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

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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**

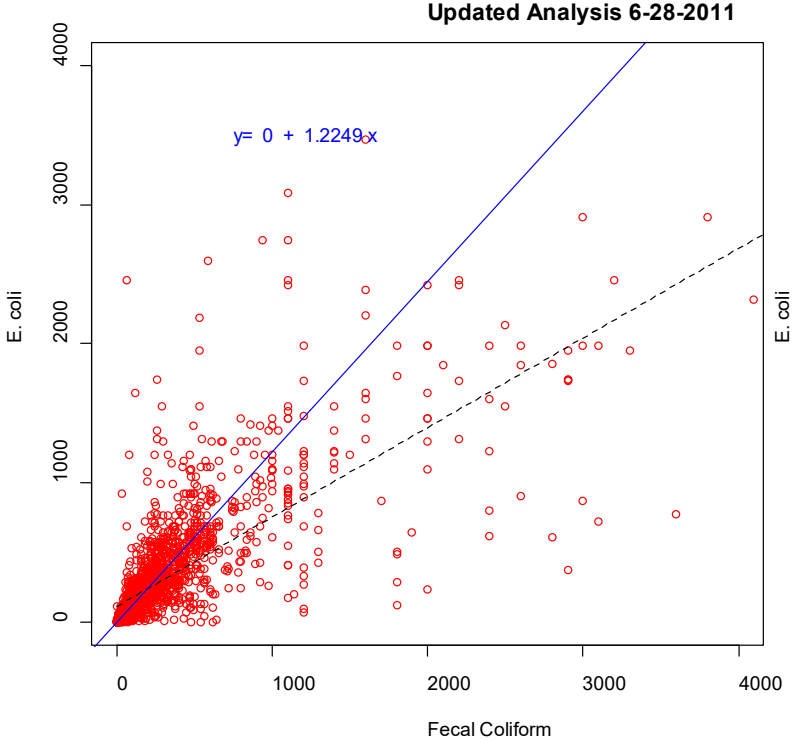


**Figure 2. Log10 Fecal Coliform vs.**
**Updated Analysis 6-28-2011**

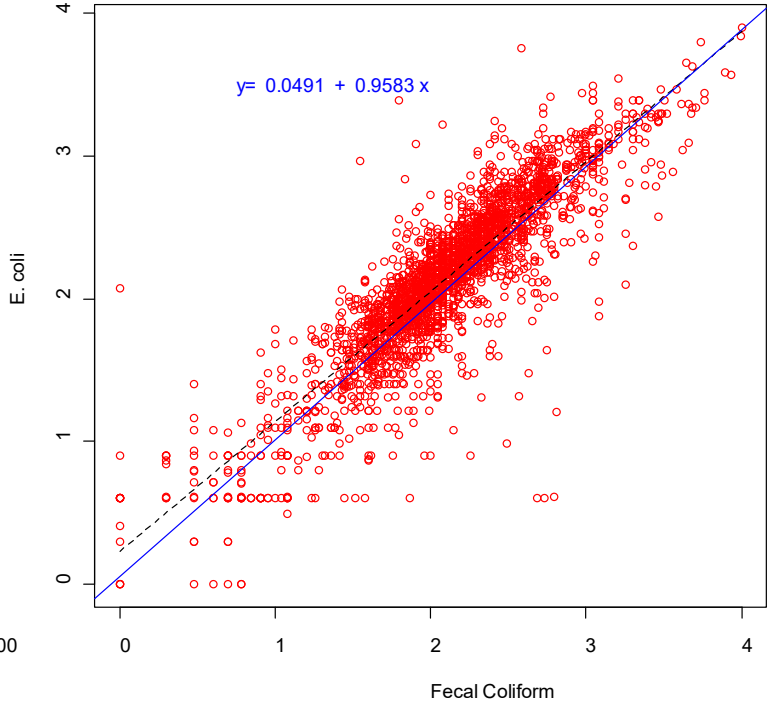


**Figure 3. Fecal Coliform vs Enteroc**

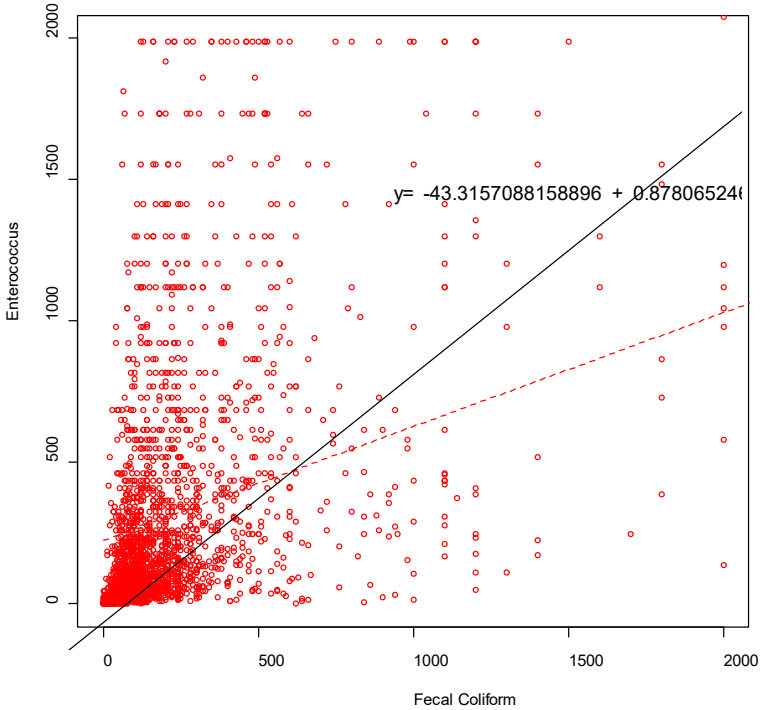


**Figure 4. Log10 Fecal Coliform vs.**

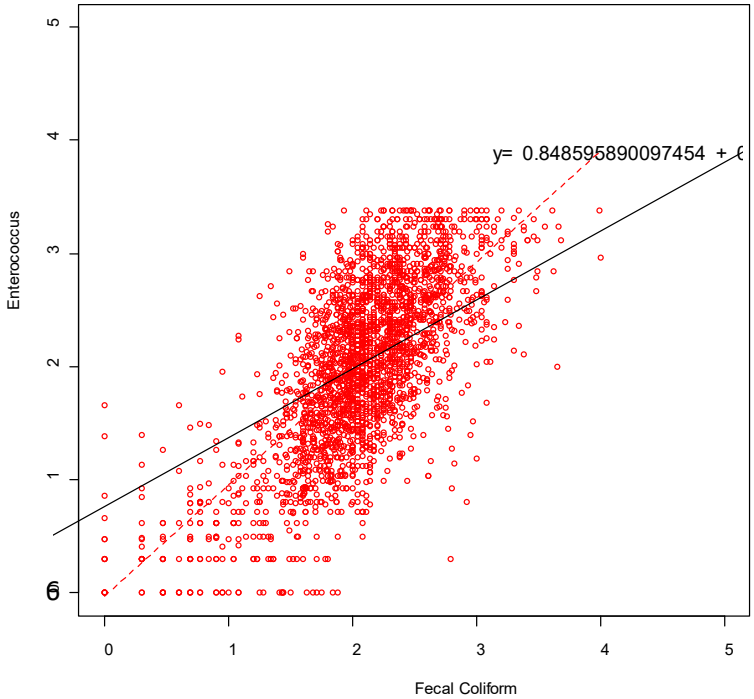

**Table 2. Recommended 2012 RWQC**

| 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

Attachment 2. Evaluation of Alternative Freshwater Pathogen Indicators

QAPP Addendum – June 1, 2009

Attachment 3. Weekly Pathogen Indicator Study Sites

Attachment 4. Weekly Pathogen Monitoring Sites Map

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

Attachment 6. EPA Approval Letter