or identifiable odor, color, toxic properties, sheen, etc and other information required by the program areas).

The field logbook may also include field instrument calibration information. If so, the information recorded should allow a person reviewing the records to recount the calibration events. Please refer to the "Guidance Document for Field Parameter Analysis".

Labels for blank or duplicate samples will be marked "blank" or "duplicate," respectively. This identifying information shall also be recorded in the bound field logbooks and on the Chain-Of-Custody Record.

2.2 Photograph Identification:

Photographs used in investigative reports or placed in the official files shall be identified on the back of the print with the following information:

- A brief, but accurate description of what the photograph shows, including the name of the facility or site and the location.
- The date and time that the photograph was taken.
- The name of the photographer.
- Reference number for complaint, project, etc.

When photographs are taken, a record of each frame exposed shall be kept in the field logbook along with the information required for each photograph. The film shall be developed with the negatives supplied uncut. The field investigator shall then enter the required information on the prints, using the photographic record from the field logbook, to identify each photograph. For criminal investigations, the negatives must be maintained with the field logbook in the project file and stored in a secured file cabinet.

Digital photographs are treated in like manner, however they must be written onto a single write CD. The time and date of writing the pictures onto the CD along with the signature of the person storing the photos

must be written on the CD. Information must be stored with the photograph computer files to accurately identify each one. Staff must be able to testify concerning the subject matter if they are used in litigation.

3. Chain-of-Custody

Chain-of-custody procedures are comprised of the following elements; 1) maintaining sample custody and 2) documentation of samples for evidence. To document chain-of-custody, an accurate record must be maintained to trace the possession of each sample from the moment of collection to its analysis.

3.1 Sample Custody:

A sample or other physical evidence is in custody if:

- it is in the actual possession of a person;
- it is in the view of a person, after being in his/her physical possession;
- it was in the physical possession of a person and then he/she secured it to prevent tampering; and/or
- it is placed in a designated secure area.

3.2 Chain-of-Custody Record:

The field Chain-Of-Custody Record is used to record the custody of all samples or other physical evidence collected and maintained by sample collectors and laboratory personnel. All samples or sample sets shall be accompanied by a Chain-Of-Custody Record. This Chain-Of-Custody Record documents transfer of custody of samples from the sample custodian to another person and/or to the laboratory. To simplify the Chain-of-Custody Record and eliminate potential litigation problems, as few people as possible should have custody of the samples. The Chain-Of-Custody Record can also serve as a sample logging mechanism for the laboratory sample custodian. A Chain-of-Custody Record will be completed for all samples collected.

Each program area must have a written procedure for sample handling. These procedures must be available to all personnel involved in any aspect of sample handling. For the purposes of litigation, it is necessary to have an accurate written record which can be used to trace the possession and handling of samples from the moment of collection through analysis.

Records must include the following:

1. Collection date and time for each sample. If the sample is a composite sample and is collected by an automatic sampler, the starting and ending dates and times of the sampling period must be documented. If the composite sample was collected manually, the date, time, and collector of each portion must be documented also.

2. Signature of sample collector(s).

3. Unique sample identification number. One sample should be entered on each line or column and a sample should not be split among multiple lines or columns.

4. Sampling location and description (if necessary).

5. Sample type - grab or composite. Although grab and composite samples might be collected from the same location at the same time, they differ in composition and must be listed separately and must have unique identification numbers.

6. Analyses required, specified for each sample.

7. Preservatives used (H_2SO_4, NaOH, ice, etc.) for each sample. This includes any dechlorination agents or other chemicals added to the bottle prior to sampling.

8. Program area

9. Sample matrix – water, waste, soil, etc.

10. Transfer signatures with dates and times for both relinquishment and laboratory receipt (the laboratory should indicate courier, FEDEX, UPS, etc. in the "relinquished to" space if applicable.

11. Receipts maintained when shipped by common carrier (FEDEX, UPS, etc.). These receipts should be attached to the pertinent chain-of-custody records.

12. The number and type of bottles used. The COC currently does not have this on the form. Until this is rectified, staff are to write this in.

The Chain-of-Custody Record, once completed, becomes an accountable document and must be maintained in the project file. The suitability of any format for chain-of-custody should be evaluated based upon its inclusion of all of the above information in a legible format. The format of the record can vary depending on the needs of the program area.

Samples should not be accepted from other sources unless the sample collection procedures used are known to be acceptable, can be documented, and the sample chain-of-custody can be established. If such samples are accepted, a standard sample label containing all relevant information and the Chain-Of-Custody Record shall be completed for each set of samples.

4. Chain-of-Custody Procedures for Samples

The success of any environmental monitoring program depends to a great degree on the capability to provide valid data and to be able to systematically demonstrate the validity of the data. It is essential that laboratories involved in the collection of primary evidence provide written procedures to be followed whenever evidence samples are collected, transferred, stored, analyzed, or destroyed. These procedures must provide for an accurate written record which systematically traces the possession of the sample within the laboratory organization from receipt of the sample to release of the data. The chain-of-custody procedures that will be discussed must be fully employed to fulfill the legal requirements of the South Carolina Department of Health and Environmental Control.

These procedures are designed to include the Regional and Central Laboratories because of their interrelationship. First, it is important that each laboratory select a primary sample custodian and a minimum of one alternate custodian. It has been demonstrated that the fewer the number of people handling a sample the better. The sample custodian is responsible for ensuring all samples received meet any required acceptance

criteria. Custodians are responsible for annotating any deficiency in the sample.

Samples sent by the Regional Laboratories may be assigned a 10-digit number. This number is logged into the Laboratory Information Management System (LIMS) as the sample reference number and can be used to access the sample in LIMS. This is also how samples are identified if the LIMS system is down for an extended period of time. The reference number is a ten-digit number coded with the date received in the laboratory and the laboratory identification code. The eighth through tenth digits (three-digit sample identification number) are ascending sequential numbers, numbers 001 thru 499 are for chemistry samples and 500 thru 999 are for microbiological samples. For example, the reference number 1212027011; the date is December 12, 2002, the laboratory identification code for Florence Regional Laboratory is 7, and 011 indicates that this sample was the 11th sample for December 12, 2002. The first sample received in the Aiken Regional Laboratory on November 15, 2002 will be identified as 1115024001 .

Regional Laboratory Code

Columbia	0
Tissue	1
Beaufort	3
Aiken	4
Charleston	5
Myrtle Beach	6
Florence	7
Greenville	8
Lancaster	9

In the Central Laboratory, the sample custodian enters each incoming sample into LIMS. A computer logbook is generated which documents the sample identification number, program charge, date and time collected, sample collector, sample location, date and time received in central lab, sample receiver, person or mode of delivery, and sample receipt comments. Each day after the sample entry has been checked and verified; the logbook is printed and signed. At the end of each month, the logbooks are bound together for archiving. The custodian numbers, dates, and initials the sample form and gives a copy to the person delivering the sample, if requested. The custodian then applies the LIMS generated sample label to the containers and places the sample(s) in an area that is secure and environmentally suitable.

The Regional sample custodian or alternate receives all incoming samples and enters the information into the appropriate log book. The custodian numbers, dates, and initials the sample forms, then numbers each container associated with that sample and places the sample in an area that is secure and environmentally suitable. The sample custodian or alternate in the Regional Laboratory also has the responsibility for data release from the laboratory after the results have been verified by another staff analyst.

4.1 Program Charge Codes:

The ARESD Laboratories support the Bureau of Drinking Water protection, the Bureau of Water Pollution Control, and the Bureau of Solid and Hazardous Waste Management. Each sample must be charged to one of these Bureaus by program designation. The following is a master list of current charge numbers; charge numbers may be added or deleted as the program requires.

WPC - Water Pollution Control

PIS - Pathogen Indicator Study (Special Study)

It is the responsibility of the sample collector/submitter to provide the information necessary to assign the Program Charge Code. For this study not only will the PIS charge be used, but WPC will be used for samples that are not only part of this study but also part of routine monitoring.

4.2 Custody of Stream Samples:

Note: Although the samples collected for the study will only be analyzed for E. Coli, Fecal Coliforms, and Enterococcus, the samples collected for WPC may require field analysis, chemistry analyses in the Regional Lab or may be shipped to ARESD in Columbia, SC for other analyses. Thus this information is included in the discussion below.

Routine monitoring samples include integrator and random stream stations. The samples are delivered to the ARESD Laboratory by the collector along with Form DHEC 2186 - Regional Laboratory Report Form for Stream and Facility Data, commonly known as the "Field Sheet". This form is a record of field data and provides spaces for Regional Lab results for pH, alkalinity, turbidity, BOD, solids, phenols, oil and grease, total coliform, fecal coliform, etc. In the event that the collector does not provide the information or proper container, the samples should be discarded and the Water Pollution Control Program is notified.

The Regional Sample Custodian logs the sample in the logbook to include sample identification number, sheet number (optional), date-time received, program charge code, station code number, sampling location, collector, date-time collected, sample submitter, tests required and receiver. The samples are analyzed for pH, alkalinity, solids, BOD, phenols, oil and grease, and microbiology parameters, if requested. The results are recorded in the appropriate workbook and then transcribed to Form DHEC 2186 and/or DHEC 1309. No sample results are released from the Regional Laboratories until the data and calculations are verified. The analyst verifying the data must initial the appropriate forms. The sample custodian records a release date and his/her initials. The Regional Laboratory results are mailed to Columbia to the Analytical Services Division upon completion of the analyses. The data is received by the Director of Analytical Services Division, reviewed, and forwarded to the appropriate Program Director.

The sample custodian next separates the samples for analyses which are only performed in the Columbia Laboratory and the appropriate forms are prepared by the sample collector. The Regional samples are shipped to the Columbia Laboratory on Monday through Thursday of each week.

In the event that no analyses are conducted in the Regional Laboratory, a sample number is still assigned and recorded in the Regional Logbook. The sample custodian records a release date and his/her initials. The forms are forwarded to the Central Laboratory each Monday through Thursday via a contracted courier service, accompanied by the samples.

The very same procedure is used for Special Compliance/Enforcement Request Samples except for the requirement of tamper-proof (Evidence Tape) seals. Each custodian has been briefed relevant to the preparation of such seals. Each bottle being transported to the Central Laboratory via courier service must be sealed, properly preserved, labeled, and submitted with full information. Samples submitted in the Central Laboratory for criminal investigation are received by Enforcement Sample Custodians (see list at the end of this section). Such samples are retained in the physical custody of the custodian until the sample containers are delivered to a secure storage area within the Laboratory.

The Regional Sample Custodian follows the same procedure with the complaint samples as utilized with the Compliance samples with the exception of tamper-proof (Evidence Tape) seals. Complaint samples do not require tamper-proof (evidence tape) seals. Analyses are conducted as usual and normal release procedures are utilized.

The Regional Sample Custodian treats special study and intensive water quality samples just as the routine monitoring samples.

The sample custodian in Columbia enters the stream and wastewater samples into the Columbia Laboratory Logbook with the chain-of-custody information previously described. The Columbia Laboratory Sample Custodian checks each sample versus each form to determine if complete sample sets as well as appropriate information is available. A sample project sheet is generated automatically when the sample is logged into LIMS for the organic parameters. This form will accompany the sample to the laboratory. The date received and custodian's signature is entered on the chain-of-custody forms. The Data Coordinator enters the information into the LIMS.

The Section Manager responsible for each Service Laboratory or designated personnel places the sample for analysis in the laboratory. After completion of the analyses and verification of data and calculations, the sample results are entered into the LIMS Computer System. The Columbia Laboratory data is released by the Division Director, any one of the four laboratory managers, or designated senior analyst.

Wastewater and Stream Monitoring Forms

Listed below are the forms used by the Wastewater and Stream Monitoring Program with an explanation of the function of the form. A completed example of each form is included.

DHEC Forms

1943 *Field Quality Control Worksheet*

All DHEC personnel performing ambient water quality or wastewater facility monitoring must maintain a permanently bound logbook for field use. All records of standardization and calibrations of field equipment, reagent replacement, meter serial numbers, monitoring equipment and other pertinent information is entered in these logbooks. All entries must be made in ink. Pages are to be numbered and should never be torn out of the logbook. The logbook is a permanent record of work performed and should be kept along with analyses records for the normal archive time of 12 years.

2186 *EQC Regional Laboratory Report Form for Stream and Facility Data - Analytical Services Division*

This form is used by personnel performing ambient water quality or wastewater facility monitoring. The form is used to record field monitoring data and request a sample analysis for parameters that are performed in the Regional Laboratory.

South Carolina Department of Health and Environmental Control

FIELD QUALITY CONTROL WORKSHEET

COLLECTOR: Ben Smith		Stream Monitoring							
WEATHER CODES: Clear 00, Fair 01, Cloudy 02, Rain 22 TIDE STAGE CODES: Ebb 2000, Flood 4000	SAMPLE I.D. →	CW-212	CW-029	RS-07208					
Time Collected	In								
(military)	Out	1010	1030	1115					
Date (yr/mo/day)		070730	070730	070730					
Weather	00041	00	00	02					
Temp., Air (°C)	00020	24.0	25.0	25.0					
% from R.B.	00002	—	—	—					
Depth (meters)	82048	0.3	0.3	0.3					
Tape Down/Staff Gage (ft.)		—	—	—					
Tide Stage	00067	—	—	—					
Field pH (su)	00400	6.91	6.98	7.04					
Field D.O. (mg/1)	00300	7.83	8.02	7.82					
Temp., Water (°C)	00010	23.0	22.0	24.8					
Chlorine, Tot. Res. (mg/1)	50060								
Salinity (ppt)	00480								
Conductivity (µmhos/cm)	00402								
Flow, Stream (cfs)	00061								
Flow, Facility (mgd)	50050								
QUALITY ASSURANCE									
pH Meter Serial No.	A1234								
pH Buffer Temp. (°C)	24.0								
pH 7.0 Buffer (su)	7.05	7.02	7.00	7.01					
Meter Adjusted to (su)	7.00	7.00		7.00					
pH 4.0 Buffer (su)	4.00								
pH 10.0 Buffer (su)	10.00								
D.O. Meter Serial No.	751			751					
Winkler D.O. (mg/1)	—			—					
Air Cal. Temp. (°C)	23.0			24.0					
Meter D.O. (mg/1)	8.45			8.50					
Meter Adjusted to (mg/1)	8.56			8.40					
Conductivity Meter Serial No.									
Std. [µmhos/cm] °C									
Std. [µmhos/cm] °C									
Chlorine Meter Serial No.									
Standard [Blank]									
Standard [0.50 mg/1]									
Standard [1.00 mg/1]									
Sample pH Adjusted to (su)									
Flow Recorder Serial No.									
Sampler Serial No.									
Comp. Frequency (Min. or Gals.)									
Ind. Sample Volume (ml)									
Number of Ind. Samples									
Comp. Temp. (°C)									
Samples Split with									

D H E C
PROMOTE PROTECT PROSPER

EQC District Laboratory Report Form for Stream and Facility Data
Analytical & Radiological Environmental Services Division

Stream: Integrator (✓) Random (✓) Special ( ) Complaint ( ) Basin ( ) Charge Code: WPC

County: York Day No.: 1 of 1

Location: Streams FCSI ☐ SCSI ☐ Samples Split: Other (1) Owner (2) None (3)

NPDES No.: — Receiving Stream: — Basin: Catawba

Date: From 07 30 07 (Month Day Year) To 07 30 07 (Month Day Year) Collector: Ben Smith All Samples Iced ☑

Weather Code: Fair 01, Clear 00, Cloudy 02, Rain 22

Laboratory Number				
District Lab ID				
Station Number		CW-212	CW-279	RS-07208
Time Collected (Military)	In			
	Out	1010	1030	1115
Sample Type (Gr or Cp)		Gr	Gr	Gr
Weather	00041	00	00	02
Temp., Air (°C)	00020	24.0	25.0	25.0
Depth (m)	82048	0.3	0.3	0.3
Tape Down/Staff Gauge (ft)		—	—	—
Field pH (su)	00400	6.91	6.78	7.04
Field D.O. (mg/L)	00300	7.83	8.02	7.82
Temp., Water (°C)	00010	23.0	22.0	24.8
Salinity (ppt)	00480			
Conductivity (umhos/cm)	00402			
Chlorine, Total Res. (mg/L)	50060			
Flow, Facility (MGD) (✓) if no access	50050			
Chlorine, Contact Chamber (mg/L)	88666			
Lab pH (su)	00403			
Tot. Alk (mg/L)	00410	X	X	X
Color (c.u.)	00080			
Turbidity (NTU)	00076	X	X	X
BOD_5 (mg/L)	00310	X	X	X
$CBOD_5$ (mg/L)	80082			
Residue Sus. (mg/L)	00530			
Residue Total (mg/L)	00500			
Residue Diss. (mg/L)	70300			
Fecal Coli/100 mL (MFC)	31616	X	X	X
Fecal Coli/100 mL (MPN)	31615			
Fecal Coli/100 mL (A-1)	31621			
Enterococci (Q-tray)	50589			
Total Coli/100 mL (MPN)	31505			
Phenols (mg/L) H_2SO_4 ☐	32730			
Oil & Grease (mg/L) H_2SO_4 ☐	00556			
Sulfides (mg/L) NaOH ☐	00745			
MBAS/Surfactants	50916			
Chlorophyll				

(✓) If form 2189 ☑ 2182 ☐ are associated List all other associated forms:

Comments:

Samples Released To: R. Baker By: Ben Smith Date: 07-30-07 Time: 1320

Samples Received in Regional Lab By: Date: Time:

Samples Released from Regional Lab By: Date:

Samples Received in ARESD Central Lab By: Date: Time:

Data released from ARESD Central Lab By: Date:

DHEC 2186 (07/2005) White - Central Office; Canary - Lab; Pink - District Office

Attachment 2. Evaluation of Alternative Freshwater Pathogen Indicators

QAPP Addendum – June 1, 2009

QAPP Addendum – June 1, 2009

Project Manager: _____ Date: 6/4/09
David Chestnut, Senior Scientist, Bureau of Water

Project Director: _____ Date: 6/4/09
David Baize, Assistant Bureau Chief, Bureau of Water

SCDHEC QA Officer: _____ Date: 6/2/09
Nydia Burdick, Manager, Office of Quality Assurance

ARESD Director: _____ Date: 06/03/09
Sandra Flemming

State Microbiologist: _____ Date: 06/02/09
Karen Suber, Manager, Microbiology Section, ARESD

This Addendum modifies Section B-4. Analytical Methods of the approved QAPP for this project.

Background

The *Escherichia coli* (*E. coli*) and *Enterococcus* tests being employed in the Evaluation of Alternative Freshwater Pathogen Indicators study uses the IDEXX Quanti-Tray/2000, a tray with 49 large and 48 small wells, for enumeration, and reagents that are selective for the targeted organism(s). The quantification of the targeted bacteria is based on the Standard Methods Most Probable Number (MPN) approach using the number of large and small wells exhibiting a positive result.

There are 2450 different combinations of positive well counts, but only 1496 different possible MPN values because multiple combinations result in the same MPN value. For example, 17 different combinations result in 51.2.

The distribution of possible MPN values shows much better resolution between values within a range near the lower values and a wider spread of numbers towards the higher end. For instance there is relatively good discriminatory power around 100, i.e. it is possible to get more values between numbers. There are 12 different positive well combinations that yield values between 99.0 and 99.9. There is only one possible way to get 260 (260.3) or 264 (264.6), and the difference between consecutive values gets larger as you get to the higher values, for example the possible values progress from

593.8, 601.5, 613.1, etc. It isn't possible to get 604. The maximum value on the MPN chart is >2419.6.

To date the *E. coli* results are producing many values in the range of 300 to 800, where the MPN table has less discriminatory power, and several values reported as >2419.6.

IDEXX recommends dilution to get within the MPN range needed, but the least dilution necessary is preferable. As part of an e-mail exchange, Krista Doucette, Water Technical Support, IDEXX Laboratories, Inc., said, "Typically, the least diluted sample which gives a readable tray with a mix of positive and negative wells (such as 80% positive/20% negative) would be the reportable MPN result. Sensitivity may be lost with each increasing dilution, but not any more so in the confidence limits. When multiplying the confidence limits by the dilution factor the range stays comparable to the range of an undiluted sample with a higher MPN value. For example if an undiluted sample gave an MPN of 920.8 95% Conf. 620.5-1282.0 and with a 1:2 dilution sample with an MPN of 452.0 after calculations would be MPN 904.0 95% Conf 715.2-1113.4. These numbers are not significantly different and as mentioned above we suggest using dilutions as per the attached reference from Methods for General and Molecular Biology, American Society for Microbiology, which references the most reliable data comes from the level in which 20 % of the cultures are negative."

If a 1 to 4 dilution using 25 ml sample, 75 ml sterile deionized (DI) water were used, then to account for the dilution, the MPN value from the table would be multiplied by 4, and the associated 95% confidence limits would also be multiplied by 4. With 49 large and 48 small wells, 80% positive wells are 39 and 38 respectively, for an MPN of 180.7. So the 1 to 4 dilution using 25 ml sample, 75 ml DI water would equate to 722.8, which allows for greater resolution of values within the ranges that have been observed.

Values of 236, 299, 409, and 576 are numbers from the 1986 EPA criteria for *E. coli.* Using the MPN table values and multiplying by 4, as in a 1 in 4 dilution (25 ml sample, 75 ml DI water), and comparing the resolution indicated by the number of combinations that give an actual number greater than 235 and less than 237, there is better resolution with the dilution results, 9 possible values within the range compared to only 4 without dilution. Similarly for values greater than 298 and less than 301, there are 12 possible values with dilution vs. 2 without dilution. Looking at values greater than 408 and less than 411, there are 8 possible values with dilution and only 2 without. For values greater than 574 and less than 579, there are 7 possible values at 1 to 4 dilution and only 1 possible value without.

With a 1 to 4 dilution (25 ml sample, 75 ml DI water) we get much better resolution in the range of values important for picking a standard within EPA acceptable ranges. While this does somewhat reduce resolution for very low values (i.e. <40) this range will be below any of the possible criteria for *E. coli.*

Considering the impact and importance of setting a new statewide pathogen standard to be protective of human health that will impact a wide range of BOW activities and the regulated community it is imperative that there is adequate resolution within the results to evaluate the different available criteria options.

Changes

Beginning with the samples collected the week of June15, 2009, a 1 to 4 dilution (25 ml of sample, 75 ml of sterile DI water) will be used for all *E. coli* samples from all sample locations for the duration of the study.

The dilution will be accomplished by pipetting 25 ml of the sample into the Colilert sample bottle and then filling the bottle to the 100 ml line with Sterile DI water.

There are no changes proposed for the *Enterococcus* analysis.

Attachment 3. Weekly Pathogen Indicator Study Sites

Weekly Pathogen Indicator Study Sites

DHEC Regional Office	Station	Approx Stream Order	Recreational Use Support	Location Description	Reason, if specified	Majority Land Use within 1/2 mile radius	Level 3 Ecoregion	Latitude	Longitude
Aiken	SV-324	2	Meets	TIMS BR AT SRP ROAD C	SRS	Developed, Medium Intensity	SOUTHEASTERN PLAINS	33.28712897950	-81.69740720610
Aiken	E-107	3	Meets	DEAN SWAMP CK AT SC 4	Region interest	Developed, Low Intensity	SOUTHEASTERN PLAINS	33.51246447210	-81.31836508450
Aiken	SV-069	3	Meets	SAND RVR AT OLD US 1 1.2 MI SE WARRENVILLE	Urban, Domestic Animals, Wildlife	Developed, High Intensity	SOUTHEASTERN PLAINS	33.55443360580	-81.78865322640
Aiken	SV-353	3	Impaired	BEAVERDAM CREEK AT FOREST SERVICE ROAD 621 OFF S-19-68	Wildlife	Scrub/Shrub	PIEDMONT	33.79981006960	-82.12331455920
Aiken	E-007	4	Meets	N FORK EDISTO RVR AT US 601 AT ORANGEBURG	Urban	Scrub/Shrub	SOUTHEASTERN PLAINS	33.48280107880	-80.87396804290
Aiken	E-059	4	Impaired	FOUR HOLE SWP AT S-38-50 5.2 MI SE OF CAMERON	Region interest	Grassland/Herbaceous	SOUTHEASTERN PLAINS	33.49044104860	-80.67995112650
Aiken	SV-325	4	Impaired	UPPER THREE RUNS AT SRP ROAD A	SRS	Pasture/Hay	SOUTHEASTERN PLAINS	33.23902931890	-81.74369715960
Aiken	SV-072	5	Meets	HORSE CK AT S-02-145	WWTP, Septic	Developed, High Intensity	SOUTHEASTERN PLAINS	33.48552047510	-81.89616937840
Aiken	SV-366	8	Meets	SAVANNAH RVR OFF JACKSON LANDING OFF END OF S-02-299	Urban	Developed, Open Space	SOUTHEASTERN PLAINS	33.27807900320	-81.84447143220
Aiken	SV-291	Lake	Meets	CLARKS HILL RESERVOIR AT US 378 7 MI SW MCCORMICK	Lake / Bridge	Mixed Forest	PIEDMONT	33.85816623450	-82.39021441110
Beaufort	RS-08260	1	Unassessed	UNNAMED TRIB TO LITTLE SALKEHATCHIE RIVER AT CULVERT ON SC 362 JUST NORTH OF S-15-465	First order stream		MIDDLE ATLANTIC COASTAL PLAIN	33.05908176070	-80.87976759890
Beaufort	CSTL-071	3	Impaired	HORSESHOE CREEK AT SC 64	Region interest	Developed, Low Intensity	MIDDLE ATLANTIC COASTAL PLAIN	32.81282517070	-80.53199215160
Beaufort	CSTL-121	3	Impaired	COOSAWHATCHIE RIVER AT SC 363	Region interest	Mixed Forest	MIDDLE ATLANTIC COASTAL PLAIN	32.85329363360	-81.16110067330
Beaufort	CSTL-122	3	Meets	CYPRESS CREEK AT S-27-108	Region interest	Developed, Low Intensity	MIDDLE ATLANTIC COASTAL PLAIN	32.69581194400	-80.98920127970
Beaufort	CSTL-109	4	Meets	COOSAWHATCHIE RVR AT S-25-27 2.5 MI SW CUMMINGS	Region interest	Emergent Herbaceous Wetland	MIDDLE ATLANTIC COASTAL PLAIN	32.76125649590	-81.02003482000
Beaufort	RS-08076	4	Unassessed	BUCKHEAD CREEK AT US 21	Region interest		MIDDLE ATLANTIC COASTAL PLAIN	33.01826926940	-80.81174363470
Beaufort	CSTL-075	Lake	Meets	LAKE WARREN, BLACK CK ARM, AT S-25-41 5 MI SW OF HAMPTON	Minor Lake	Pasture/Hay	MIDDLE ATLANTIC COASTAL PLAIN	32.82681229170	-81.18030838490
Catawba	CW-088	1	Impaired	GRASSY RUN BR AT SC 72 1.6 MI NE CHESTER	Urban	Cultivated Crops	PIEDMONT	34.71771468050	-81.19228425150
Catawba	CW-036	4	Impaired	SUGAR CREEK AT S-46-36	Urban, NC	Developed, Low Intensity	PIEDMONT	34.95077641670	-80.86868032940
Catawba	CW-016	7	Meets	CATAWBA RVR AT SC 9 AT FT LAWN	Major river below NC	Scrub/Shrub	PIEDMONT	34.70832342570	-80.86756144250
WQM & ABS	C-076	3	Unassessed	CEDAR CK CANOE ACCESS OFF S-40-1288 (SO CEDAR CK RD)	Background/National Park		SOUTHEASTERN PLAINS	33.81840198270	-80.78798276390
WQM & ABS	C-077	3	Unassessed	CEDAR CK - BRIDGE B	Background/National Park		SOUTHEASTERN PLAINS	33.81988312540	-80.82308357150
Cent Mid	B-123	1	Impaired	WINNSBORO BR AT US 321-AB WINNSBORO MILLS OUTFALL	Urban, Golf Course	Woody Wetlands	PIEDMONT	34.37128783600	-81.08935624380
Cent Mid	C-021	1	Impaired	MILL CK AT SC 262	Suburban	Pasture/Hay	SOUTHEASTERN PLAINS	33.98014695560	-80.91460740420
Cent Mid	B-280	2	Impaired	SMITH BR AT N MAIN ST (US 21) IN COLA	Urban, no NPDES	Pasture/Hay	SOUTHEASTERN PLAINS	34.02723011690	-81.04197905580
Cent Mid	S-287	2	Impaired	RAWLS CREEK AT S-32-107	Suburban	Scrub/Shrub	PIEDMONT	34.05384144410	-81.18636514470
Cent Mid	S-306	3	Impaired	HOLLOW CK AT S-32-54	Livestock	Barren Land	PIEDMONT	33.99208232800	-81.46496652760
Cent Mid	C-001	4	Impaired	GILLS CK AT BRDG ON US 76 (GARNERS FERRY ROAD)	Urban	Scrub/Shrub	SOUTHEASTERN PLAINS	33.98965502440	-80.97411392710
Cent Mid	S-298	5	Meets	SALUDA RVR AT USGS GAGING STATION, 1/2 MI BELOW I-20	Urban runoff, rec use area	Scrub/Shrub	PIEDMONT	34.01385513060	-81.08780920450
Cent Mid	C-068	Lake	Meets	FOREST LAKE AT DAM	Lake/ Suburban	Mixed Forest	SOUTHEASTERN PLAINS	34.02199837450	-80.96255680580
Cent Mid	CW-208	Lake	Meets	LK WATEREE AT S-20-101 11 MI ENE WINNSBORO	Major Lake	Developed, High Intensity	PIEDMONT	34.42192264290	-80.86743212470
Cent Mid	S-213	Lake	Meets	LAKE MURRAY AT S-36-15	Major Lake	Scrub/Shrub	PIEDMONT	34.12514632320	-81.43367351170
Charleston	CSTL-043	2	Impaired	SAWMILL BR AT SC 78 E OF SUMMERVILLE	Suburban	Deciduous Forest	MIDDLE ATLANTIC COASTAL PLAIN	33.02228925090	-80.16348542910
Charleston	RS-01056	2	Impaired	CEDAR CREEK AT CNTY RD 857 HAMPTON PLANTATION STATE PARK	Region interest		MIDDLE ATLANTIC COASTAL PLAIN	33.19401262670	-79.45328603470
Charleston	RS-02461	2	Impaired	WADBOO SWAMP AT S-08-447 THIRD BRIDGE FROM WEST	Wildlife	Developed, Open Space	MIDDLE ATLANTIC COASTAL PLAIN	33.30186924210	-79.91208159830
Charleston	RS-05572	2	Impaired	GUM BRANCH AT S-18-167 4.9 MI SE OF ST GEORGE	Domestic Animals	Open Water	MIDDLE ATLANTIC COASTAL PLAIN	33.13206998170	-80.52191265790
Charleston	CSTL-063	3	Meets	WASSAMASSAW SWP AT US 176	Wildlife	Cultivated Crops	MIDDLE ATLANTIC COASTAL PLAIN	33.15092640890	-80.17538101080
Charleston	CSTL-113	3	Meets	WADBOO SWP AT SC 402	Wildlife	Developed, Medium Intensity	MIDDLE ATLANTIC COASTAL PLAIN	33.19531049050	-79.95155117230

DHEC Regional Office	Station	Approx Stream Order	Recreational Use Support	Location Description	Reason, if specified	Majority Land Use within 1/2 mile radius	Level 3 Ecoregion	Latitude	Longitude
Charleston	E-016	3	Meets	POLK SWP AT UNIMP RD S-18-180 2 MI S OF ST GEORGE	Domestic Animals	Mixed Forest	MIDDLE ATLANTIC COASTAL PLAIN	33.15478934790	-80.58505492930
Charleston	E-032	3	Impaired	INDIAN FIELD SWAMP AT S-18-19	Domestic Animals	Grassland/Herbaceous	MIDDLE ATLANTIC COASTAL PLAIN	33.09142760530	-80.51275626720
Charleston	E-109	3	Impaired	POLK SWAMP AT S-18-19	Domestic Animals	Open Water	MIDDLE ATLANTIC COASTAL PLAIN	33.08921301340	-80.52144934860
Charleston	RS-02467	3	Impaired	ECHAW CK AT PITCH LANDING FRANCIS MARION NATL FOREST	Wildlife	Pasture/Hay	MIDDLE ATLANTIC COASTAL PLAIN	33.24736710690	-79.57740951040
Charleston	RS-03333	3	Impaired	CANE GULLEY BRANCH AT S-08-97 6.1 MI NE OF MONCKS CORNER	Wildlife	Grassland/Herbaceous	MIDDLE ATLANTIC COASTAL PLAIN	33.21918222580	-79.91319486020
Charleston	CSTL-112	4	Meets	WAMBAW CK AT EXTENSION OF S-10-857 (BRIDGE NEAR BOAT LANDING)	Wildlife	Developed, Open Space	MIDDLE ATLANTIC COASTAL PLAIN	33.20833204390	-79.46903540410
Florence	PD-065	1	Impaired	GULLEY BR AT S-21-13, TIMROD PARK	Urban	Mixed Forest	SOUTHEASTERN PLAINS	34.18339141950	-79.76975666380
Florence	PD-258	1	Impaired	SNAKE BR AT RR AVE IN HARTSVILLE	Urban	Emergent Herbaceous Wetland	SOUTHEASTERN PLAINS	34.37370233310	-80.06077039840
Florence	PD-167	3	Impaired	WILLOW CREEK AT S-21-57	Urban - Looks Ag to us	Developed, Low Intensity	SOUTHEASTERN PLAINS	34.11052697700	-79.59418241890
Florence	PD-230	3	Impaired	MIDDLE SWP AT SC 51 3.5 MI SSE OF FLORENCE	Urban, Swamp	Developed, High Intensity	SOUTHEASTERN PLAINS	34.15195293970	-79.73473349950
Florence	PD-256	3	Impaired	JEFFRIES CK AT S-21-112 4.8 MI W OF FLORENCE	AG	Emergent Herbaceous Wetland	SOUTHEASTERN PLAINS	34.18613706210	-79.85134708150
Florence	PD-346	3	Impaired	CAMP BRANCH AT S-21-278	Urban	Open Water	MIDDLE ATLANTIC COASTAL PLAIN	33.88170522410	-79.76394561040
Florence	PD-353	3	Meets	BLACK RIVER AT S-43-57	AG	Mixed Forest	SOUTHEASTERN PLAINS	33.95034608990	-80.17971577290
Florence	PD-035	4	Meets	JEFFERIES CK AT SC 327 AT CLAUSSEN	Urban		SOUTHEASTERN PLAINS	34.13580514330	-79.63162556920
Florence	PD-367	4	Meets	THREE CREEKS AT SC 38, S OF BLENHEIM	AG	Emergent Herbaceous Wetland	SOUTHEASTERN PLAINS	34.50019416950	-79.64991137960
Florence	PD-027	5	Meets	BLACK CK AT S-16-35 5.5 MI SE DARLINGTON	AG	Grassland/Herbaceous	SOUTHEASTERN PLAINS	34.27074444190	-79.78645668570
Florence	PD-041	5	Meets	LYNCHES RVR AT US 52 NEAR EFFINGHAM	WWTP, AG	Developed, High Intensity	MIDDLE ATLANTIC COASTAL PLAIN	34.05149422450	-79.75360879280
Florence	PD-042	6	Meets	LITTLE PEE DEE RVR AT US 501, GALIVANT'S FERRY	WWTP, AG	Deciduous Forest	MIDDLE ATLANTIC COASTAL PLAIN	34.05705586880	-79.24781846830
Florence	PD-337	7	Meets	GREAT PEE DEE RVR AT US 301/76	WWTP, AG	Emergent Herbaceous Wetland	SOUTHEASTERN PLAINS	34.20357138570	-79.54927014080
Florence	CL-077	Lake	Meets	LAKE ASHWOOD, FOREBAY MOVED TO CATWALK NEAR DAM	Lake / AG	Mixed Forest	SOUTHEASTERN PLAINS	34.09966293210	-80.31662730190
Florence	PD-081	Lake	Meets	PRESTWOOD LK AT US 15	Lake / Urban		SOUTHEASTERN PLAINS	34.38633662720	-80.07679206260
Greenville	BE-035	2	Impaired	BRUSHY CK AT HOWELL RD (S-23-273/335) APPROX 5 MI NE OF GREENVILLE (BIO B-798)	Urban	Mixed Forest	PIEDMONT	34.87876816270	-82.33074176680
Greenville	RS-02330	2	Meets	ADAMS CK AT UNPVD RD FROM SC 8 AND END OF S-39-34	Rural	Mixed Forest	PIEDMONT	34.98722804850	-82.65660682860
Greenville	SV-341	2	Impaired	LITTLE EASTATOE CREEK AT S-39-49	Blue Ridge ecoregion	Scrub/Shrub	BLUE RIDGE	34.94919541370	-82.83309687680
Greenville	B-246	3	Impaired	BEAVERDAM CK AT S-30-97, 7 MI NE OF GRAY COURT	Septic, Livestock, SPST	Developed, Medium Intensity	PIEDMONT	34.64623594580	-81.99552644730
Greenville	B-317	3	Impaired	MUSH CK AT SC 253 BL TIGERVILLE	SPST	Open Water	PIEDMONT	35.05487919140	-82.36721541670
Greenville	S-319	3	Impaired	REEDY RVR AT RIVERS ST, DOWNTOWN GREENVILLE	Urban, Local Interest	Deciduous Forest	PIEDMONT	34.84489991260	-82.40167687050
Greenville	SV-230	3	Meets	EASTATOE CREEK AT S-39-143	Reference	Scrub/Shrub	BLUE RIDGE	34.95812944850	-82.85319035840
Greenville	SV-342	3	Impaired	CANE CREEK AT S-37-133	Local Interest, upper Piedmont ecoregion	Mixed Forest	PIEDMONT	34.76653493770	-83.02571045320
Greenville	SV-343	3	Impaired	LITTLE CANE CREEK AT S-37-133	Local Interest, upper Piedmont ecoregion	Mixed Forest	PIEDMONT	34.76926882230	-83.01150169190
Greenville	BL-001	4	Impaired	LAWSONS FORK CK AT S-42-108	Local Interest	Developed, Low Intensity	PIEDMONT	34.94370037520	-81.78854862910
Greenville	S-004	4	Impaired	N SALUDA RVR AT BRDG AB JCT WITH SALUDA RVR E OF SC 186	SPST, Local Interest	Scrub/Shrub	PIEDMONT	34.97890320770	-82.52201756330
Greenville	B-339	Lake	Meets	LAKE BOWEN 0.3 MI W OF SC 9	Lake/ Bridge	Mixed Forest	PIEDMONT	35.11285121980	-82.04553096510
Greenville	RL-02307	Lake	Meets	LAKE OOLENOY SAMPLED FROM S SIDE OF SC 11 BRIDGE	Minor Lake	Scrub/Shrub	PIEDMONT	35.02157963190	-82.69494984370
Greenville	RL-04461	Lake	Meets	LAKE BLALOCK AT US 221	Minor Lake	Barren Land	PIEDMONT	35.06693019040	-81.87737030480
Greenville	SV-288	Lake	Meets	LK HARTWELL, SENECA RVR ARM AT USACE BUOY BTWN MRKRS S-28A & S-29	Major Lake	Mixed Forest	PIEDMONT	34.52647023750	-82.81533736630

Attachment 4. Weekly Pathogen Monitoring Sites Map


B-339
B-317
RL-02307
RL-04461
SV-341
S-004
BE-001
SV-230
S-319
SV-342
BE-025
SV-343
B-246
CW-036
CW-088
CW-016
SV-288
CW-208
B-123
PD-367
PD-081
PD-027
PD-258
PD-256
PD-337
PD-035
CL-074
PD-041
PD-042
PD-167
PD-353
PD-346
S-213
S-298
CL-068
C-021
S-287
C-001
S-306
SV-291
SV-353
C-076
C-077
SV-072
E-107
E-007
E-059
SV-366
SV-324
SV-325
RS-02461
RS-02467
CSTL-112
CSTL-063
RS-03333
E-109
E-032
RS-08260
E-016
CSTL-043
RS-01056
RS-08076
CSTL-121
CSTL-075
CSTL-071
CSTL-122
CSTL-108
Weekly Pathogen Monitoring Sites
WeeklyStats
DISTRICT
Aiken
Beaufort
Catawba
Cent Mid
Charleston
Florence
Greenville
WQM & ABS

Attachment 5. Statistical Evaluation Summary and R Script and 349 Calculation

Evaluation of Freshwater Recreational Uses and Bacteria
Evaluation of Alternative Indicators
Updated 6/28/2011

Note: The fecal coliform vs. E. coli regressions (Figures 1 and 2) were recalculated on 6/28/2011 using the final QA/QC'd public export raw data (link below and included on the enclosed CD). The R script used to conduct the actual correlation and regression analyses is included in Appendix A.

Introduction

For an overview, introduction, and background of this effort, please visit http://www.scdhec.gov/environment/water/fwater.htm.

The project Quality Assurance Project Plan can be found at http://www.dhec.sc.gov/environment/water/docs/fw_EAFPI.pdf and the Addendum to the plan is available at http://www.scdhec.gov/environment/water/docs/fw_PISQAPP.pdf.

A list of the sampling sites can be found at http://www.dhec.sc.gov/environment/water/docs/fw_weekly.pdf, with a general map of the sampling locations at http://www.dhec.sc.gov/environment/water/docs/fw_weeklym.pdf.

The raw data is available via a link from http://www.scdhec.gov/environment/water/fw_PIS.htm.

Results and Data Evaluation

Weekly sampling for three pathogen indicators: fecal coliform bacteria, *Escherichia coli*, and *Enterococcus*, was conducted at 73 locations during 2009. From January 5, 2009 through December 30, 2009, there were a total of10, 922 analyses conducted of which: 3,717 were for fecal coliform bacteria, 3,602 for *Escherichia coli*, and 3,603 for *Enterococcus*.

Statistical analyses of the resulting data were performed using R (2009, R Development Core Team, http://www.R-project.org). The statistical analyses excluded censored data as discussed below.

For microbial analyses, dilution of the sample is often necessary to obtain concentrations within a quantifiable range. With different dilution factors, this can result in a variety of different "Less Than" or "Greater Than", or "Estimated" values when the resulting value is not within the quantifiable range. Censored data are those where an individual number is not known, but it is known that the value is below or above a threshold ("Less Than", "Greater Than", or "Estimated").

Correlation or regression of data where censored data are present can alter the variation from what would have occurred in nature and introduce error in the estimates of the relationships between the variables being compared. Therefore, all of the analytical analyses presented and discussed below are based on only the uncensored data with all values reported as Greater Than (GT), Less Than (LT), or Estimated excluded.

Bacteria commonly reproduce by an asexual kind of cell division called binary fission, whereby a single bacterial cell divides into two identical cells. Under favorable conditions, this results in logarithmic population growth, a very rapid form of growth where the population initially doubles, then quadruples, then grows to 8 times the original number, then16 times, 32 times, etc. Arithmetic evaluations of such populations are often improved by transforming the raw data to logarithmic values prior to statistical analyses. Therefore, all of the analyses were conducted using both raw values and log base 10 transformed data.

Correlations were evaluated using Pearson's Product-Moment Correlations for fecal coliform bacteria vs. *Escherichia coli*, and fecal coliform bacteria vs. *Enterococcus* (Tables 1.).

To examine relationships between the different indicators tested, regressions were performed on the same data sets. Because the measures of all the indicators compared have associated measurement errors, simple linear regression is not suitable. A more appropriate regression method in such situations is the orthogonal least squares regression. To illustrate this difference, in Figures 1-4 a simple linear regression line is also included as the red dashed line.

Table 1. Pearson Product-Moment Correlation Results, Uncensored Data Only

Comparison	Correlation Coefficient	Lower 95th Percent Confidence Interval	Upper 95th Percent Confidence Interval
Fecal coliform vs. *E. coli*	0.8102	0.7967	0.8230
Log10 Fecal coliform vs. Log10 *E. coli*	0.8765	0.8673	0.8851
Fecal coliform vs. *Enterococcus*	0.3826	0.3488	0.4154
Log10 Fecal coliform vs. Log10 *Enterococcus*	0.6930	0.6722	0.7128

Figure 1. Fecal Coliform vs. E. Coli
Updated Analysis 6-28-2011

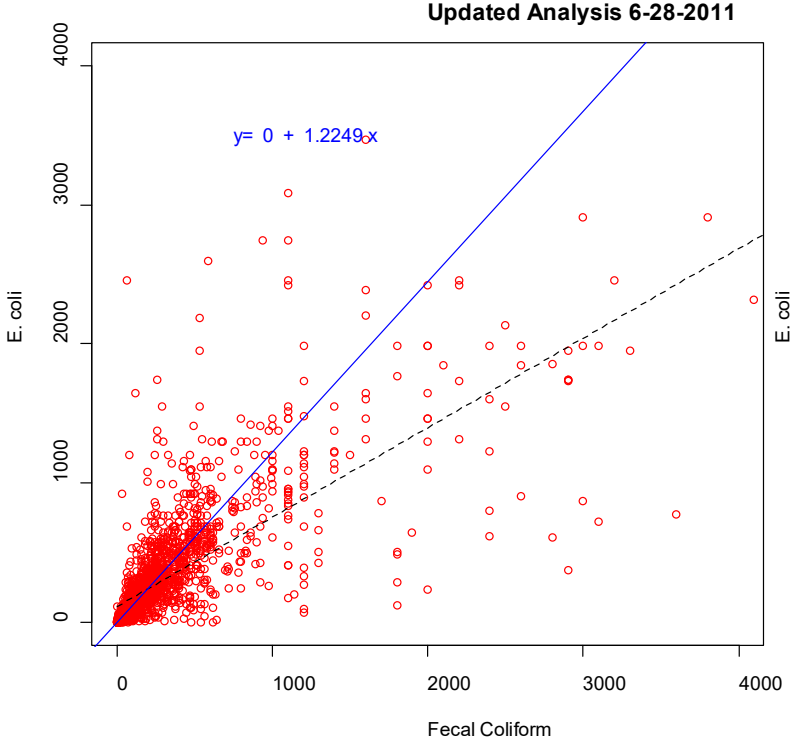


Figure 2. Log10 Fecal Coliform vs.
Updated Analysis 6-28-2011

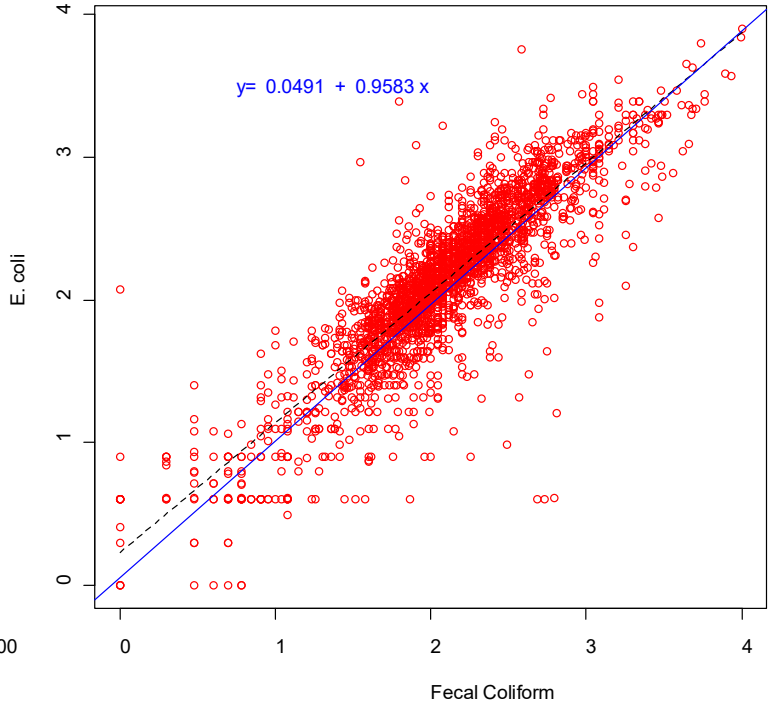


Figure 3. Fecal Coliform vs Enteroc

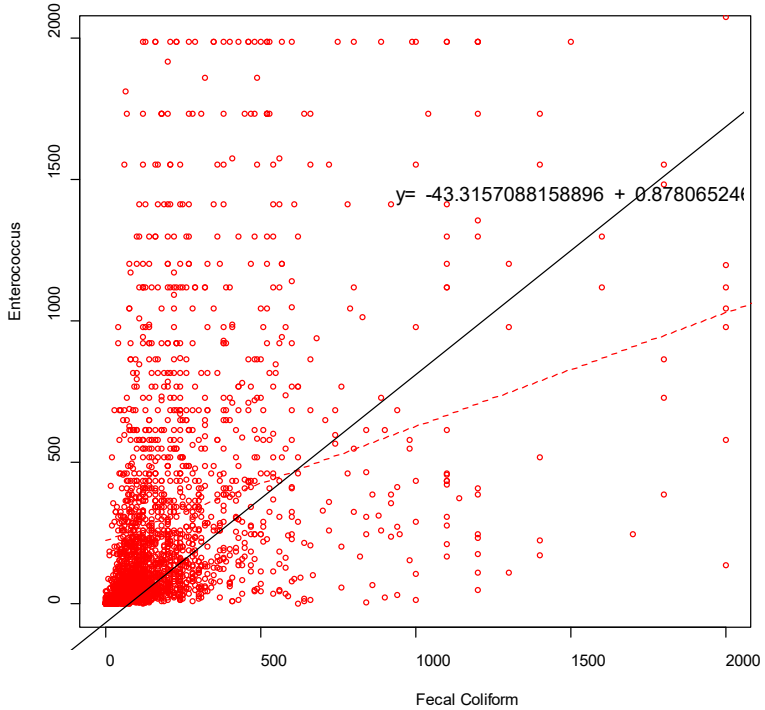
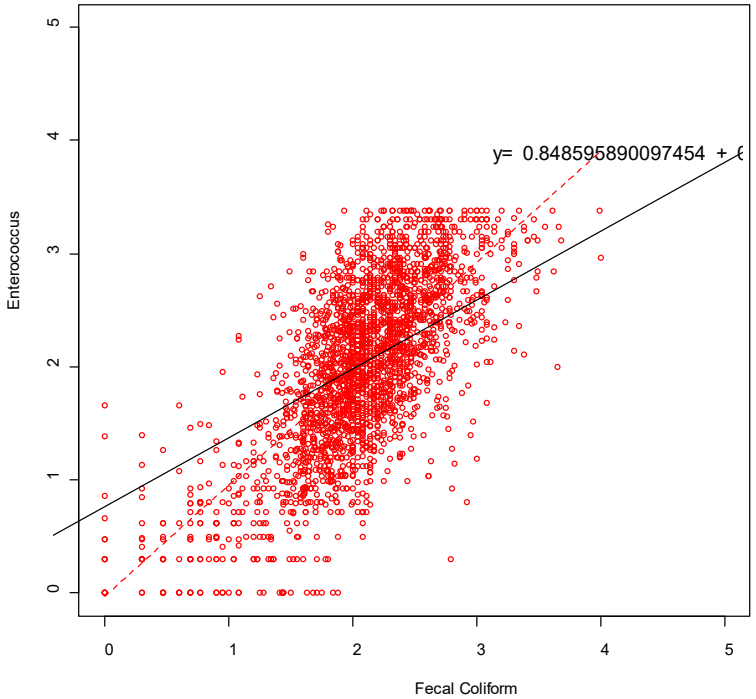


Figure 4. Log10 Fecal Coliform vs.



Discussion

In its 1986 Ambient Water Quality Criteria for Bacteria (EPA440/5-84-002) http://www.epa.gov/waterscience/beaches/files/1986crit.pdf, EPA's results of freshwater illness studies indicated that both *E. coli* and *Enterococcus* showed strong relationships to the occurrence of gastroenteritis in swimmers, although none of the indicators tests showed relationships to other illnesses investigated.

EPA concluded that the similarities in the relationships of *E. coli* and enterococci to swimming associated gastroenteritis in freshwater indicate that these two indicators are equally efficient for monitoring water quality in freshwater. The correlation coefficient for *E. coli* (0.80) was slightly greater than that for enterococci, (0.74) however, statistical analysis indicated that the two values were not significantly different.

EPA also stated that *E. coli* is the most fecal specific of the coliform indicators.

A cursory survey of State water quality standards available online gives the following picture of current freshwater regulatory pathogen indicators:

Table 2. Summary of Freshwater Pathogen Indicators in State Water Quality Standards

Indicator(s)	Number of States
E. coli	23
E. coli & Fecal coliform (several in transition to *E. coli* only)	7
Enterococcus	2
Enterococcus & *E. coli*	3
Enterococcus & Fecal coliform	1
Fecal coliform	11
Fecal coliform in general, *E. coli* waterbody specific	1
Fecal coliform – unclear about other indicators	1

The correlation analyses for this study (Table 1.) indicate that of the evaluated alternative pathogen indicators, *E. coli* is most closely correlated with the historic fecal coliform bacteria indicator. This is also supported by the regression analyses. Figures 1 and 2, illustrate better relationships between the current fecal coliform indicator and *E. coli* than between fecal coliforms and *Enterococcus* (Figs. 3 and 4).

Derivation of a Single Sample Maximum Allowable Density

EPA considers that illness rates of 10 illnesses per 1000 swimmers (1.0%) or less for fresh waters are as protective of human health as the 1986 bacteria criteria (Water Quality Standards For Coastal Recreation Waters, Considerations for States as They Select Appropriate Risk Levels, EPA September 2006, EPA-823-F-06-012 (http://www.epa.gov/waterscience/beaches/rules/bacteria-risk-level-factsheet.htm#whatdoes); Implementation Guidance for Ambient Water Quality Criteria for Bacteria, USEPA November 2003 Draft; Federal Register: November 16, 2004 (Volume 69, Number 220),Rules and Regulations, pp 67217-67243, page 67232 (http://www.epa.gov/EPA-WATER/2004/November/Day-16/w25303.htm). All of the illness rates cited below are considered by the USEPA to protect primary contact recreation and support the swimmable goal of the Clean Water Act.

- 8 illnesses per 1000 swimmers
- 9 illnesses per 1000 swimmers
- 10 illnesses per 1000 swimmers

An *E. coli* concentration equivalent to a fecal coliform bacteria density of 400 per 100 ml (the current maximum for fecal coliform bacteria not to be exceeded by more than 10% of the total samples during any 30 day period) was calculated using the regression formula from Figure 2 as below.

Log10(Y)=0.0491+0.9583Log10(x)
Log10(E. coli)=0.0491+0.9583Log10(Fecal Coliform)
Log10(E. coli)=0.0491+0.9583Log10(400)
Log10(E. coli) =2.5426
E. coli $=10^{2.5426}$
E. coli =348.8
E. coli 349= Fecal Coliform 400

The single sample maximum allowable *E. coli* density of 349 per 100 ml falls between the 1986 criteria single sample maxima for Moderate and Lightly Used Full Body Contact Recreation at 8 illnesses per 1000 swimmers. This value had a great deal of consensus support from the stakeholder community that participated in the discussions leading to the pathogen indicator change. It is also based on the most restrictive illness rate suggested by the EPA of 8 illnesses per 1000 swimmers, as is the current fecal coliform standards.

Appendix A
R Script Used For Correlation and Regression Analyses

```
################################################################################
#
#R Code to do the Orthogonal Regression anlysis
#Data set used was the public export that is avaliable on the SCDHEC website
with
#      duplicate dates, times, and results removed via Microsoft Access
#      In two instances there were two fecal values for a given station, date,
#      and time CSTL-109 4/15/2009 and SV-325 12/7/2009 the lower of each value
#      was deleted.  Also created were separate fields for date and time.  This
#      way the joins were done correctly with out any manipulation in R.
#
#Last Modified by
#Bryan Rabon (raboneb@dhec.sc.gov)
#on 6/28/2011
#This code was run under R version 2.10.1 (2009-12-14)
################################################################################
#

################################################################################
#
#Adding the required libraries
library(xlsReadWrite)
library(car)

################################################################################
#
#Function used to rename a known column in an unknown location in a dataframe
ReName <- function(x, replace) {
      replacement <-  replace[names(x)]
      names(x)[!is.na(replacement)] <- replacement[!is.na(replacement)]
      x
}

################################################################################
#
#Dataset is read in using the xlsReadWrite library
ALLDATA <- read.xls("PubExp.xls", colNames = TRUE,
                          colClasses = c('character',
'character','isodatetime',
                          'character','character','numeric','character',
                          'character','character'))

################################################################################
#
#Remove all data that is not an actual value,
#i.e. EST or LT or GT
ALLDATA <- subset(ALLDATA, REMARK == "")

################################################################################
#
#Create the fecal dataset
FECALALL <- subset(ALLDATA, PARAMETER_CODE == "31616",
  select=c(STATION,DATE_ONLY,TIME_ONLY,RESULT, REMARK))
```

```
################################################################################
#
#Create the Ecoli dataset
ECOLIALL <- subset(ALLDATA, PARAMETER_CODE == "31633",
  select=c(STATION,DATE_ONLY,TIME_ONLY,RESULT, REMARK))

################################################################################
#
#Calculate the Log10 values for each record in a new field
FECALALL$logfecal <- with(FECALALL, log10(RESULT))
ECOLIALL$logecoli <- with(ECOLIALL, log10(RESULT))

################################################################################
#
#Rename the result column in each of the datasets to the name of the parameter
#in the dataset
FECALALL <- ReName(FECALALL,c(RESULT="FECAL"))
ECOLIALL <- ReName(ECOLIALL,c(RESULT="ECOLI"))

################################################################################
#
#Join the two datasets together based on the records that are matched by
#          Station, date, and time
ECOLIFECAL <- merge(FECALALL, ECOLIALL)

################################################################################
#
#Start the file to hold the correlation
sink(file= "pathresults.txt")

################################################################################
#
#Correlation between Fecal and Ecoli
cor.test(ECOLIFECAL$FECAL, ECOLIFECAL$ECOLI)

################################################################################
#
#Correlation between the Log10 of Fecal and Ecoli
cor.test(ECOLIFECAL$logfecal, ECOLIFECAL$logecoli)

################################################################################
#
#Close the file holding the output
sink()


################################################################################
#
#Doing the orthogonal regression for Log10 Fecal and Ecoli

#The two datasets this section will use
fecal <- subset(ECOLIFECAL, select = c(FECAL,logfecal))
ecoli <- subset(ECOLIFECAL, select = c(ECOLI,logecoli))

################################################################################
#
```

```
#seting the starting point for the optimization
x <- c(0,1)

################################################################################
#
#The orthogonal regression function for the log10 data
elllog <- function(x)
           {sum(((fecal$logfecal - (x[1] +
x[2]*ecoli$logecoli))^2)/(1+x[2]^2))}

################################################################################
#
#Running the optimiztion
elllogopt <- optim(x,elllog,lower =c(0,0), hessian = TRUE, method = "L-BFGS-B")

################################################################################
#
#LOG10 PLOT

################################################################################
#
#Creating the graphic device to store the plot
win.metafile("FecalvsEcoliLogNoLTGTEST.wmf")

################################################################################
#
#Attaching the dataset making it easier to work with the columns of data so the
#data can be called by just the column name and not DATAFRAME$COLUMNNAME
attach(ECOLIFECAL)

################################################################################
#
#Creating the scatter plot with a linear least squares regression plotted as a
#                dashed line
scatterplot(logecoli~logfecal, reg.line=lm, lty = 2, smooth=FALSE,
labels=FALSE,
              boxplots=FALSE, span=0.5, xlab="Fecal Coliform", ylab="E. coli",
              grid = FALSE)

################################################################################
#
#Adding the Title
title(
"Figure 2. Log10 Fecal Coliform vs. Log10 E. coli\nUpdated Analysis 6-28-2011"
)

################################################################################
#
#Adding the optimized orthogonal regression line to the plot as a blue line
abline(elllogopt$par[1],elllogopt$par[2], col = "blue")

################################################################################
#
#Adding the formula to the plot as blue text
equaplot <- paste("y= ",round(elllogopt$par[1],4), " + ",
                        round(elllogopt$par[2],4), "x")
text(.75, 3.5, equaplot, col = "blue")
```

```
#############################################################################
#
#Closing the graphic device so the plot is saved
dev.off()

#############################################################################
#
#Doing the orthogonal regression for untransformed Fecal and Ecoli

#############################################################################
#
#The orthogonal regression function for the untransformed data
ell <- function(x)
         {sum(((fecal$FECAL - (x[1] + x[2]*ecoli$ECOLI))^2)/(1+x[2]^2))}

#############################################################################
#
#Running the optimiztion
ellopt <- optim(x,ell,lower =c(0,0), hessian = TRUE, method = "L-BFGS-B")

#############################################################################
#
#Creating the graphic device to store the plot
win.metafile("FecalvsEcoliNoLTGTEST.wmf")

#############################################################################
#
#Creating the scatter plot with a linear least squares regression plotted as a
#                dashed line
scatterplot(ECOLI~FECAL, reg.line=lm, lty = 2, smooth=FALSE, labels=FALSE,
               boxplots=FALSE, span=0.5, xlab="Fecal Coliform", ylab="E. coli",
               xlim = c(0,4000), ylim = c(0,4000),grid = FALSE)

#############################################################################
#
#Adding the Title
title("Figure 1. Fecal Coliform vs. E. Coli\nUpdated Analysis 6-28-2011")

#############################################################################
#
#Adding the optimized orthogonal regression line to the plot as a blue line
abline(ellopt$par[1],ellopt$par[2], col = "blue")

#############################################################################
#
#Adding the formula to the plot as blue text
equaplot2 <- paste("y= ",round(ellopt$par[1],4), " + ",
                         round(ellopt$par[2],4), "x")
text(750, 3500, equaplot2, col = "blue")

#############################################################################
#
#Detaching the dataset for clean up
detach(ECOLIFECAL)
```

```
################################################################################
#
#Closing the graphic device so the plot is saved
dev.off()
```

Attachment 6. EPA Approval Letter

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 4
ATLANTA FEDERAL CENTER
61 FORSYTH STREET
ATLANTA, GEORGIA 30303-8960

FEB 28 2013

Mr. David Wilson, Chief
Bureau of Water
South Carolina Department of Health and
Environmental Control
2600 Bull Street
Columbia, South Carolina 29201-1708

Dear Mr. Wilson:

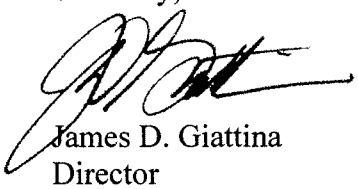
The purpose of this letter is to notify you that the U.S. Environmental Protection Agency is approving revisions to South Carolina Regulation 61-68, *Water Classifications and Standards*, and Regulation 61-69, *Classified* Waters, as revisions to South Carolina's water quality standards. These revisions were duly promulgated by the Board of the Department of Health and Environmental Control (DHEC) and became effective for purposes of State law upon publication in the State Register on June 22, 2012. These revisions include the removal of the fecal coliform indicator and adoption of the *E. coli* indicator for recreational uses in Freshwaters of the State and other editorial revisions.

These revisions were submitted for the EPA review by letter dated July 2, 2012 from W. Marshall Taylor, Jr., General Counsel for the South Carolina DHEC to Gwendolyn Keyes Fleming, Regional Administrator of the EPA's Region 4 Office. The State's request for review included a certification by the DHEC's General Counsel that the revisions were duly adopted pursuant to State law.

In accordance with Section 303(c) of the Clean Water Act (CWA) and 40 Code of Federal Regulations Part 131, I am hereby approving these revisions to the State water quality standards with the following exceptions. EPA is not taking action on the following revisions because they fall outside the scope of Section 303(c) of the Act and are not considered water quality standards: R.61-68.E.14.c(8)-(14) inclusive, R.61-68.E.14.d(4), and R.61-68.H.8-10 inclusive. The first two sets of revisions constitute permit limit derivation/compliance determinations or assessment procedures for Section 303(d) listing and have been referred to the appropriate programs in the Region for further review. The revisions to Section H are related to the State groundwater classifications and outside the scope of the Clean Water Act. The EPA is additionally not taking action on the following language in R.61-68.G.10.f: '…based on at least four samples collected from a given sampling site…' as this language is determined to be related to assessment procedures within the State and not to be a water quality standard. Finally, EPA published updated primary contact recreation criteria in November 2012 and the State is encouraged to review this criterion and associated implementation recommendations during its next triennial review. The EPA will monitor the implementation of all of the approved revisions to ensure consistency with the CWA and the appropriate implementing regulations.

These revisions to the State's water quality standards represent your Department's continuing efforts to protect and enhance the quality of South Carolina's waters. You and your staff are to be congratulated on your efforts and accomplishments reflected in the adopted water quality standards.

Sincerely,



James D. Giattina
Director
Water Protection Division