Contract 17-04
Anguilla Brook Bacteria Trackdown
and Watershed-Based Plan

Eastern Connecticut Conservation District
January 28, 2020

Task 1d – Conduct Water Quality Sampling
Introduction

The Eastern Connecticut Conservation District (ECCD) has received funding from the Connecticut Department of Energy and Environmental Protection (CT DEEP) through the Clean Water Act Section 319 Nonpoint Source program to conduct water quality sampling of perennial streams in North Stonington and Stonington, Connecticut that discharge to Wequetequock Cove and develop a watershed-based plan for the Anguilla Brook watershed (Fig. 1).

Wequetequock Cove is an embayment of Little Narragansett Bay, located at the outfall of the Pawcatuck River at the boundary between the States of Connecticut and Rhode Island. The Inner Wequetequock Cove estuary (CT_E1_003), located in Stonington, is listed in the State of Connecticut’s biennial Integrated Water Quality Report to Congress as not meeting its designated uses for habitat for marine fish, other aquatic life and wildlife, recreation, and direct consumption of shellfish due to estuarine bioassessments, excess algal growth, and high levels of enterococcus and fecal coliform bacteria, respectively (CT DEEP, 2017). Potential pollutant sources include stormwater, agricultural activities, and other unidentified upstream sources.

ECCD conducted bacteria sampling from June to August of 2019. Sampled streams include Anguilla Brook (CT2101-00_01), Wheeler Brook (CT2101-01_01), an unnamed tributary to Wheeler Brook (CT32101-02_01), and Donahue Brook (CT2101-03_01). The purpose of the bacteria sampling was to quantify fecal bacteria levels in the streams in order to determine if watershed sources are contributing to fecal bacteria documented in Inner Wequetequock Cove. The bacteria data, along with other project data, will be used to develop a watershed-based plan for the Anguilla Brook watershed.
**Procedure**

**Monitoring Plan and Quality Assurance Project Plan**

ECCD developed a monitoring plan (Attachment A) for the Anguilla Brook bacteria trackdown in consultation with DEEP TMDL Program staff and a review and incorporation of recommendations made in the Stonington Estuary Bacteria Total Maximum Daily Load (TMDL) Summary (Appendix 12) (CT DEEP, 2013). The monitoring plan outlined the procedure that ECCD would use to conduct bacteria sampling and identified bacteria sampling sites. The monitoring plan was approved by CT DEEP on March 28, 2019. In order to ensure that water quality data would be collected using the generally accepted sample collection protocols, ECCD revised a previously approved Bacteria Sampling Quality Assurance Project Plan (QAPP) (Attachment B). ECCD received approval for the Bacteria Sampling QAPP (EPA Tracking # RFA 19088) from CT DEEP on 4/29/19 and EPA on 6/10/19.

**Volunteer Recruitment and Training**

In cooperation with The Last Green Valley Volunteer (TLGV) Water Quality Monitoring program, ECCD held a bacteria sampling workshop for water quality volunteers on May 20, 2019 at ECCD’s Norwich office. Volunteers were solicited from among the Anguilla Brook Trackdown Project stakeholder group and the TLGV Water Quality Monitoring program. During the workshop, volunteers were trained in the sample collection method outlined in the Anguilla Brook Bacteria Sampling QAPP to ensure that all samples would be directly comparable.

**Bacteria Sample Collection**

Prior to the commencement of bacteria sample collection, ECCD identified eleven sites along the Anguilla Brook and its tributaries to be sampled (Table 1 and Fig. 2). The sampling sites were selected to quantify bacteria levels in watershed streams based in part on a review of local land use (Fig. 3). ECCD also considered recommendations made in the (Appendix 12) of *A Statewide Total Maximum Daily Load Analysis for Stonington Estuary Summary Bacteria Impaired Waters* (CT DEEP, 2012). The sampling sites were numbered sequentially, beginning with the designation of the downstream-most site on each stream as ‘01’ and proceeding numerically upstream. Named tributaries were identified by their initials (e.g. Wheeler Brook was called ‘WB’). The single unnamed tributary was designated as ‘UN’. For example, the downstream-most site on Anguilla Brook was designated AB-01; the upstream-most site was designated AB-04, and the two additional sampling sites in between were designated AB-02 and AB-03.
Table 1. Anguilla Brook Bacteria Trackdown project bacteria sampling sites.

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Stream Name</th>
<th>Location/Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-01</td>
<td>Anguilla Brook</td>
<td>Off Trolley Crossing – at outlet of Wequetequock Pond. Downstream-most site; above salt-water limit</td>
</tr>
<tr>
<td>AB-02</td>
<td>Anguilla Brook</td>
<td>At RT 1 Handlebar Plaza – same as UConn PATH sampling site – downstream of agricultural and commercial uses</td>
</tr>
<tr>
<td>AB-03</td>
<td>Anguilla Brook</td>
<td>End of Anguilla Brook Road – downstream of residential, agriculture and recreational uses</td>
</tr>
<tr>
<td>AB-04</td>
<td>Anguilla Brook</td>
<td>AT RT 184 – upstream-most site; downstream of agricultural uses</td>
</tr>
<tr>
<td>DB-01</td>
<td>Donahue Brook</td>
<td>US of confluence with Oxocossett Brook above salt-water limit; downstream of agricultural uses; tributary to Wequetequock Cove</td>
</tr>
<tr>
<td>DB-02</td>
<td>Donahue Brook</td>
<td>At Barnes Road – upstream-most site</td>
</tr>
<tr>
<td>WB-01</td>
<td>Wheeler Brook</td>
<td>Upstream of the confluence with Anguilla Brook at end of Miner Pentway; downstream of agricultural uses</td>
</tr>
<tr>
<td>WB-02</td>
<td>Wheeler Brook</td>
<td>At Taugwonk Road – upstream of the confluence with Stony Brook; downstream of agricultural uses</td>
</tr>
<tr>
<td>WB-03</td>
<td>Wheeler Brook</td>
<td>At RT 184 – upstream-most site; downstream of residential use</td>
</tr>
<tr>
<td>UN-01</td>
<td>Unnamed stream</td>
<td>Upstream of confluence with Wheeler Brook; downstream of agricultural uses</td>
</tr>
<tr>
<td>UN-02</td>
<td>Unnamed stream</td>
<td>At Stony Brook Rd – upstream-most site</td>
</tr>
</tbody>
</table>

Water samples were collected once a week for ten weeks, beginning June 24th and ending August 26th, utilizing the QAPP protocols in accordance with the approved monitoring plan. Water samples were collected by hand or via an extension pole, using sterilized 125 ml Nalgene collection bottles provided by the CT Department of Public Health. In order to ensure quality control, on each sampling day one duplicate and one blank sample was collected for every ten samples. The locations of the duplicate and blank sample sites were determined using a random number generator. Duplicate samples were collected side-by-side to ensure they were accurately representative of the same water condition. Butterfield’s buffer solution was used for the blank sample. Water samples were placed on ice in a cooler during the sampling process. Water samples were delivered to Ledge Light Health District (LLHD) in New London, CT., where they were picked up by a Connecticut Department of Public Health (DPH) courier and delivered to the DPH Laboratory in Rocky Hill, CT., for processing. Water samples from all the sampling sites were analyzed for *Escherichia coli* (E. coli). Additionally, water samples collected at the lowermost site on Anguilla Brook (AB-01), were analyzed for fecal coliform and Enterococcus. Bacteria analysis results were reported to Ledge Light Health District and relayed to ECCD by LLHD staff. Bacteria results were tabulated and evaluated by ECCD. Data was submitted to CT DEEP in October 2019 for consideration in DEEP’s 2020 Integrated Water Quality Assessment.
Figure 2. Fecal bacteria sampling sites in the Anguilla Brook watershed.
Figure 3. Land cover type and land use in the vicinity of the bacteria sampling sites (2010 land cover data from the Center for Landuse Education and Research, 2012).
Results

*E. coli* bacteria sampling results for Anguilla Brook and its tributary streams are summarized in Table 2. A geometric mean was calculated for each sample set. Bacteria levels listed in **bold** font in Table 2 exceed the established water quality limits. Bacteria samples with \( D = n \) indicate a duplicate sample was collected at that site on that sampling day. Table 2 also notes whether the sample was collected during wet (a rainfall in excess of 0.1 inches within 24 hours) or dry conditions. Bacteria results are graphically depicted in Figure 4. A simple statistical distribution of the *E. coli* bacteria sampling results was prepared, using a box and whisker plot of the data set (Fig. 5).

For comparison to established water quality standards, the 2012 Connecticut Water Quality Standards for freshwater is presented in Fig. 6. *Escherichia coli* is the preferred indicator bacteria for freshwater sampling. Indicator bacteria are easily quantified surrogates for other, more harmful bacteria and pathogens that may be present in water. The designated use for this project was *Recreation – all other uses*. Water quality criteria for that use are a single sample maximum of 576 colony-forming units (cfu) per 100 milliliters of water and a maximum sample set geometric mean of less than 126 cfu/100 ml.

In saltwater, fecal coliform is the indicator bacteria for the consumption (direct and indirect) of shellfish, and enterococcus is the indicator bacteria for recreation. Fecal coliform and enterococcus bacteria results from site AB-01 are presented in Table 3. There are no water quality criteria for fecal coliform or enterococcus in freshwater; these data were collected to determine if freshwater sources contributed to bacteria levels in the estuary. However, for reference, the Connecticut Water Quality Standards for saltwater are presented in Fig. 7.

Summaries of bacteria sampling results for each individual sampling site are provided below, following Figure 7.
### Table 2. Anguilla Brook watershed *E. Coli* bacteria sampling results.

<table>
<thead>
<tr>
<th>Site</th>
<th>6/24/19</th>
<th>7/1/19</th>
<th>7/8/19</th>
<th>7/15/19</th>
<th>7/22/19</th>
<th>7/29/19</th>
<th>8/5/19</th>
<th>8/12/19</th>
<th>8/19/19</th>
<th>8/26/19</th>
<th>Geomean</th>
</tr>
</thead>
<tbody>
<tr>
<td>X01</td>
<td>98</td>
<td>160</td>
<td>31</td>
<td>41 (10)</td>
<td>31</td>
<td>130</td>
<td>10</td>
<td>41 (&lt;10)</td>
<td>280</td>
<td>31</td>
<td>42</td>
</tr>
<tr>
<td>X02</td>
<td>180</td>
<td>110</td>
<td>120 (180)</td>
<td>75</td>
<td>95</td>
<td>170</td>
<td>63</td>
<td>52</td>
<td>540</td>
<td>74</td>
<td>119</td>
</tr>
<tr>
<td>X03</td>
<td>150</td>
<td>150</td>
<td>96</td>
<td>160</td>
<td>200</td>
<td>97</td>
<td>20</td>
<td>52</td>
<td>420</td>
<td>86 (75)</td>
<td>106</td>
</tr>
<tr>
<td>X04</td>
<td>120</td>
<td>400</td>
<td>2,200</td>
<td>280</td>
<td>420</td>
<td>41</td>
<td>52</td>
<td>41 (&lt;10)</td>
<td>108</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X01</td>
<td>DNS</td>
<td>260</td>
<td>85</td>
<td>63</td>
<td>20,000</td>
<td>210</td>
<td>270</td>
<td>&lt;10</td>
<td>510</td>
<td>10</td>
<td>163</td>
</tr>
<tr>
<td>X02</td>
<td>120</td>
<td>200 (170)</td>
<td>220</td>
<td>260</td>
<td>230</td>
<td>200 (86)</td>
<td>51</td>
<td>1,300</td>
<td>450</td>
<td>310</td>
<td>213</td>
</tr>
<tr>
<td>X03</td>
<td>250</td>
<td>460</td>
<td>110</td>
<td>590</td>
<td>3,700</td>
<td>250</td>
<td>86</td>
<td>290</td>
<td>1,100</td>
<td>96</td>
<td>336</td>
</tr>
<tr>
<td>X01</td>
<td>31 (41)</td>
<td>63</td>
<td>260</td>
<td>180</td>
<td>96 (85)</td>
<td>31</td>
<td>52</td>
<td>52</td>
<td>340</td>
<td>20</td>
<td>71</td>
</tr>
<tr>
<td>X02</td>
<td>74</td>
<td>52</td>
<td>63</td>
<td>120</td>
<td>130</td>
<td>62</td>
<td>20</td>
<td>DNS</td>
<td>360</td>
<td>360</td>
<td>95</td>
</tr>
<tr>
<td>X01</td>
<td>DNS</td>
<td>97</td>
<td>4,400</td>
<td>310</td>
<td>260</td>
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<td>86</td>
<td>170</td>
<td>1,700</td>
<td>280</td>
<td>313</td>
</tr>
<tr>
<td>X02</td>
<td>97</td>
<td>180</td>
<td>110</td>
<td>DNS</td>
<td>DNS</td>
<td></td>
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<td></td>
<td></td>
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<td>124</td>
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<tr>
<td>X02a</td>
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<tr>
<td>Notes:</td>
<td>1 MPN/100 ml - <em>E. coli</em> is measured as the most probable number (MPN) of colonies per 100 ml water sample.</td>
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</tr>
<tr>
<td>Notes:</td>
<td>2 Two branches of Donahue Brook emerged from a wooded wetland/stream complex north of Barnes Road. The west branch dried up; sampling switched to the east branch which had adequate flow.</td>
<td></td>
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</tr>
<tr>
<td>Notes:</td>
<td>DNS - did not sample.</td>
<td></td>
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</tr>
<tr>
<td>Notes:</td>
<td>Number in parentheses is a duplicate sample collected for quality control purposes.</td>
<td></td>
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</tr>
<tr>
<td>Notes:</td>
<td>Single sample limit for <em>E. coli</em> is 576 colony-forming units (CFU)/100 ml - CFU = MPN.</td>
<td></td>
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<tr>
<td>Notes:</td>
<td>Geometric mean limit is &lt;126 CFU/100 ml.</td>
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<td></td>
</tr>
</tbody>
</table>
### Table 3. Site AB01 Enterococcus and fecal coliform bacteria data.

<table>
<thead>
<tr>
<th>Date</th>
<th>Enterococcus (MPN/100 ml)</th>
<th>Fecal Coliform (MPN/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/24/2019</td>
<td>97</td>
<td>110</td>
</tr>
<tr>
<td>7/1/2019</td>
<td>41</td>
<td>200</td>
</tr>
<tr>
<td>7/8/2019</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>7/15/2019</td>
<td>&lt;10 (10)</td>
<td>41 (20)</td>
</tr>
<tr>
<td>7/22/2019</td>
<td>41</td>
<td>31</td>
</tr>
<tr>
<td>7/29/2019</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>8/5/2019</td>
<td>74</td>
<td>31</td>
</tr>
<tr>
<td>8/12/2019</td>
<td>10 (10)</td>
<td>&lt;10 (41)</td>
</tr>
<tr>
<td>8/19/2019</td>
<td>300</td>
<td>428</td>
</tr>
<tr>
<td>8/26/2019</td>
<td>&lt;10</td>
<td>31</td>
</tr>
</tbody>
</table>

### Water Quality Standards Criteria

<table>
<thead>
<tr>
<th>Single sample limit: 104/100ml</th>
<th>90% of sample less than 31/100ml</th>
<th>= 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geomean: less than 35/100ml</td>
<td>Geomean: less than 14/100ml</td>
<td>= 44</td>
</tr>
</tbody>
</table>

**Notes:**

- **MPN/100 ml** – Fecal coliform and enterococcus are measured as the most probable number (MPN) of colonies per 100 ml water sample.
- The number in parentheses is a duplicate sample collected for quality control purposes.
- Enterococcus is the indicator bacteria for the consumption (direct and indirect) of shellfish.
- Fecal coliform is the indicator bacteria for recreation.
Figure 4. Anguilla Brook watershed *E. coli* bacteria sampling results. A green dot indicates the site met established water quality criteria for the geometric mean; an orange dot indicates that the site failed to meet the geometric mean criteria.
Figure 5. Statistical distribution of bacteria results by sampling site.
<table>
<thead>
<tr>
<th>Designated Use</th>
<th>Indicator</th>
<th>Criteria by classification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drinking water supply</strong></td>
<td>Total Coliform</td>
<td>Monthly moving average less than 100/100 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Single sample maximum 500/100 ml</td>
</tr>
<tr>
<td><strong>Recreation</strong></td>
<td>Escherchia coli</td>
<td>Geometric mean less than 126/100 ml</td>
</tr>
<tr>
<td>Designated swimming</td>
<td></td>
<td>Single sample maximum 235/100 ml</td>
</tr>
<tr>
<td><strong>Recreation</strong></td>
<td>Escherchia coli</td>
<td>Geometric mean less than 126/100 ml</td>
</tr>
<tr>
<td>Non Designated Swimming</td>
<td></td>
<td>Single sample maximum 410/100 ml</td>
</tr>
<tr>
<td><strong>Recreation</strong></td>
<td>Escherchia coli</td>
<td>Geometric mean less than 126/100 ml</td>
</tr>
<tr>
<td>All other uses</td>
<td></td>
<td>Single sample maximum 576/100 ml</td>
</tr>
</tbody>
</table>

Figure 6. Indicator Bacteria for Freshwater (CT Water Quality Standards, CT DEEP, 2012).

<table>
<thead>
<tr>
<th>Designated Use</th>
<th>Indicator</th>
<th>Criteria by classification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shell fishing</strong></td>
<td>Fecal coliform</td>
<td>Geometric mean less than 14/100 ml</td>
</tr>
<tr>
<td>Direct Consumption</td>
<td></td>
<td>90% of samples less than 31/100 ml</td>
</tr>
<tr>
<td><strong>Shell fishing</strong></td>
<td>Fecal coliform</td>
<td>Geometric mean less than 88/100 ml</td>
</tr>
<tr>
<td>Indirect consumption</td>
<td></td>
<td>90% of samples less than 260/100 ml</td>
</tr>
<tr>
<td><strong>Recreation</strong></td>
<td>Enterococci</td>
<td>Geometric mean less than 35/100ml</td>
</tr>
<tr>
<td>Designated swimming</td>
<td></td>
<td>Single sample maximum 104/100 ml</td>
</tr>
<tr>
<td><strong>Recreation</strong></td>
<td>Enterococci</td>
<td>Geometric mean less than 35/100 ml</td>
</tr>
<tr>
<td>All other uses</td>
<td></td>
<td>Single sample maximum 500/100 ml</td>
</tr>
</tbody>
</table>

Figure 7. Indicator Bacteria for Saltwater (CT Water Quality Standards, CT DEEP, 2012).
Bacteria Sampling Results by Sampling Site

**AB01 – Anguilla Brook at the outlet of Wequetequock Pond:**

![Graph of bacteria sampling results at AB01](image1)

*Figure 8.* Graph of bacteria sampling results at AB01; upstream view of Anguilla Brook at the sampling location; and an aerial (Google Earth) image of the sampling site location and vicinity.

AB01 is located at the outlet of Wequetequock Pond (aka Cheeseborough Pond), upstream of the saltwater limit of Anguilla Brook. This is the downstream-most sampling site on Anguilla Brook and is located approximately 760 feet upstream of the top of Wequetequock Cove. This site was selected to document bacteria levels in Anguilla Brook prior to its discharge into Wequetequock Cove. Nearby land cover includes residential and commercial uses.

Twelve water samples were collected and analyzed for *E. coli* at this site, including two duplicates. All of the *E. coli* samples met the single sample Connecticut water quality standard of 576 cfu/100ml. The geometric mean for this site is 42, which is within the allowable geometric mean of less than 126 cfu/100 ml. No bacteria reduction is required at this site.
In order to determine if terrestrial sources from the Anguilla Brook watershed were contributing to bacteria levels that have been documented in Wequetequock Cove by CUSH and others, water samples collected at AB01 were analyzed for enterococcus and fecal coliform in addition to *E. coli*. Although there is no protocol for the analysis of either bacteria in freshwater samples, the presence of either or both could indicate that terrestrial sources are contributing to the bacteria loading of Wequetequock Cove.

Eleven of the twelve samples analyzed for enterococcus, the indicator bacteria for recreation in saltwater, met the single sample water quality limit of 104 cfu/100ml. The geometric mean of the sample set was 29 cfu/100ml, which is within the allowable geometric mean of 35 cfu/100ml.

There is no single sample limit for fecal coliform, the indicator bacteria for the consumption of shellfish. Instead, 90% of the sample must be less than 31 cfu/100ml. Six of the twelve samples (50%) analyzed for fecal coliform exceeded the standard of 31 cfu/100ml. The sample set exceeded the geometric mean limit of less than 14 cfu/100ml at 44 cfu/100ml.
AB02 – Anguilla Brook at State Route 1:

**Figure 10.** Graph of bacteria sampling results at AB02; upstream view of Anguilla Brook (towards RT 1) at the sampling location; and an aerial image of the sampling site location and vicinity.

AB02 is located at the downstream side of the crossing of Anguilla Brook and South Broad Street (State Route 1), at the Handlebar Plaza. This site is downstream of a forested wetland and agricultural and commercial uses. Bacteria samples were collected at this site by the University of Connecticut in 2017 for an NRCS Regional Conservation Partnership Program project (PATH to Reduce Pathogens in CT Agricultural Runoff).

Eleven water samples were collected at this site, including one duplicate sample. All of the samples (100%) met the single sample Connecticut water quality standard of 576 cfu/100ml. The geometric mean for this site is 119, which is within the allowable geometric mean of less than 126 cfu/100 ml. No bacteria reduction is required at this site.
AB03 – Anguilla Brook at Anguilla Brook Road:

**Figure 11.** Graph of bacteria sampling results at AB03; downstream view of Anguilla Brook at the sampling location; and an aerial image of the sampling site location and vicinity.

AB03 is located on Anguilla Brook at the end of Anguilla Brook Road. This site is downstream of a forested wetland and residential, agricultural and recreational uses.

Eleven water samples were collected at this site, including one duplicate sample. All of the samples (100%) met the single sample Connecticut water quality standard of 576 cfu/100ml. The geometric mean for this site is 106, which is within the allowable geometric mean of less than 126 cfu/100 ml. No bacteria reduction is required at this site.
AB04 – Anguilla Brook at State Route 184:

**Figure 12.** Graph of bacteria sampling results at AB04; upstream view of Anguilla Brook at Route 184 and an aerial image of the sampling site location and vicinity.

AB04 is located at the downstream side of the crossing of Providence-New London Turnpike (State Route 184). This is the upstream-most sampling site on Anguilla Brook and is located just south of a forested wetland which forms the Anguilla Brook headwaters. Land cover upstream of this site is comprised primarily of forest land, with a small amount of agricultural land.

Eleven water samples were collected at this site, including one duplicate sample. Ten of the eleven samples (91%) met the single sample Connecticut water quality standard of 576 cfu/100ml. The geometric mean for this site is 108, which is within the allowable geometric mean of less than 126 cfu/100 ml. No bacteria reduction is required at this site.
WB01 – Wheeler Brook off Miner Pentway:

Figure 13. Graph of bacteria sampling results at WB01; upstream view of Wheeler Brook at the sampling location; and an aerial image of the sampling site location and vicinity.

WB01 is located approximately 1,400 feet upstream of the confluence with Anguilla Brook off Miner Pentway. Upstream land cover/uses include forest land and agriculture.

Nine water samples were collected at this site. Eight of the samples (89%) met the single sample Connecticut water quality standard of 576 cfu/100ml. The geometric mean for this site is 163, which exceeds the allowable geometric mean of less than 126 cfu/100 ml. A 23% bacteria reduction is required at this site.
**WB2 – Wheeler Brook at Taugwonk Road:**

![Graph of bacteria sampling results at WB02; downstream view of Wheeler Brook at the sampling location; and an aerial image of the sampling site location and vicinity.](image)

**Figure 14.** Graph of bacteria sampling results at WB02; downstream view of Wheeler Brook at the sampling location; and an aerial image of the sampling site location and vicinity.

WB02 is located at the downstream side of the crossing of Wheeler Brook at Taugwonk Road. This site is approximately halfway along the length of Wheeler Brook and is downstream of forest and agricultural uses.

Twelve water samples were collected at this site, including two duplicates. Eleven of the samples (92%) met the single sample Connecticut water quality standard of 576 cfu/100ml. The geometric mean for this site is 213, which exceeds the allowable geometric mean of less than 126 cfu/100 ml. A 41% bacteria reduction is required at this site.
**WB03 – Wheeler Brook at State Route 184:**

![Graph of bacteria sampling results at WB03](image1)

![Downstream view of Wheeler Brook at the sampling location](image2)

![Aerial image of the sampling site location and vicinity](image3)

**Figure 15.** Graph of bacteria sampling results at WB03; downstream view of Wheeler Brook at the sampling location; and an aerial image of the sampling site location and vicinity.

WB03 is located downstream of the Providence-New London Turnpike (State Route 184) crossing of Wheeler Brook. This is the upstream-most site on Wheeler Brook approximately 1,000 feet downstream of the Wheeler Brook headwaters. The surrounding area is rural residential, agricultural and forested.

Ten water samples were collected at this site. Seven of the samples (70%) met the Connecticut water quality standard of 576 cfu/100ml for single samples. The geometric mean for this site is 335, which exceeds the allowable geometric mean of less than 126 cfu/100 ml. A 62% bacteria reduction is required at this site.
UN01—Unnamed brook off Taugwonk Road:

Figure 16. Graph of bacteria sampling results at UN01; upstream view of the unnamed tributary to Wheeler Brook at the sampling location; and an aerial image of the sampling site location and vicinity.

UN01 is the downstream-most sampling site on an unnamed brook that originates in a wetland north of State Route 184. The stream is a tributary to Wheeler Brook. UN01 is located approximately 200 feet upstream of the confluence with Wheeler Brook. Land cover upstream of UN01 is primarily forested with rural residential and agricultural uses.

Twelve water samples were collected at this site, including two duplicate samples. All the samples (100%) met the single sample Connecticut water quality standard of 576 cfu/100ml. The geometric mean for this site is 71, which is within the allowable geometric mean of less than 126 cfu/100 ml. No bacteria reduction is required at this site.
UN02 – Unnamed brook at Stony Brook Road:

Figure 17. Graph of bacteria sampling results at UN02; downstream view of the unnamed tributary to Wheeler Brook at the sampling location; and an aerial image of the sampling site location and vicinity.

UN02 is located at the downstream crossing of the unnamed tributary to Wheeler Brook at Stony Brook Road. Land-use upstream of UN02 is primarily forest with scattered rural residential land use.

Nine water samples were collected at this site. All the samples (100%) met the Connecticut water quality standard of 576 cfu/100ml for single samples. The geometric mean for this site is 95, which is within the allowable geometric mean of less than 126 cfu/100 ml. No bacteria reduction is required at this site.
DB01 – Donahue Brook at 711 Stonington Road:

Figure 18. Graph of bacteria sampling results at DB01; downstream view of Donahue Brook at the sampling location; and an aerial image of the sampling site location and vicinity.

DB01 is located upstream of the saltwater limit along Donahue Brook, approximately 1,425 feet upstream of the confluence with Oxocosset Brook. Oxocosset Brook is a tributary to Wequetequock Cove. Land cover upstream of DB01 includes forest and agricultural uses.

Nine water samples were collected at this site. Seven samples (78%) met the Connecticut water quality standard of 576 cfu/100 ml for single samples. The geometric mean for this site is 313, which exceeds the allowable geometric mean of less than 126 cfu/100 ml. A 60% bacteria reduction is required at this site.
**DB02/DB02a– Donahue Brook at Barnes Road:**

**Figure 19.** Graph of bacteria sampling results at DB02; view of Donahue Brook downstream of the sampling site; and an aerial image of the sampling site locations and vicinity.

DB02 and DB02a are located along Donahue Brook on the downstream side of the Barnes Road crossing. DB02 drains the east side of a forested wetland located north of Barnes Road. DB02a is located approximately 300 feet west of DB02, and drains the west side of the same forested wetland. The two branches of Donahue Brook rejoin approximately 300 feet downstream of the road crossing. Sampling commenced at DB02a on 7/29/19 when diminished flow at DB02 made sampling at the site not possible.

Since both sites discharged from the same forested wetland, the bacteria results were combined. Nine water samples were collected at this site, including one duplicate sample. Eight of the samples (89%) met Connecticut water quality standard of 576 cfu/100 ml for single samples. The geometric mean for this site is 198, which exceeds the allowable geometric mean of less than 126 cfu/100 ml. A 36% bacteria reduction is required at this site.
Discussion

E. coli bacteria levels in Anguilla Brook and an unnamed tributary to Wheeler Brook met Connecticut water quality standards for their designated recreational use, while Wheeler Brook and Donahue Brook failed to meet Connecticut water quality standards for their designated recreational use. All sampling sites on Wheeler and Donahue Brooks failed to meet the geometric mean for recreation (less than 126 cfu/100 ml) and all sampling sites except for WB02 had one or more single sample exceedances. A brief analysis of enterococcus and fecal coliform bacteria levels at AB01 indicates that while enterococcus levels were within allowable levels established by the Connecticut Water Quality Standards, fecal coliform levels exceeded allowable levels. While fresh water flowing into Wequetequock Cove from the Anguilla watershed may not be contributing significant levels of enterococcus to the Cove, it is possible that unidentified terrestrial sources in the watershed may be contributing to the fecal coliform load.

A review of the E. coli bacteria results under dry and wet (rainfall of 0.1 inch or more within 24 hours of the sampling period) conditions (Fig. 20) indicates that bacteria levels generally increased after rainfalls, although the increases varied by site and in most cases did not exceed the single sample limit of 576 cfu/100ml. Over the ten-week sampling period, two samples were collected during wet periods. Westerly State Airport, which is located approximately 4.5 miles from the Anguilla watershed, documented 0.12 inches of rain on 7/01/19 and 1.71 inches of rain on 8/18/19. Elevated bacteria levels are noted on 7/1/19 and 8/19/19 on Figure 20. In general, an increase in bacteria levels after rainstorms indicates that stormwater runoff rather than baseflow (groundwater) is a more significant vector for the mobilization of non-point source pollution including fecal bacteria into waterways.

There are several high dry weather data points on Figure 20, including results associated with AB04 and DB01 (on 7/8/19), WB03 (on 7/22/19), and WB02 and DB02 (on 8/12/19). An additional data point of 20,000 cfu/100ml at WB01 on 7/22/19 is not depicted on Figure 20. It is difficult to determine if those results are representative of water quality conditions or are the result of sampler error related to low water levels due to the general lack of rain over the sampling period. Because these high dry weather data points were not repeated at any of the sites, it is not likely that they are indicative of high levels of bacteria in baseflow.
In order to compare wet weather to dry weather stream bacteria levels, wet and dry weather *E. coli* averages were calculated (Table 4). To account for potential sampler error, an adjusted dry weather average, which excludes the outliers identified in Figure 20, was calculated. In general, wet weather *E. coli* averages were greater than dry weather levels, again indicating that stormwater is the most likely source of fecal bacteria in the streams.

Table 4. Comparison of wet weather to dry weather *E. coli* averages (cfu/100ml) for each sampling site.

<table>
<thead>
<tr>
<th>Site</th>
<th>AB01</th>
<th>AB02</th>
<th>AB03</th>
<th>AB04</th>
<th>WB01</th>
<th>WB02</th>
<th>WB03</th>
<th>UN01</th>
<th>UN02</th>
<th>DB01</th>
<th>DB02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet</td>
<td>220</td>
<td>325</td>
<td>285</td>
<td>226</td>
<td>385</td>
<td>318</td>
<td>775</td>
<td>202</td>
<td>206</td>
<td>899</td>
<td>273</td>
</tr>
<tr>
<td>Dry</td>
<td>48</td>
<td>107</td>
<td>107</td>
<td>395</td>
<td>2,581</td>
<td>329</td>
<td>672</td>
<td>90</td>
<td>104</td>
<td>703</td>
<td>252</td>
</tr>
<tr>
<td>Adj.</td>
<td>137</td>
<td>108</td>
<td>191</td>
<td>239</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>204</td>
<td>123</td>
</tr>
</tbody>
</table>

* Outliers were removed from the calculation of the dry weather average to eliminate potential sampler error.

Non-point source pollution (NPS) is the type of pollution most associated with stormwater runoff. NPS is composed of a wide variety of pollutants distributed across the ground surface.
that are immobile until they are mobilized by rain or snowmelt and transported into nearby waterways. NPS includes sediment, lawn and garden chemicals, vehicular chemicals, trash, yard waste, animal waste/manure, and underperforming or failing septic systems.

A review of land cover and landuse in the Wheeler Brook and Donahue Brook watersheds (Figure 21) indicates several potential sources of fecal bacteria. These include agricultural and residential land uses (brown and yellow areas, respectively). Agricultural activities that could contribute to bacteria loads includes the stockpiling of manure, the spreading of manure on agricultural fields and manure produced by grazing livestock. Bacteria in residential areas could derive from pet waste, manure from backyard livestock such as chickens, and effluent from underperforming or failing septic systems.

**Figure 21.** Bacteria sampling sites relative to land use in the Wheeler Brook watershed (left) and Donahue Brook watershed (right). Geometric means for each site are depicted next to the site identification code. Green indicates forest, aqua indicates forested wetlands, blue indicates open water, yellow indicates turf grass such as lawns or athletic fields, brown indicates agricultural fields, red indicates impervious surfaces such as roads, parking lots or large buildings, and orange indicates utility rights-of-way (CLEAR, 2012).
Conclusion

From June to August of 2019, ECCD and TLGV water quality monitoring volunteers collected water samples from eleven sites along Anguilla Brook and its tributaries in Stonington and North Stonington, Connecticut. The water samples were analyzed by the CT Department of Public Health’s Microbiology Laboratory for fecal bacteria (E. coli, enterococcus and fecal coliform) content. A review of the bacteria data indicates that Wheeler Brook (CT3300-10) and Donahue Brook (CT3300-05) do not currently meet State of Connecticut water quality standards for recreational use and that the watershed may be a source of fecal coliform to Wequetequock Cove. A comparison of E. coli data to rainfall records collected by the Westerly State Airport indicates that that stormwater runoff rather than baseflow may be a more significant vector for the mobilization of fecal bacteria in the Anguilla Brook watershed. ECCD will incorporate the results of the Anguilla Brook bacteria sampling into the development of a watershed management plan for the Anguilla Brook watershed.


References


Attachment A

Anguilla Brook Bacteria Trackdown and Watershed Based Plan

Water Quality Monitoring Plan
Contract 17-04
Anguilla Brook Bacteria Trackdown and Watershed-Based Plan

Eastern Connecticut Conservation District
March 21, 2019 (Revised 6/11/19)

Water Quality Monitoring Plan

This project is funded in part by CT DEEP through a US EPA Clean Water Act §319 Nonpoint Source Program grant.

17-04 Anguilla Brook Bacteria Trackdown and Watershed-Based Plan
Task 1c – Develop a Water Monitoring Plan

www.ConserveCT.org/eastern
**Introduction:**

The Eastern Connecticut Conservation District (ECCD) has received funding from the Connecticut Department of Energy and Environmental Protection (CT DEEP) through the Clean Water Act Section 319 Nonpoint Source program to conduct a water quality investigation in the Anguilla Brook watershed (CT2101) in Stonington and North Stonington, Connecticut (Fig. 1). The water quality of Anguilla Brook has not been assessed; however, Wequetequock Cove, the embayment to which Anguilla Brook discharges, does not meet water quality standards for its designated uses, including aquatic habitat, recreation and direct consumption of shellfish. In order to identify potential sources of pollution that may be contributing to the water quality impairments, ECCD will conduct an assessment of the Anguilla Brook watershed. The assessment will include freshwater stream sampling for *Escherichia coli* (*E. coli*) as an indicator for fecal bacteria and other pathogens that may be entering Wequetequock Cove from terrestrial sources, stream corridor condition assessments and a visual land use assessment. In addition, the water sample from the lowermost site on Anguilla Brook will be analyzed for Enterococci and Fecal Coliform. The data collected through this assessment will be used to develop a watershed-based plan for the Anguilla Brook watershed.

ECCD has developed this Water Quality Monitoring Plan to provide a framework for the freshwater stream fecal indicator bacteria sampling. No estuarine or marine fecal indicator bacteria sample collection is proposed.

**Anguilla Brook Watershed:**

The Anguilla Brook watershed is a 12.3-square mile coastal watershed located in Stonington and North Stonington, Connecticut. Anguilla Brook is the primary tributary discharging to Wequetequock Cove. A second tributary, Donahue Brook, discharges to Wequetequock Cove from the west. Wheeler Brook discharges to Anguilla Brook in the northwest part of the watershed. An un-named tributary discharges to Wheeler Brook in the same area. Anguilla Brook is brackish/tidal to the outlet of Wequetequock Pond, located along Route 1 upstream of Wequetequock Cove. Donahue Brook is brackish/tidal through a saltmarsh located on the north side of Route 1 (Fig. 2).
Figure 2. Perennial streams in the Anguilla Brook watershed are depicted in relation to their local watersheds. Approximate saltwater limits are depicted by the short green lines.

Land use and land cover types in the Anguilla Brook watershed are mixed (Fig. 3). The primary land cover is forest (~45%), and agricultural fields (~15%) and developed land (~13%) are the predominant land uses (Table 1). Agricultural activity is scattered throughout the watershed. Development is prevalent along major corridors, including the Route 1 and RT 234 corridors in the southern part of the watershed, the Route 184 corridor in the north part of the watershed, and in the Pawcatuck section of Stonington, located in the southeastern part of the watershed.

Figure 3. The distribution of land use/land cover in the Anguilla Brook Watershed (Center for Land Use Education and Research, 2018).

Table 1. Land use/land cover types and acreage (AREA_AC), and percent of each type (PRCT_LC) in the Anguilla Brook Watershed (Center for Land Use Education and Research, 2018).

<table>
<thead>
<tr>
<th>CLASS_NAME</th>
<th>AREA_AC</th>
<th>PRCT_LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developed</td>
<td>1048.128</td>
<td>13.296</td>
</tr>
<tr>
<td>Turf &amp; Grass</td>
<td>762.626</td>
<td>9.867</td>
</tr>
<tr>
<td>Other Grasses</td>
<td>149.219</td>
<td>1.891</td>
</tr>
<tr>
<td>Agricultural Field</td>
<td>1208.382</td>
<td>15.291</td>
</tr>
<tr>
<td>Deciduous Forest</td>
<td>3445.622</td>
<td>43.677</td>
</tr>
<tr>
<td>Coniferous Forest</td>
<td>81.726</td>
<td>1.036</td>
</tr>
<tr>
<td>Water</td>
<td>113.407</td>
<td>1.437</td>
</tr>
<tr>
<td>Non-forested Wetland</td>
<td>115.932</td>
<td>1.469</td>
</tr>
<tr>
<td>Forested Wetland</td>
<td>807.851</td>
<td>10.24</td>
</tr>
<tr>
<td>Tidal Wetland</td>
<td>76.448</td>
<td>0.989</td>
</tr>
<tr>
<td>Barren Land</td>
<td>21.579</td>
<td>0.274</td>
</tr>
<tr>
<td>Utility Corridor</td>
<td>59.229</td>
<td>0.751</td>
</tr>
</tbody>
</table>
**Water Quality Impairment:**

Inner Wequetequock Cove (CT-E1_003), located from the railroad crossing at the south end of the Cove upstream to the saltwater limit adjacent to State Route 1, is listed in the 2016 *State of Connecticut Integrated Water Quality Report* as impaired for its designated uses. These include habitat for marine life, other aquatic life and wildlife, recreation, and the direct consumption of shellfish, due to estuarine bioassessments and excess algal growth related to pollutant sources such as stormwater, agricultural activities and other unidentified upstream sources (Fig. 4 & 5). The 2016 *Integrated Water Quality Report* does not provide causes of the shellfish impairment in inner Wequetequock Cove. Suspected sources include marina/boating sanitary on-vessel discharges, municipal point sources, onsite treatment systems, residential districts, urban runoff/storm sewers, waterfowl (Katie O’Brien-Clayton, personal communication, March 6, 2019). The Total Maximum Daily Load (TMDL) for Estuary 12: Stonington Appendix (CT DEEP, 2013) to the Statewide Total Maximum Daily Load (TMDL) for Bacteria-Impaired Waters (CT DEEP, 2012) cites illicit discharges, failing septic systems, marinas, stormwater runoff and nuisance wildlife/pets as potential bacteria sources to the impaired segments in the Stonington estuary.

![Table 2-5](image.png)

**Figure 4.** The 305b water quality assessment of inner Wequetequock Cove, from the State of Connecticut 2016 Integrated Water Quality Report (CT DEEP, 2017).
### Table 3-4. Connecticut Impaired Waters List (EPA Category 5)

<table>
<thead>
<tr>
<th>Waterbody Segment ID</th>
<th>Waterbody Name</th>
<th>Impaired Designated Use</th>
<th>Cause</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT-E1_002-SB</td>
<td>LIS EB Inner - Pawaquack River (02), Stonington</td>
<td>Habitat for Marine Fish, Other Aquatic Life and Wildlife</td>
<td>Excess Algal Growth</td>
<td>Potential sources include stormwater, agricultural activities, upstream sources</td>
</tr>
<tr>
<td>CT-E1_002-SB</td>
<td>LIS EB Inner - Pawaquack River (02), Stonington</td>
<td>Habitat for Marine Fish, Other Aquatic Life and Wildlife</td>
<td>Nutrient/ Eutrophication Biological Indicators</td>
<td>Potential sources include stormwater, agricultural activities, upstream sources</td>
</tr>
<tr>
<td>CT-E1_002-SB</td>
<td>LIS EB Inner - Pawaquack River (02), Stonington</td>
<td>Recreation</td>
<td>Estuarine Bioassessments</td>
<td>Potential sources include stormwater, agricultural activities, upstream sources</td>
</tr>
<tr>
<td>CT-E1_002-SB</td>
<td>LIS EB Inner - Pawaquack River (02), Stonington</td>
<td>Recreation</td>
<td>Excess Algal Growth</td>
<td>Potential sources include stormwater, agricultural activities, upstream sources</td>
</tr>
<tr>
<td>CT-E1_003</td>
<td>LIS EB Inner - Inner Wequetequock Cove, Stonington</td>
<td>Habitat for Marine Fish, Other Aquatic Life and Wildlife</td>
<td>Estuarine Bioassessments</td>
<td>Potential sources include stormwater, agricultural activities, upstream sources</td>
</tr>
<tr>
<td>CT-E1_003</td>
<td>LIS EB Inner - Inner Wequetequock Cove, Stonington</td>
<td>Habitat for Marine Fish, Other Aquatic Life and Wildlife</td>
<td>Excess Algal Growth</td>
<td>Potential sources include stormwater, agricultural activities, upstream sources</td>
</tr>
<tr>
<td>CT-E1_003</td>
<td>LIS EB Inner - Inner Wequetequock Cove, Stonington</td>
<td>Recreation</td>
<td>Estuarine Bioassessments</td>
<td>Potential sources include stormwater, agricultural activities, upstream sources</td>
</tr>
<tr>
<td>CT-E1_001</td>
<td>LIS EB Inner - Inner Wequetequock Cove, Stonington</td>
<td>Recreation</td>
<td>Excess Algal Growth</td>
<td>Potential sources include stormwater, agricultural activities, upstream sources</td>
</tr>
</tbody>
</table>

*Figure 5.* Listed causes of the impairments of inner Wequetequock Cove (CT-E1-003), from the State of Connecticut 2016 Integrated Water Quality Report (CT DEEP, 2017).

**Project Partners:**

ECCD will conduct *E. coli* sampling in the Anguilla Brook watershed in partnership and coordination with the Towns of North Stonington and Stonington, Ledge Light Health District (LLHD), the Connecticut Department of Public Health (CT DPH), and the Connecticut Department of Energy and Environmental Protection. ECCD will partner with The Last Green Valley (TLGV) Volunteer Water Quality Monitoring Program, which developed the *E. coli* sampling protocol that ECCD will use. TLGV will assist with recruiting water quality monitoring volunteers and provide volunteer training. ECCD will also partner with local organizations, including CUSH (Clean Up Sounds and Harbors), the Stonington Land Trust, the Avalonia Land Conservancy, and area residents to develop this water quality monitoring plan and conduct water quality sampling (Table 2).
Table 2. Anguilla Brook water quality monitoring project partners

<table>
<thead>
<tr>
<th>Project Partner</th>
<th>Role/Responsibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECCD</td>
<td>Project management, monitoring plan development, data collection</td>
</tr>
<tr>
<td>Town of North Stonington</td>
<td>Key stakeholder, project support, monitoring plan development</td>
</tr>
<tr>
<td>Town of Stonington</td>
<td>Key stakeholder, project support, monitoring plan development</td>
</tr>
<tr>
<td>Ledge Light Health District</td>
<td>Bacteria sample conveyance to DPH lab</td>
</tr>
<tr>
<td>CT Department of Public Health</td>
<td>Bacteria sample analysis</td>
</tr>
<tr>
<td>CT Department of Energy and Environmental Protection</td>
<td>Project funding, oversight &amp; guidance</td>
</tr>
<tr>
<td>The Last Green Valley</td>
<td>E. coli sampling protocol, volunteer recruitment and training</td>
</tr>
<tr>
<td>CUSH</td>
<td>Monitoring plan development, data collection volunteers</td>
</tr>
<tr>
<td>Stonington Land Trust</td>
<td>Monitoring plan development, data collection volunteers</td>
</tr>
<tr>
<td>Avalonia Land Conservancy</td>
<td>Monitoring plan development, data collection volunteers</td>
</tr>
<tr>
<td>Area residents</td>
<td>Data collection volunteers</td>
</tr>
</tbody>
</table>

**Water Quality Monitoring Plan:**

This water quality monitoring plan has been prepared to present the process ECCD will utilize to collect fecal indicator bacteria samples from freshwater streams in the Anguilla Brook watershed. This plan was prepared in conformance with the *Anguilla Brook Watershed-Based Plan Bacteria Sampling Quality Assurance Project Plan* (RFA #19088) approved by CT DEEP and US EPA (Region 1) on June 10, 2019.

ECCD will conduct *E. coli* sampling in the Anguilla Brook watershed in order to quantify fecal indicator bacteria levels in Anguilla Brook and its perennial tributaries. In addition, the water sample collected at the lowermost site on Anguilla Brook will be analyzed for Enterococci and Fecal Coliform. The collected fecal indicator bacteria data will be evaluated to determine if water quality in the streams complies with allowable limits established in the Connecticut Water Quality Standards (CT DEEP, 2013) for freshwater recreation (‘all other uses’ designation). The data will be used to identify source areas that may contribute to the impairment of inner Wequetequock Cove. The data will also be used to develop a nine-element watershed-based plan for Anguilla Brook.
In order to identify optimal bacteria sampling sites, ECCD reviewed available information, including the Total Maximum Daily Load (TMDL) for Estuary 12: Stonington Appendix (CT DEEP, 2013) to the Statewide Total Maximum Daily Load (TMDL) for Bacteria-Impaired Waters (CT DEEP, 2012). ECCD reviewed the results of bacteria sampling conducted in 2017 by the UConn PATH project (unpublished) and data collected by CUSH from 2009 – 2018 (Sally Cogan, personal communication, January 6, 2019). ECCD also evaluated land use/land cover data (CLEAR, 2018) to identify land uses in the watershed most likely to contribute to bacteria loading. Based on this evaluation, ECCD selected eleven (11) sites in the Anguilla Brook watershed at which to collect water samples. ECCD reviewed the proposed sites with watershed stakeholders at a meeting in January 2019. Based on their comments, the locations of several sites were adjusted. The sampling sites are described in detail in Table 3 and are shown on the map in Attachment A.

**Table 3. Stream E. coli Sampling Site Descriptions**

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Stream Name</th>
<th>Location/Justification</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-01</td>
<td>Anguilla Brook</td>
<td>Off Trolley Crossing – at outlet of Wequetequock Pond. Downstream-most site; upstream of salt-water limit</td>
<td>41°21'39.75&quot;N</td>
<td>-71°52'29.66&quot;W</td>
</tr>
<tr>
<td>AB-02</td>
<td>Anguilla Brook</td>
<td>At RT 1 – same as UConn PATH sampling site</td>
<td>41°21'56.21&quot;N</td>
<td>-71°51'57.75&quot;W</td>
</tr>
<tr>
<td>AB-03</td>
<td>Anguilla Brook</td>
<td>At Anguilla Brook Road– midway in watershed; downstream of residential, agriculture and recreational uses</td>
<td>41°23'0.72&quot;N</td>
<td>-71°52'30.40&quot;W</td>
</tr>
<tr>
<td>AB-04</td>
<td>Anguilla Brook</td>
<td>AT RT 184 – upstream-most site; downstream of agricultural uses</td>
<td>41°25'20.19&quot;N</td>
<td>-71°52'47.18&quot;W</td>
</tr>
<tr>
<td>DB-01</td>
<td>Donahue Brook</td>
<td>US of confluence with Oxocossett Brook above salt-water limit; downstream of agricultural uses; tributary to Wequetequock Cove</td>
<td>41°21'11.70&quot;N</td>
<td>-71°53'44.45&quot;W</td>
</tr>
<tr>
<td>DB-02</td>
<td>Donahue Brook</td>
<td>At Barnes Road – upstream-most site</td>
<td>41°22'0.45&quot;N</td>
<td>-71°53'49.32&quot;W</td>
</tr>
<tr>
<td>WB-01</td>
<td>Wheeler Brook</td>
<td>Upstream of the confluence with Anguilla Brook</td>
<td>41°24'25.38&quot;N</td>
<td>-71°52'38.49&quot;W</td>
</tr>
<tr>
<td>WB-02</td>
<td>Wheeler Brook</td>
<td>At Taugwonk Road – upstream of the confluence with Stony Brook; downstream of agricultural uses</td>
<td>41°24'15.21&quot;N</td>
<td>-71°53'41.11&quot;W</td>
</tr>
<tr>
<td>WB-03</td>
<td>Wheeler Brook</td>
<td>At RT 184 – upstream-most site; downstream of residential use</td>
<td>41°24'57.29&quot;N</td>
<td>-71°54'19.33&quot;W</td>
</tr>
<tr>
<td>UN-01</td>
<td>Unnamed stream</td>
<td>Upstream of confluence with Wheeler Brook; downstream of agricultural uses</td>
<td>41°24'24.91&quot;N</td>
<td>-71°53'24.36&quot;W</td>
</tr>
<tr>
<td>UN-02</td>
<td>Unnamed stream</td>
<td>At Stony Brook Rd – upstream-most site</td>
<td>41°24'52.62&quot;N</td>
<td>-71°53'49.06&quot;W</td>
</tr>
</tbody>
</table>
In April and May 2019, ECCD will recruit volunteers to participate in bacteria sampling. In May or June 2019, ECCD and TLGV will conduct a half-day bacteria sample collection workshop for volunteers. Volunteers will be instructed in the water sample collection methodology outlined in the Anguilla Brook Bacteria Trackdown and Watershed-Based Plan Bacteria Field Sampling Manual included in the QAPP.

Upon completion of the training workshop, water quality monitoring volunteers will collect water samples from eleven (11) freshwater stream sites throughout the Anguilla Brook watershed once a week for 10 consecutive weeks. Sampling will be conducted in June, July and August to coincide with the 2019 recreational swimming season (Memorial Day to Labor Day). Ten samples will be collected at the downstream-most site on Anguilla Brook (site AB-01 on the attached map) in order to evaluate water quality for this investigation and to obtain a sufficient sample set for the completion of a recreation use assessment by DEEP. The remaining 10 sites will be sampled an adequate number of times to establish if they meet or do not meet water quality standards. Sampling may be discontinued if sites demonstrate consistently good water quality; additional sites may be added to bracket E. coli levels as data is received from the DPH lab. Samples will be delivered to Ledge Light Health District in New London, CT and transported via the DPH courier service within the 6-hour hold period to the State of Connecticut Department of Public Health (CT DPH) Microbiology Lab in Rocky Hill for analysis. Because the ECCD sampling schedule will be developed to coincide with the courier service pick-up date for Ledge Light Health District, the schedule will not be adjusted to target wet or dry-day sampling. It is anticipated that over the course of the ten-week collection period, a representative number of both wet and dry-weather samples will be collected.

Samples will be collected by hand by wading into the stream, if the stream is easily accessible, using sterile single-use 120-ml polystyrene collection bottles provided by DPH. If the stream is not easily accessible, the sample will be collected using a sampling bottle attached to an extension pole. Water samples will not be collected if conditions are considered to be unsafe or if water levels are too low to allow sample collection. Samples will not be collected from stagnant water. The samples will be labeled and placed in a cooler on ice to be kept at a temperature of 10ºC or less until delivered to Ledge Light Health District. Volunteers will also collect air and water temperature data at each site and will note general stream conditions such as relative water level (high, moderate, low), water conditions such as odors or discoloration, and any recreational uses nearby, such as boating or fishing, on a field form. For quality control purposes, volunteers will collect one duplicate sample and prepare one blank sample (using a buffered solution provided by DPH) on each sampling date. The sampling sites for the blank and duplicate samples will be selected using a random number generator.

Data results will be reviewed by ECCD as they are received from CT DPH (typically within three to four days of the sample collection date). The monitoring plan will be evaluated and adjusted as needed in response to the data review, to ensure that the data being collected are providing the maximum information necessary to achieve the goals of this investigation. Upon completion of
sampling, ECCD will review the data to ensure it has been transcribed correctly from the DPH reports to an Excel spreadsheet provided by DEEP prior to being submitted to CT DEEP for review and incorporation in the biannual water quality assessment.

References


Attachment A – Proposed Bacteria Sampling Sites in the Anguilla Brook Watershed

Legend
- Sampling Sites
- Anguilla Streams

Anguilla Brook Watershed - Proposed Sampling Sites
Attachment B

Anguilla Brook Watershed Based Plan
Bacteria Sampling Quality Assurance Project Plan
Anguilla Brook Watershed-Based Plan
Bacteria Sampling QAPP
Title and Approval Sheet
EPA Tracking # RFA 19088

ECCD Project Manager

Signature

ECCD Quality Assurance Officer

Signature

CT DEEP Technical Project Reviewer

Signature

USEPA-NE Quality Assurance Reviewer

Signature

USEPA-NE Project Officer

Signature

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Date 6/11/19

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Date 6/12/19

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Date 6/11/2019

RFA #19088 Approved 6/10/19
Anguilla Brook Watershed-Based Plan
Bacteria Sampling QAPP
Title and Approval Sheet
EPA Tracking # RFA 19088

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Signature _________________________________ Date__________________

RFA #19088  Approved 6/10/19
Quality Assurance Project Plan

Bacteria Source Tracking

Anguilla Brook Bacteria Trackdown and Watershed-Based Plan

Eastern Connecticut Conservation District

Final June 10, 2019
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- Table 3. Field Sampling Methods
- Table 4. DPH Laboratory Analytical Method for Indicator Bacteria in Fresh Surface Waters
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- Appendix 2: Sample Data Forms

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## Distribution List

The following list identifies the agencies, organizations and personnel that will receive a copy of the approved QAPP and any subsequent revisions.

<table>
<thead>
<tr>
<th>Organization</th>
<th>Personnel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern Connecticut Conservation District:</td>
<td>Judy Rondeau</td>
</tr>
<tr>
<td></td>
<td>Dan Mullins</td>
</tr>
<tr>
<td></td>
<td>Jean Pillo</td>
</tr>
<tr>
<td></td>
<td>Maura Robie</td>
</tr>
<tr>
<td>Connecticut Department of Energy and Environmental Protection:</td>
<td>Eric Thomas</td>
</tr>
<tr>
<td></td>
<td>Erik Bedan</td>
</tr>
<tr>
<td></td>
<td>Katie O’Brien-Clayton</td>
</tr>
<tr>
<td>US Environmental Protection Agency (Reg. 1):</td>
<td>Nora Conlon</td>
</tr>
<tr>
<td></td>
<td>Steven Winnett</td>
</tr>
<tr>
<td>Connecticut Department of Public Health:</td>
<td>Kimberly Holmes-Talbot</td>
</tr>
<tr>
<td>Ledge Light Health District:</td>
<td>Stephen Mansfield</td>
</tr>
<tr>
<td>Town of Stonington:</td>
<td>Robert Simmons</td>
</tr>
<tr>
<td>Town of North Stonington:</td>
<td>Michael Urgo</td>
</tr>
</tbody>
</table>
Project Task Organization

Key personnel associated with the project are identified in Figure 1. Judy Rondeau, the Project Manager for the Eastern Connecticut Conservation District, will be directly responsible for coordination of the project, and will be assisted by other Eastern Connecticut Conservation District (ECCD) staff, volunteers and local project partners (e.g. Connecticut Department of Energy and Environmental Protection (CT DEEP), municipalities, and other data users).

The Eastern Connecticut Conservation District would like to specially acknowledge the Connecticut River Coastal Conservation District for providing their Quality Assurance Project Plan and Volunteer Handbook as models for this project.
Element 5

Problem Definition/Background

The water quality of Anguilla Brook has not been assessed; however, Inner Wequetequock Cove, the embayment to which Anguilla Brook discharges, does not meet water quality standards for its designated uses. Inner Wequetequock Cove (CT-E1_003), located from the railroad crossing at the south end of the Cove upstream to the saltwater limit adjacent to State Route 1, is listed in the 2016 *State of Connecticut Integrated Water Quality Report* as impaired for its designated uses. These include habitat for marine life, other aquatic life and wildlife, recreation, and the direct consumption of shellfish, due to estuarine bioassessments and excess algal growth related to pollutant sources such as stormwater, agricultural activities and other unidentified upstream sources (Fig. 4 & 5). The 2016 *Integrated Water Quality Report* does not provide causes of the shellfish impairment in inner Wequetequock Cove. Suspected sources include marina/boating sanitary on-vessel discharges, municipal point sources, onsite treatment systems, residential districts, urban runoff/storm sewers, waterfowl (Katie O’Brien-Clayton, personal communication, March 6, 2019). The *Total Maximum Daily Load (TMDL) for Estuary 12: Stonington Appendix* (CT DEEP, 2013) to the *Statewide Total Maximum Daily Load (TMDL) for Bacteria-Impaired Waters* (CT DEEP, 2012) cites illicit discharges, failing septic systems, marinas, stormwater runoff and nuisance wildlife/pets as potential bacteria sources to the impaired segments in the Stonington estuary.

The purpose of this project is to conduct an investigation of the Anguilla Brook watershed, including the collection of water quality data from Anguilla Brook and its perennial tributaries, in order to develop a watershed-based plan for the Anguilla Brook watershed. The Anguilla Brook Watershed-Based Plan (the Plan) will follow the EPA’s Nine Element format, including the identification of potential pollutant source areas (including fecal bacteria), estimation of pollutant loads, development of specific water quality improvement action items, and estimation of load reductions for specific water quality action items. ECCD will work with the watershed municipalities and other local stakeholders to conduct the water quality investigation and prepare the Plan.

ECCD will work closely with CT Department of Energy and Environmental Protection (DEEP) staff, including DEEP’s TMDL staff, and a large group of local, regional and state stakeholders, to plan and conduct the water quality investigation and prepare the Plan. Project partners and stakeholders include Town of Stonington and Town of North Stonington planning, engineering and public works staff and land-use and shellfish commissions, The Last Green Valley Water Quality Monitoring Program, CUSH (Clean Up Sounds and Harbors), the Stonington Land Trust, the Avalonia Land Conservancy and local businesses and residents.

Element 6

Project/Task Description

The Eastern Connecticut Conservation District (ECCD), in cooperation with CT DEEP, will conduct a water quality study in the Anguilla Brook watershed in order to develop a watershed-
based plan that will lead to an improvement of the water quality of Anguilla Brook and Inner Wequetequock Cove.

The goals of the water quality study include:
- quantification of *E. coli* concentrations in Anguilla Brook
- identification of areas contributing to bacteria loading in Anguilla Brook and Inner Wequetequock Cove
- increase in public awareness of water quality issues and human impacts on waterways and Long Island Sound
- preparation of an EPA nine-element format watershed-based plan for the Anguilla Brook watershed that will provide targeted implementable actions intended to improve the water quality of Anguilla Brook and Inner Wequetequock Cove.

In 2019, ECCD will recruit volunteers from the community to assist with the collection of water samples for *E. coli* analysis. Samples will be collected from various locations on Anguilla Brook and its perennial tributaries. Additionally, the water sample collected from the lower-most site on Anguilla Brook will be analyzed for fecal coliform and enterococcus to determine if Anguilla Brook is contributing to levels of these fecal indicator bacteria that have been observed in Wequetequock Cove. This monitoring strategy will help to determine whether, and to what extent, the selected tributaries are contributing to bacteria levels documented by CT DEEP, DABA and others in Wequetequock Cove. Water sample collection will be conducted between Memorial Day and Labor Day to coincide with the recreational bathing season. Water samples will be submitted to the State of Connecticut Department of Public Health Laboratory for *E. coli* analysis. ECCD staff and trained volunteers will also make and record measurements of air and water temperature, and observations of water levels, color and odor at each site. The monitoring data will be used in planning future assessment and improvement activities. All data will be entered in a computerized data management system as results are received, and preliminary reviews will be performed. Data summaries with narrative and other analyses will be produced as each type of activity is completed during the course of the sampling season. Press releases and/or social media posts will be issued periodically throughout the year as part of the public outreach and education component of the project.

**Proposed Plan of Work - Item/Task Dates**
2. March - April 2019 - Present monitoring plan to CT DEEP for approval.
6. October 2019 - Compile and interpret water quality data.
7. Issue press releases and promote activities through social media and other outlets continuously throughout the project period.
Data Quality Objectives and Criteria

Sampling design and methods are described in this document (Quality Assurance Project Plan) and the Anguilla Brook Bacteria Trackdown and Watershed-Based Plan Bacteria Field Sampling Manual (Appendix 1.B). The following table summarizes Data Quality Objectives.

Table 1. Data Quality Objectives

<table>
<thead>
<tr>
<th>DQO Indicator</th>
<th>Measurement Performance Criteria</th>
<th>QC Sample or Activity</th>
<th>Action if DQO is Exceeded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>RDP &lt;30%</td>
<td>Duplicate sample</td>
<td>Review sample collection procedure with volunteers for errors. Consult DPH lab QA/QC for errors.</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Media performs correctly, positive controls are positive for enterococci, negative controls are negative.</td>
<td>Positive and negative control samples and sterile field blanks.</td>
<td>If controls out, invalidate corresponding batch. If field blank is contaminated, review sample collection procedure with volunteers for errors.</td>
</tr>
<tr>
<td>Representativeness</td>
<td>Sampling station locations are selected in conformance with standard guidelines.</td>
<td>Samples are collected at the same locations.</td>
<td>Review sampling station locations to ensure they adequately reflect water conditions throughout the watershed.</td>
</tr>
<tr>
<td>Comparability</td>
<td>Samples are collected at the same sites.</td>
<td>Sample sites are clearly marked with surveyor’s tape.</td>
<td>Review site locations with samplers.</td>
</tr>
<tr>
<td>Completeness</td>
<td>Provide data for 90% of the samples submitted to the lab.</td>
<td>Total number of results reported vs. total number of samples collected.</td>
<td>Review sample collection procedures for errors. Recollect sample if necessary. Reschedule cancelled sampling events.</td>
</tr>
</tbody>
</table>

Precision and Accuracy

A field duplicate is collected at one sampling location during each weekly sample collection. A relative percent difference value of 30% is used as a precision threshold for field duplicates. If the RPD exceeds the allowable limit, the data will be qualified.

The Relative Percent Difference (RPD), where V1 is the sample value and V2 is the duplicate value, is calculated for duplicate samples as follows:

$$\text{RPD} = \left( \frac{(V1 - V2)}{(V1 + V2)/2} \right) \times 100$$

One sterile field blank is prepared per weekly sample collection. The state laboratory provides sealed bottles containing sterile dilution water for QA field blank samples. To prepare a field blank sample, volunteers are trained to: 1) prepare the blank field sample at the pre-designated sampling station; 2) remove the seal on the dilution bottle and open the bottle; 3) open the sterile field sample
collection bottle provided by the state laboratory; 4) pour the sterile dilution water into the field sample collection bottle; and 5) screw the top tightly onto the field collection bottle.

The state laboratory tests one positive and one negative control on a daily basis. The data quality objective for blanks is zero percent. An analytic method check is performed on each day of analysis. Positive controls should be positive. Negative controls should be negative. If these conditions are not met the corresponding batch of data is invalidated.

Representativeness

The sampling sites were selected at key locations along the target streams throughout the watershed in order to accurately represent water conditions. For each stream being sampled, an upstream sampling site was selected to represent unimpacted water conditions and a downstream site was selected to represent any cumulative impacts. Additional sampling sites along Anguilla Brook (the mainstem waterbody) were selected at locations downstream of significant land uses, such as agriculture and residential developments, to represent impacts from those specific uses.

Comparability

The bacteria sampling method that will be employed during this project follows standard procedures for sample collection and analysis. Standard Operating Procedures associated with this project are located in Appendix 1 – SOP Guides. All volunteers are trained to utilize the same sample collection procedure. Sampling sites are marked with surveyor’s tape and samples will be collected at the same location throughout the duration of the sampling period. All samples are analyzed using EPA approved methodologies to ensure results are comparable.

Completeness

ECCD data are intended for use in an informational, advisory capacity, not in any regulatory or other legal proceedings or for any compliance purposes. A minimum of ten samples are recommended for statistical purposes. It is expected that samples will be collected from at least 90% of sites on each sample day unless weather conditions prevent sampling due to unsafe conditions or low water levels make sampling not possible. We expect to collect 130 samples, including 10 duplicates and 10 blank samples. If a sampling event needs to be cancelled due to weather or other unforeseen circumstance, it will be rescheduled.

Training Requirements/Certification

Volunteers participate in training sessions conducted by ECCD staff to learn how to perform monitoring activities. Water sampling volunteers are trained to collect water samples in a training workshop prior to the beginning of the sampling program. Sampling procedures are demonstrated and volunteers are encouraged to collect water samples. Volunteers are also trained to complete the water sample collection field sheet. Volunteers will receive instruction on the sampling procedure and will be provided a copy of ECCD Bacteria Field Sampling Manual at the training workshop.
session. A list of volunteers trained at each session is kept to document all volunteers who have been trained.

It is important to have all the volunteers participate in the same training activities and to provide everyone with written procedures to foster consistency in approach. Performance is evaluated through careful monitoring of activities through periodic field inspections and/or monthly data review by ECCD staff. If problems arise, staff will conduct side-by-side water sampling with volunteers to evaluate sampling technique.

Element 9

Documentation and Records

ECCD staff and/or water sampling volunteers must complete a Field Sheet for Bacteria Monitoring for water samples at the time of sample collection. Volunteers record their names, the date, the weather (current and previous 3 days), and for each site, the site number, type of samples collected and bottle number (where applicable), time samples collected, air and water temperature, observations by a code system for water level, water odor, water color, observed use, and any other observations. The Field Sheet for Bacteria Monitoring is delivered with the samples. These forms are used by ECCD and kept on file in the office. A copy of the Field Sheet for Bacteria Monitoring can be found in Appendix B - Sample Documents.

For water samples analyzed at the State Laboratory, an Environmental Microbiology Fresh Surface Water Examination form must be completed by volunteers and/or program staff and included with each sample set. Information to be filled out includes date and time of collection, site number, collector’s name, title and phone, source of sample (e.g. stream name), address of collection site (or GPS coordinates), and requested surface water tests. A label identifying the contact person (health director-sanitarian) and address of the requisitioning municipality must also be included1.

At the State Laboratory, a laboratory accession number is given to each sample and a copy of the label put on the form. Copies of these forms are made and given to ECCD, and are kept on file in the office. Processed water quality results are returned to ECCD, and kept on file in the office as well. Hard copies of all data as well as back-up disks of digital data are maintained by ECCD and will be stored for 3 years.

Element 10

Sampling Process Design

Discrete grab samples will be collected by ECCD staff and/or volunteers before noon from water sampling sites weekly between Memorial Day and Labor Day utilizing methodology outlined in the Anguilla Brook Bacteria Trackdown and Watershed-Based Plan Bacteria Field Sampling Manual (Appendix 1.D). Water temperature will be measured at the site, and samples will be

1 This municipal contact information must be included to obtain services of the State Laboratory free of charge, which all municipalities receive automatically.
analyzed by the Connecticut State Laboratory for freshwater fecal indicator bacteria (E. coli). Additionally, the water sample collected from the lower-most site on Anguilla Brook (AB-01) will be analyzed for fecal coliform and enterococcus to determine if Anguilla Brook is contributing to levels of these fecal indicator bacteria that have been observed in Wequetequock Cove. Sampling sites, which are listed in Table 3 below and shown on Figure 2, have been selected to address the study goals. These proposed stations have been submitted to the CT DEEP Total Maximum Daily Load Program staff for review and may be adjusted by their recommendation. As mentioned in Element 6, Project/Task Description, samples will be collected from Anguilla Brook and perennial tributaries to help determine whether, and to what extent, the selected tributaries are contributing to bacteria levels documented in the main stem. New sites may be added if initial sampling results indicate that additional assessment may bracket bacterial sources. Sites may be discontinued if they demonstrate consistently low (background) or acceptable E. coli levels.

Where necessary, permission to cross private property is sought to access sites. Volunteers are advised to consider their safety and not collect samples if safety is in question.

**Table 2. Proposed Monitoring Stations for Bacteria Source Identification**

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Stream Name</th>
<th>Site Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-01</td>
<td>Anguilla Brook</td>
<td>Off Trolley Crossing – at outlet of Wequetequock Pond. Downstream-most site; upstream of salt-water limit</td>
</tr>
<tr>
<td>AB-02</td>
<td>Anguilla Brook</td>
<td>At RT 1 – same as UConn PATH sampling site</td>
</tr>
<tr>
<td>AB-03</td>
<td>Anguilla Brook</td>
<td>At Anguilla Brook Road – midway in watershed; downstream of residential, agriculture and recreational uses</td>
</tr>
<tr>
<td>AB-04</td>
<td>Anguilla Brook</td>
<td>AT RT 184 – upstream-most site; downstream of agricultural uses</td>
</tr>
<tr>
<td>DB-01</td>
<td>Donahue Brook</td>
<td>US of confluence with Oxocossett Brook above salt-water limit; downstream of agricultural uses; tributary to Wequetequock Cove</td>
</tr>
<tr>
<td>DB-02</td>
<td>Donahue Brook</td>
<td>At Barnes Road – upstream-most site</td>
</tr>
<tr>
<td>WB-01</td>
<td>Wheeler Brook</td>
<td>Upstream of the confluence with Anguilla Brook</td>
</tr>
<tr>
<td>WB-02</td>
<td>Wheeler Brook</td>
<td>At Taugwonk Road – upstream of the confluence with Stony Brook; downstream of agricultural uses</td>
</tr>
<tr>
<td>WB-03</td>
<td>Wheeler Brook</td>
<td>AT RT 184 – upstream-most site; downstream of residential use</td>
</tr>
<tr>
<td>UN-01</td>
<td>Unnamed stream</td>
<td>Upstream of confluence with Wheeler Brook; downstream of agricultural uses</td>
</tr>
<tr>
<td>UN-02</td>
<td>Unnamed stream</td>
<td>At Stony Brook Rd – upstream-most site</td>
</tr>
</tbody>
</table>
Figure 1. Proposed Monitoring Stations for Bacteria Source Identification
Element 11

Sampling Methods Requirements

The methods utilized in the Anguilla Brook Bacteria Trackdown and Watershed-Based Plan project are included in Appendix 1, Standard Operating Procedure Guides. The volunteer manual contains detailed information about sampling protocols and equipment. The following tables provide a summary of this information.

Table 3. Field Sampling Methods

<table>
<thead>
<tr>
<th>Analyte/Parameter</th>
<th>Sample Matrix</th>
<th>Total # Samples</th>
<th>Sample Volume Needed</th>
<th>Sample Container</th>
<th>Method of Sample Preservation</th>
<th>Maximum Allowable Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli Bacteria*</td>
<td>River water 6-10” deep, or mid-depth</td>
<td>11 freshwater samples per sampling event, for a total of 110 samples</td>
<td>100 mL</td>
<td>Single-use sterilized 125 ml polystyrene bottle with a screw cap</td>
<td>Cooler with non-toxic re-freezable ice packs to &lt;10ºC</td>
<td>6 hours</td>
</tr>
<tr>
<td>Stream Temperature</td>
<td>River water 6-10” deep, or mid-depth</td>
<td>Three measurements will be collected and averaged</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Sampling site AB-01 (the lower-most site on Anguilla Brook) will also be analyzed for fecal coliform and Enterococci at the request of CT DEEP.

Element 12

Sample Handling and Custody Requirements

Sample containers used for analysis of indicator bacteria at the Connecticut State Laboratory are pre-labeled with blank labels. The monitoring volunteer records the date, time, and site number on the labels using a permanent marker, and also records the sample collection information on the Field Sheet for Bacteria Monitoring. At the end of the sampling event, a second volunteer checks the samples and Field Sheet for Bacteria Monitoring to ensure that all the samples are
collected and information is recorded properly. The contact name and phone number of a team member involved in the sampling is also included on the **Field Sheet for Bacteria Monitoring** in case there are questions from the lab during their analysis of the samples. A sample checklist listing all site numbers is referenced to ensure all samples have been collected on each collection date. Water samples will be iced or refrigerated at a temperature of <10°C during transit to the laboratory. Use of insulated containers ensures proper maintenance of storage temperature. Care is taken to ensure sample bottles are tightly closed after the sample is collected and are not immersed in water during transit or storage.

An ECCD staff person or monitoring volunteer completes a State Laboratory form to be submitted with each sample (Bureau of Laboratories, **Environmental Microbiology Fresh Surface Water Examination**). Information to be filled out includes date, collector’s name, town, sampling location (stream name/site location), time of collection, rainfall, and collector’s number (site number). The contact name and phone number of a team member involved in the sampling is also included on the **Environmental Microbiology Fresh Surface Water Examination** form in case there are questions from the lab during their analysis of the samples. Samples are transported to the State Laboratory either by an ECCD staff person, the volunteer Team Leader, or by a CT Department of Public Health courier. At the State Laboratory, each sample is labeled with a unique laboratory accession number. A duplicate copy of the accession number label is put on the form. Copies of these forms are given to ECCD for reference and tracking.

### Element 13

**Analytical Methods Requirements**

Analytical methods used by the DPH Laboratory and ECCD are summarized below. Standard operating procedures for the Connecticut State Laboratory are detailed in Appendix 1.

**Table 4. DPH Laboratory Analytical Method for Indicator Bacteria in Fresh Surface Waters**

<table>
<thead>
<tr>
<th>Indicator Organism</th>
<th>E. coli</th>
<th>Fecal coliform</th>
<th>Enterococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method Reference</td>
<td>Colilert (1)</td>
<td>Colilert (1)</td>
<td>Enterolert (1)</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>10 ml diluted to 100 ml</td>
<td>10 ml diluted to 100 ml</td>
<td>10 ml diluted to 100 ml</td>
</tr>
<tr>
<td>Incubation time/temperature</td>
<td>18-22 hrs at 35°C ±0.5°C</td>
<td>18-22 hrs at 44.5°C ±0.2°C</td>
<td>24/28 hrs @41° ±0.5°C</td>
</tr>
<tr>
<td>Detection Limit (at 1:10 dilution)</td>
<td>10 MPN/100 ml</td>
<td>10 MPN/100 ml</td>
<td>10 MPN/100 ml</td>
</tr>
<tr>
<td>Comments</td>
<td>Lab uses Colilert-18 media</td>
<td>Lab uses Colilert-18 media</td>
<td>Fresh water sample</td>
</tr>
</tbody>
</table>

1. Federal Register March 26, 2007 using quanti-tray format, 40 CFR Part 136.3
Table 5. ECCD Analytical Method

<table>
<thead>
<tr>
<th>Analyte/Parameter</th>
<th>Sample Matrix &amp; Quantity Analyzed</th>
<th>Analytical Method</th>
<th>QA/QC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>In-stream measurement</td>
<td>Pocket Thermometer</td>
<td>3 field measurements taken and averaged</td>
</tr>
</tbody>
</table>

**Element 14**

**Quality Control Requirements**

A variety of field and laboratory checks are used by ECCD to ensure the quality and reliability of data. Specific quality control procedures for each type of lab analysis are summarized in the “Analytical Procedures” table included in Element 13. Additional field and laboratory checks are described below. The State Laboratory is a Federal EPA-certified lab and has an approved QA/QC plan on file.

Training: Program volunteers are trained in proper water sampling technique prior to actual sampling, and are provided with a written manual of procedures for reference.

Quality Control Samples: Quality control samples for bacteria are collected as a check for both field sampling and lab procedures.

1. Duplicate Samples: Duplicate samples for bacteria are collected at no less than 10% of the sites each sample day. The duplicate samples are marked on the bottle and field sheet with a site number, followed by a “D,” and transported with the rest of the samples to the Connecticut State Laboratory for analysis.

2. Field Blanks: Field blanks for bacteria are submitted to the Connecticut State Laboratory for analysis along with regular samples at a frequency of 10%. Volunteers will be provided with a container of sterilized buffered solution and an extra sample bottle. At the site, volunteers will fill one sample bottle with sterilized buffered solution. The field blanks are marked on the bottle and field sheet with a site number, followed by a “B,” and transported with the rest of the samples to the lab for analysis.

**Element 15**

**Instrument/Equipment Testing, Inspection, and Maintenance Requirements**

Sample Bottles: Sterile, single-use 120-ml polystyrene bacteria collection bottles are provided by the Connecticut State Laboratory. Extra bottles are available if bottles become lost, damaged or contaminated.
Coolers: Coolers and ice packs used for transporting samples are cleaned and inspected for leaks or cracks before being used. Coolers and ice packs should be cleaned and wiped with alcohol before use to reduce the potential for accidental contamination.

Thermometers: Alcohol-filled thermometers are used to measure water and air temperature in the field. They are inspected before use to make sure that they are not frozen, cracked or contain air bubbles. Extra thermometers are available should a thermometer become lost or damaged.

**Element 16**

**Instrument Calibration and Frequency**

The alcohol-filled thermometers are checked annually against each other and an electronic thermometer which has been calibrated against a NIST-traceable thermometer at room temperature and near freezing to ensure the results are comparable and within allowable tolerances (1° F).

**Element 17**

**Inspection/Acceptance Requirements for Supplies**

All equipment and supplies are purchased under the supervision of the Project Manager (ECCD), or are on loan from project partners, according to program specifications.

**Sample Bottles:** Sterilized single-use 120 mL polystyrene screw-cap bottles are provided by the State of Connecticut Department of Public Health Microbiology Laboratory. All sample bottles are inspected for cracks, broken plastic seals, loose tops and possible leaks by program staff prior to acceptance. If the bottles do not meet standards, they are replaced.

**Coolers/Cold Packs:** Coolers and cold packs are purchased locally and are used to preserve, store and transport sampling equipment in the field, and to the laboratory. Coolers must be large enough to hold four frozen cold packs, and as many 125-mL sample bottles as required to sample all sites (including blanks and duplicates). Coolers and cold packs are inspected prior to use by program staff to ensure they are not defective and meet specifications.

**Other Equipment:** In addition to coolers and sample bottles, monitoring volunteers will be provided an alcohol thermometer, permanent marker, a clip board with storage, pencils and a data sheet.

**Element 18**

**Data Acquisition Requirements**

External data and reference material used by ECCD to design this project and interpret water quality data are described below.
• State of Connecticut Water Quality Standards (revised 10-10-13) - to evaluate program data
• Geographic Information System:
  o Center for Land-use Education and Research (CLEAR) - 2015 Land Cover
  o CT DEEP:
    ▪ Hydrography_shp
    ▪ Local_basin_shp
    ▪ Routes_shp
  o USDA\NAIPbyCounty\Ortho_1-1_1m_j_ct011_2008_2.jp2
  o Town of Stonington Geographic & Property Information Application - http://gis.stonington-ct.gov/ags_map/
  o GoogleEarth 2018

Element 19

Data Management

All sampling data are stored on computers at the ECCD Brooklyn, CT office. Hard copies of all collected data are also kept on file at this office for ten years. Computers to be used are all PCs, and Microsoft Excel is used to compile and analyze data. This program was chosen because it is capable of storing data and producing documents in formats that are effectively universal, and is compatible with the computer systems of most organizations.

Program staff and volunteers will collect data in the field on data sheets that are provided to them prior to the sample date. These field sheets require certain information in order to keep records accurate.

Samplers’ names, current weather conditions as well as the weather conditions of the previous 3 days are entered on the sheet along with current air and water temperature, site designation, bottle #, and time the sample was taken.

After sampling is complete, the field data sheets are reviewed for completeness while volunteers are still available to complete any omissions or correct any misinformation. When all samples and data sheets are completed and accounted for, program staff or volunteers transport the samples to the State Laboratory or pre-arranged pick-up location for analysis. The original field data sheets are filed at the ECCD Brooklyn, CT office for reference. Samples analyzed at the State Laboratory are assigned a laboratory accession number by the State Laboratory at the time they are checked in. Results are reported by accession number and by ECCD-assigned site number (labeled on the bottle). A hard copy of the results is sent to the cooperating local health department office by DPH. An electronic copy is emailed to ECCD by DPH. All results are entered into the computer system by ECCD staff using Microsoft Office Excel. After data are entered into the database, they are reviewed and compared against the original hard copies by other program staff for omissions, typographical errors, and duplication. Electronic and hard copies of the data are stored at the ECCD Brooklyn, CT office.
Element 20

Assessments and Response Actions

The project manager (ECCD) is responsible for reviewing field activities and water sample analysis results, in consultation with the QA Officer. Water sampling volunteers are trained annually in sampling procedures. Volunteers are observed by ECCD staff in the field to ensure they are following sampling protocols correctly. They are corrected in the field if they are not following sampling protocols correctly. In addition, program staff perform visual checks of samples at the time of drop off. If, for example, samples appear unusually turbid, volunteers are questioned about conditions in the stream or sampling methods used. If sampling methods are questionable, the sample may be discarded and a new sample collected. If the sample is submitted for analysis, a note will be made on the data collection form. The results may be qualified or discarded if they seem disparate from other data from the same site or conditions on the sampling day. Volunteers are also requested to collect duplicate samples and field blanks on a rotating schedule; processing of duplicates and field blanks provide additional checks on sampling protocols. If data quality objectives for duplicate and field blank samples are not met (and discrepancies cannot be ascribed to analytical methods), volunteers are questioned about protocols used, observed in the field, and, if necessary, re-trained in procedures.

Element 21

Reports

Water quality sampling results are compiled, analyzed and summarized in a summary report upon completion of water quality sampling. The sampling report summarizes water sampling and analysis activities including a description of the activity (type of sampling, site descriptions, frequency, timing, sample analysis), a narrative discussion of the results, including findings and conclusions, and data tables. The raw water quality data is transcribed into a spreadsheet provided by DEEP and is submitted to DEEP Water Quality Monitoring and TMDL staff upon completion of sampling. The sampling report is distributed to CT DEEP Watershed Management staff, EPA, municipalities, program volunteers, partners, and other potential data users at the local level. The Project Manager (ECCD) is responsible for report production and distribution.

Element 22

Data Review, Validation and Verification

The Project Manager and QA Officer (ECCD) will review field and laboratory data to determine if QAPP data objectives are being met as data is received and at the completion of sampling activity. The review of laboratory data as it is processed and received by ECCD staff may result in adjustments to the monitoring plan. For example, a site may be discontinued if it demonstrates consistently low FIB levels, while additional sites may be added to bracket a site with consistently high FIB levels. In addition, CT DEEP personnel and other resource professionals with a particular
expertise may be consulted as needs arise. The Project Manager and QA Officer (ECCD) make
the final determination about whether results will be accepted, rejected, or qualified.

Element 23

Verification and Validation Methods

The Project Manager (ECCD) will review all data as it is received from the field and the laboratory
to ensure all samples were received and analyzed within the allowable hold time. If all samples
were not received by the lab and/or analyzed within the allowable hold time, the Project Manager
will contact the local health district to which the samples were delivered for pick-up by the lab
courier service and the lab to determine where errors in the chain of custody may have occurred.
The consistency of water sampling data will be evaluated throughout the sampling season. Results
of analyses performed by the Connecticut State Laboratory are reviewed and any questions or
concerns about inconsistencies are raised with the director of the microbiology lab. All data will
be checked against the acceptance criteria included in Elements 7, 11 and 13 of this QAPP in order
to determine whether the data quality is sufficient for this project. If data are in question, results
will not be used or will be qualified. Once entered in the computer, data are checked against the
original data sheets. Errors in data entry are corrected.

Element 24

Reconciliation with Data Quality Objectives

Quality assurance samples are checked against acceptance criteria, and if necessary, corrective
actions taken. The Connecticut State Laboratory monitors and reconciles its own data quality
objectives as part of its standard operating procedures. If duplicate analyses appear inconsistent
and do not meet data quality objectives, results are discussed with the director of the microbiology
lab, and data may be qualified or not used. In all cases, the problem will be investigated and the
source of the error, whether related to sampling protocol, analytical method, or equipment failure,
determined and corrected.
Appendix 1
Standard Operating Procedures

1.A. Connecticut Department of Public Health Laboratory Standard Operating Procedure for Determination of *Escherichia coli* in Bathing Waters

1.B. Connecticut Department of Public Health Laboratory Standard Operating Procedure for Determination of Enterococci in Bathing Waters

1.C. Colilert Test Kit Procedure for Determination of Total Coliforms and *E. coli* in freshwater

1.D. Anguilla Brook Bacteria Trackdown and Watershed Based Plan Bacteria Field Sampling Manual
Appendix 1.A.

Connecticut Department of Public Health Laboratory Standard Operating Procedure for Determination of *Escherichia coli* in Bathing Waters
Connecticut Department of Public Health
Division of Laboratory Services
Environmental Microbiology Section

Procedure:

Colilert Bathing Water Enumeration Method for *E. coli*

Chromogenic Substrate Coliform Test

For Bathing Water Analysis
Environmental Microbiology

PREPARED BY: __________________________ DATE: ____________

REVIEWED BY: _________________________ DATE: ____________

APPROVED BY: _________________________ DATE: ____________
1.0 PRINCIPLE:

1.1 This procedure provides instructions for the enumeration of *Escherichia coli* (*E. coli*), the indicator organism in Fresh Bathing Water samples. The Colilert® reagents are used for the simultaneous detection and confirmation of total coliforms and *E. coli* in water. It is based on IDEXX’s patented Defined Substrate Technology and utilizes a nutrient indicator that produces color (for Total coliform) and/or fluorescence (for *E. coli*) when metabolized by total coliforms and *E. coli*. When the reagent is added to the sample and incubated, it can detect these bacteria at 1 MPN/100ml within 18 hours with as many as 2 million heterotrophic bacteria present. The IDEXX Quanti-trays are designed to give quantitative bacterial counts of 100 ml samples using IDEXX Defined Substrate Technology reagent products. Add the reagent/sample mixture to a Quanti-tray, seal it in a Quanti-tray Sealer and incubate per reagent directions. Then count the number of positive wells and use the MPN table to determine the Most Probable Number (MPN).

1.2 *Escherichia coli*: A substrate such as the fluorogenic substrate 4-methylumbelliferyl-ß-D-glucuronide (MUG) is used to detect the enzyme ß-glucuronidase, which is produced by *E. coli*. The ß-glucuronidase enzyme hydrolyzes the substrate and produces a fluorescent product when viewed under long-wavelength (366-nm) ultraviolet (UV) light. The number of fluorescent wells are counted and the MPN table is used to determine the Most Probable Number (MPN).

2.0 SAMPLE:

2.1 The chromogenic substrate coliform test is recommended for the analysis of Fresh Bathing water samples.


2.3 Guidelines for rejecting samples are found in the “QA Manual”, Connecticut Department of Public Health, Division of Laboratory Services.

3.0 SAFETY:

3.1 Treat all biological material as potentially infective.

3.2 Wash hands with soap immediately if they become contaminated. Wash hands with soap after removing lab coat. Wash hands with soap before leaving the laboratory.
3.3 No mouth pipeting. No eating, smoking, or chewing gum in the laboratory.

3.4 Always wear a laboratory coat, apron or gown when working in the laboratory. Remove uniforms before leaving the laboratory.

3.5 Decontaminate laboratory work surfaces at least daily with freshly prepared chemical germicide when work activities are completed.

3.6 Immediately decontaminate fluid culture spills.

4.0 EQUIPMENT:

4.1 Temperature Monitoring Device - Use glass thermometers

4.1.1. Thermometers are graduated in 0.5°C increments or less (0.1°C increments for tests incubated at 44.5°C)

4.1.2 There should be no separation in fluid column of glass thermometer.

4.1.3 Calibrate thermometers annually at the temperature used, against a NIST certified thermometer or one meeting the requirements of NBS Monograph SP 250-23.

4.1.4 Check NIST certified thermometer annually for accuracy by ice point determination. Record and maintain results.

4.1.5 Record calibration checks in quality control (QC) record and on thermometer. Mark thermometer with identification, NBS calibration correction factor, calibration temperature, calibration date, on each thermometer.

4.1.6 All working thermometers must be appropriately immersed.

4.1.7 Discard thermometer if off by more than 1°

4.1.8 Record daily temperature checks in the daily temperature book and keep for at least five years. Continuous recording devices are not used to monitor temperatures.

4.2. Incubator

4.2.1 Maintain temperature at 35°C ± 0.5°C.

4.2.2 Place thermometers on top and bottom shelves of use area.

4.2.3 If partially-submersible glass thermometer is used, bulb and stem must be immersed in water to the mark on the stem.

4.2.4 Check and record calibration corrected temperature twice per day with readings separated by at least 4 hours.

4.2.5 Waterbath must be equipped with gable cover and pump used to circulate water.
4.3 Autoclave
The purpose of the autoclave in this procedure is to decontaminate infectious material. The Support Services division has responsibility for the autoclave operation.

4.3.1 Autoclave has internal heat source, a temperature gauge with a sensor on the exhaust, pressure gauge, and operational safety valve.
4.3.2 Autoclave maintains sterilization temperature during the sterilizing cycle and completes an entire cycle within 45 minutes when a 15-minute sterilization period is used.
4.3.3 Autoclave should depressurize slowly to ensure media does not boil over and bubbles do not form in inverted tubes.
4.3.4 Record date, contents, sterilization time, temperature, total cycle time, and analysts initials for each cycle.
4.3.5 Establish service contract or internal maintenance protocol, and maintain records. Conduct maintenance weekly with records of most recent service performed. Keep door seals and drain screen clean.
4.3.6 Use maximum-temperature-registering thermometer during each autoclave media run. Record temperature.
4.3.7 Avoid overcrowding.
4.3.8 Use spore strips weekly.
4.3.9 Check automatic timing mechanism with stopwatch quarterly.

4.4 Long wavelength (366 nm) ultraviolet lamp.

4.5 Quanati-Tray Sealer
4.5.1 Purchase from IDEXX Laboratories, Inc

5.0 MATERIALS:

5.1 Substrate

5.1.1 Purchase Colilert® Reagent from IDEXX Laboratories, Inc. in snap packs for 100 mL water samples for the Enumeration Procedure. Fresh Water Bathing water samples are diluted at least ten-fold with sterile Butterfield’s buffer water (see procedures 7.0)

5.1.2 Store at 4-25°C away from light. Avoid prolonged exposure of substrate to direct sunlight.

5.2 Quanti-tray 51 well

5.2.1 Purchase the 51 Well Quanti-tray from IDEXX Laboratories, Inc.

5.3 Butterfield’s dilution blanks, 90 ml
5.4 Pipets, 10 ml.

5.5 Colilert® Quanti-tray Comparator
Purchase from Idexx Laboratories, Inc.

6.0 QUALITY CONTROL:

6.1 Check each lot of Colilert® Reagent for autofluorescence and color change on receipt.

Add 100 ml. of sterile distilled water to a sterile vessel. Add Colilert reagent, shake to mix and wait until reagent is dissolved. If reagent exhibits a color change before incubation, lot is unacceptable. Check the reagent with 366-nm ultraviolet light with 6 watt bulb. If reagent exhibits faint fluorescence, the reagent lot is unacceptable; reject lot. Record results.

6.2 Test each lot of Colilert® Reagent for sterility on receipt.

6.2.1 Add 100 ml. of sterile distilled water to a sterile vessel. Add Colilert reagent, shake to mix and incubate at 35° ± 0.5° C for 24 hrs (18 hrs. for Colilert-18) and check for growth. Record as acceptable if no growth and not acceptable if growth is present. Reject the lot if contamination is indicated.

6.3 Test each lot of Colilert reagent when received for proper reactions.

6.3.1 Label three sterile vessels "Escherichia coli", "Klebsiella pneumoniae" and "Pseudomonas aeruginosa".

6.3.2 Add 100 ml. of sterile distilled water to each of three vessels, then add Colilert reagent and mix thoroughly.

6.3.3 Aseptically inoculate the respective vessels with growth from 18-24 hr. slants of each organism.

6.3.4 Incubate inoculated vessels at 35° ± 0.5° C for 24 hrs (18 hrs. for Colilert-18). Read and record results.

6.3.5 Reject lot if results do not match the following:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Yellow and Fluorescence</td>
<td>Positive for <em>Escherichia coli</em></td>
</tr>
<tr>
<td><em>Klebsiella Pneumoniae</em></td>
<td>Yellow And No Fluorescence</td>
<td>Positive For Total Coliform</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Colorless or slight tinge</td>
<td>Negative for <em>E. coli</em> and Total Coliform</td>
</tr>
</tbody>
</table>

6.4 Each day of Bathing Water Testing, perform the Colilert® Test on a known positive, negative and sterile sample. Use the following ATTC (American Type Culture Collection) organisms recommended by the manufacturer IDEXX Laboratories, inc. For Positive samples use ATCC 25922
or 11775 (*E. coli*), for Negative samples use ATCC 31488 Klebsiella pneumoniae and use the 90 ml Butterfield’s buffer for the sterile sample.

6.4.1 Label three Butterfield’s buffer vessels “EC” (*E. coli*), “KP” (Klebsiella pneumoniae) and “Sterility”

6.4.2 Add Colilert® Reagent to each marked vessel.

6.4.3 Aseptically inoculate the respective vessels with ATCC growth from 18-24 hours. Slants of each organism. Do not inoculate the vessel marked “sterility”

6.4.4 After sample has been inoculated and after Colilert® has dissolved, pour into the 51 Well Quanti-tray and seal. Incubate inoculated trays and sterility tray at 35° ± 0.5° C for minimum of 18 hours but not more than 22 hours. Read and record results.

7.0 PROCEDURE: Quanti-tray Enumeration Procedure

7.1 Label 90 ml Butterfield’s dilution blank with accession number and test “C-18”.

7.2 label 51 well Quanti-tray with accession number, date and test “C-18”

7.3 Aseptically open a pack of Colilert® reagent by snapping back the top of the scoreline and add the contents of the Colilert® to the labeled 90 ml Butterfield’s dilution blank.

7.4 Shake the Bathing water sample bottle vigorously (25 times in one foot arc in 7 seconds.)

7.5 Aseptically pipet 10 ml of the mixed Bathing water sample to the labeled 90ml sterile Butterfield’s buffer dilution blank containing the Colilert® Reagent.

7.6 Aseptically cap the dilution blank and mix.

7.7 Once the reagent is dissolved, pour this sample directly into the 51- well Quanti-tray avoiding contact with the foil tab.

7.8 Seal tray in Quanti-tray sealer that has been pre-heated and label tray with the time sealed and incubated.

7.9 Incubate the sealed Quanti-tray at 35° ± 0.5° for 18-22 hours.

7.9.1. Colilert®-18 results are definitive at 18-22 hours. Do not read before 18 hours of incubation or after 22 hours of incubation.

7.10 Read the results at 18-22 hours by placing a 6-watt, 365 nm wavelength UV light within 5 inches of the 51 well Quanti-tray in a dark environment. Be sure the light is facing away from your eyes and towards the Quanti-tray. Fluorescence indicates the presence of *E. coli*. 
7.10.1 Count of number of fluorescent Quanti-tray wells and refer to the 51 well Quanti-tray MPN table to find the Most probable number (MPN). Multiply the number on the chart by 10 to obtain the MPN/100ml (see 8.0 Reporting).

7.10.2 Autoclave Colilert® Quanti-tray vessel as infectious waste for proper disposal.

8.0 REPORTING:

8.1 Record the number of positive wells (fluorescence) and the corresponding MPN values for E.coli on the sample invoice. To obtain the MPN value, multiply the MPN value on the chart by the dilution factor, 10 to obtain the proper quantitative result.

8.2 Report results in the computer by worklist.

8.3 Report results as MPN/100ml

8.4 Call the submitter and relevant agencies (Department of Environmental Protection, Local Health Departments and Laboratories) for every E.coli result over the value of 235/100ml.

   8.4.1 Record on the laboratory invoice the name of person, date and time called.

8.5 Submit the completed sample invoice form to the supervisor or designee to check results.

8.6 Report checked results in computer by workboard.

   8.6.1 Report results as per 100 ml.
   8.6.2 Review results for accuracy before filing.

9.0 REFERENCES:

2004, Connecticut Department of Public Health, Division of Laboratory Services QA Manual


IDEXX, Inc., Colilert® product inserts.

(REFERENCES continued)

# IDEXX 51-Well Quanti-Tray® MPN Table

<table>
<thead>
<tr>
<th>No. of wells giving positive reaction</th>
<th>MPN per 100 ml sample</th>
<th>95% Confidence Limits Lower</th>
<th>Upper</th>
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<tbody>
<tr>
<td>0</td>
<td>&lt;1.0</td>
<td>0.0</td>
<td>3.7</td>
</tr>
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Appendix 1.B.

Connecticut Department of Public Health Laboratory Standard Operating Procedure for Determination of Enterococci in Bathing Waters
Procedure:

Enterolert®

Enterococci Defined Substrate Test

For Marine Bathing Water Analysis
Environmental Microbiology

PREPARED BY: Aristea Kinney        DATE: 02/07/07

REVIEWED BY:                      DATE: __________

APPROVED BY:                      DATE: __________
1. PRINCIPLE .................................................................................. 3

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5. QUALITY CONTROL ................................................................. 7-10

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7. RESULTS .................................................................................. 12

8. REFERENCES ........................................................................... 12
1.0 PRINCIPLE:

1.1 The Enterolert® Reagent is used for the detection of enterococci such as E. faecium and E. faecalis in water. It is based on IDEXX’s patented Defined Substrate Technology and utilizes a nutrient indicator substrate, 4-methyl-umbelliferyl β-D-glucoside that fluoresces when metabolizes by enterococci enzyme B-glucosidase. When the reagent is added to the sample and incubated, it can detect these bacteria at 1 MPN/100ml within 24 hours. The IDEXX Quantitrays are designed to give quantitative bacterial counts of 100 ml samples using IDEXX Defined Substrate Technology reagent products. Add the reagent/sample mixture to a Quanti-Tray, seal it in a Quanti-Tray Sealer and incubate per the reagent directions. Then count the number of positive wells and use the MPN table to determine the Most Probable Number (MPN)/100 ml of sample.

2.0 SAMPLE:

2.1 The Enterolert test is used for the analysis of Marine bathing water samples.

2.2 Sampling, transport, and holding time procedures are found in 40 CFR Part 136. Samples must be analyzed within 8 hours of collection or the sample is not tested and reported as “Unsatisfactory for Examination”.

2.3 Guidelines for rejecting samples are found in the “Quality Manual” Connecticut Department of Public Health, Division of Laboratory Services.
3.0 SAFETY:

3.1 Treat all biological material as potentially infective.

3.2 Wash hands with soap immediately if they become contaminated. Wash hands with soap after removing lab coat. Wash hands with soap before leaving laboratory.

3.3 No mouth pipeting. No eating, smoking, or chewing gum in the laboratory.

3.4 Always wear a laboratory coat or gown when working in the laboratory. Remove Laboratory coats of gowns before leaving the laboratory.

3.5 Decontaminate laboratory work surfaces with freshly prepared chemical germicide when work activities are completed.

3.6 Refer to Connecticut Department of Public Health Laboratory Safety Manual-Laboratory Health and Safety Plan for additional safety information.

4.0 EQUIPMENT:

4.1 Temperature Monitoring Device - Use glass thermometers

4.1.1 Thermometers are graduated in 0.5°C increments.

4.1.2 There should be no separation in fluid column of glass thermometer.

4.1.3 Calibrate thermometers annually at the temperature used, against a NIST certified thermometer or one meeting the requirements of NBS Monograph SP 250-23.

4.1.4 Check NIST certified thermometer annually for accuracy by ice point determination. Record and maintain results.
4.1.5 Record calibration checks in quality control (QC) record and on thermometer. Mark thermometer with identification, NBS calibration correction factor, calibration temperature, calibration date, on each thermometer.

4.1.6 Use partial Immersion Thermometer.

4.1.7 Discard thermometer if off by more than 1°.

4.2 Incubator

4.2.1 Maintain temperature at 41°C ± 0.5°C.

4.2.2 Place thermometers on top and bottom shelves of use area.

4.2.3 If partially-submersible glass thermometer is used, bulb and stem must be immersed in water to the mark on the stem.

4.2.4 Check and record calibration corrected temperature twice per day with readings separated by at least 4 hours.

4.2.5 Record daily temperature checks in the daily temperature book and keep for at least five years.

4.3 Autoclave

The purpose of the autoclave in this procedure is to decontaminate infectious material. The Support Services Division has responsibility for the autoclave operation.

4.3.1 Autoclave has internal heat source, a temperature gauge with a sensor on the exhaust, pressure gauge, and operational safety valve.
4.3.2 Autoclave maintains sterilization temperature during the sterilizing cycle and completes an entire cycle within 45 minutes when a 15 minute sterilization period is used.

4.3.3 Record date, contents, sterilization time, temperature, total cycle time, and analysts initials for each cycle.

4.3.4 Establish service contract or internal maintenance protocol, and maintain records. Conduct maintenance weekly with records of most recent service performed. Keep door seals and drain screen clean.

4.3.5 Avoid overcrowding.

4.3.6 Test spore strips weekly. Record results on sterilization records. Take corrective action if positive.

4.3.7 Check automatic timing mechanism with stopwatch quarterly.

4.4 Ultraviolet Lamp Box with a 365 nm and 6 watt.

4.4.1 Maintenance

4.4.2 QA

5.0 MATERIALS:

5.1 Substrate

5.1.1 Purchase Enterolert® Reagent from Idexx Laboratories, Inc. (Catalog # WENT200) in snap packs for 100 mL water samples for the Quanti-Tray Enumeration Procedure.
5.1.2 Marine water samples must be diluted ten-fold with sterile Butterfield’s Buffer. See 7.3. Store at 4-25°C away from light. Avoid prolonged exposure of substrate to direct sunlight.

5.1.3 Discard expired substrate.

5.2 Quanti-Tray 51 well

5.2.1 Purchase the 51 Well Quanti-Tray from Idexx Laboratories, Inc. (Catalog #WQT-100)

5.3 Butterfield’s dilution blanks, 90 ml

5.3.1 Purchase Butterfield’s dilution blanks from Biotrace (Redmond, WA. Catalog # FT-BFD-99.)

5.4 Quanti-Tray Sealer

5.4.1 Purchase from IDEXX Laboratories, Inc. (Catalog # WQTS2X-115)

5.5 Pipets, 10 ml

5.5.1 Purchase from Fisher Scientfic (Catalog # 13-678-14A).

5.6 Sterile vessel, Disposable

5.6.1 Purchase from IDEXX Laboratories, Inc. (Catalog # WV12SB-200)

6.0 QUALITY CONTROL:

6.1 Check each lot of Enterolert® Reagent for autofluorescence and color change on receipt.
6.1.1 Add 100 ml. of sterile distilled water to a sterile vessel (see 5.6). Add Enterolert reagent, shake to mix and wait until reagent is dissolved. If reagent exhibits a color change before incubation, reject as unacceptable. Check the reagent mixture by placing in a Ultraviolet Light box (365-nm ultraviolet light with 6 watt bulb). If reagent exhibits faint fluorescence, the reagent lot is unacceptable; reject lot. Record results in Water QC book.

6.2 Test each lot of Enterolert® Reagent for sterility on receipt.

6.2.1 Add 100 ml. of sterile distilled water to a sterile vessel. Add Enterolert reagent, shake to mix and incubate at 41°C ± 0.5°C for 24 hrs and check for growth. Record in Water QC Book as acceptable if no growth and not acceptable if growth is present. Reject the lot if contamination is indicated.

6.3 Test each lot of Enterolert reagent when received for proper reactions. Use the following organisms recommended by the manufacturer Idexx Laboratories, Inc: Enterococcus faecium, Serratia marcescens and Aerococcus viridans.

6.3.1 Label three sterile vessels "EF", "SM" and "AV".

6.3.2 Add 100 ml. of Butterfield’ Buffer to each of three vessels, then add Enterolert reagent and mix thoroughly.

6.3.3 Aseptically inoculate the respective vessels with growth from 18-24 hr. slants of each organism.

6.3.4 After sample has been inoculated and Enterolert has dissolved, pour into 51 Well Quanti-Tray, seal and incubate inoculated Quanti-trays at 41°C ± 0.5°C for 24 hrs. (see 7.7).
6.3.5 Read and record results after incubation time by placing Quanti-Tray in Ultraviolet Light box, looking for fluorescence.

6.3.6 Reject lot if results do not match the following:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecium</td>
<td>Fluorescence</td>
<td>Positive for enterococcus</td>
</tr>
<tr>
<td>Serratia Marcescens</td>
<td>No Fluorescence</td>
<td>Negative For Enterococcus</td>
</tr>
<tr>
<td>Aerococcus viridans</td>
<td>No fluorescence</td>
<td>Negative for enterococcus</td>
</tr>
</tbody>
</table>

6.4 Each day of Bathing Water Testing, perform the Enterolert test on a known positive, negative and sterile sample. Use the following ATTC organisms recommended by the manufacturer Idexx Laboratories, Inc for the Positive and Negative sample: *Enterococcus faecium*, and *Serratia marcescens*, and use the 90ml Butterfield’s Buffer for the sterile sample.

6.4.1 Label three sterile vessels "EF", "SM" and "Sterility"

6.4.2 Add 100 ml. of Buttersfield’s buffer to each sterile vessel. Then add Enterolert reagent and mix thoroughly.

6.4.3 Aseptically inoculate the respective vessels with growth from 18-24 hr. slants of each organism. Do not inoculate the vessel marked “Sterility”

6.4.4 After sample has been inoculated and after Enterolert has dissolved, pour into 51 Well Quanti-Tray and seal (see 7.7) Incubate inoculated trays and Sterility tray at 41ºC ± 0.5ºC for 24 hrs.
6.4.5 Read and record results in Water QC book after incubation time by placing Quanti-Tray in Ultraviolet Light box, looking for fluorescence.

6.4.6 Reject all results for that day that do not match the following results:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecium</td>
<td>Fluorescence</td>
<td>Positive for enterococcus</td>
</tr>
<tr>
<td>Serratia Marcescens</td>
<td>No Fluorescence</td>
<td>Negative For Enterococcus</td>
</tr>
<tr>
<td>Sterility</td>
<td>No fluorescence</td>
<td>Negative for enterococcus</td>
</tr>
</tbody>
</table>
7.0 PROCEDURE: Quanti-Tray Enumeration Procedure

7.1 Label 90 ml Butterfield’s dilution blank with accession number and test (ENT).

7.2 Label 50 well Quanti-Tray with accession number, date, and test (ENT).

7.3 Aseptically open a pack of Enterolert reagent by snapping back the top at the scoreline and add the contents of the Enterolert to the labeled 90 ml Butterfield’s dilution blank.

7.2 Shake the Bathing water sample bottle vigorously (25 times in one foot arc in 7 seconds).

7.3 Aseptically pipet 10ml of the sample to the labeled 90ml sterile Butterfield’s dilution blank containing the Enterolert reagent.

7.4 Aseptically cap the dilution blank and mix.

7.6 Once the reagent is dissolved, aseptically pour this sample directly into the labeled 51-well Quanti-Tray avoiding contact with the foil tab.

7.7 Seal Quanti-Tray in a preheated Quanti-Tray Sealer by placing Quanti-tray in the rubber insert, aligning holes to fit Quanti-Tray and gently pushing until the rubber insert is grabbed and is drawn into the sealer.

7.8 Once sealed, the Quanti-Tray will be partially ejected from rear of the Sealer. Remove the rubber insert and Quanti-tray from the rear of the sealer and mark the time on the tray.

7.9 Incubate the sealed Quanti-Tray at 41 ±0.5°C for 24 hours.

7.9.1 Enterolert results are definitive at 24-28 hours.

7.10 Read the results at 24 hours by placing the Quanti-Tray in the Ultraviolet Light box with the 6 watt, 365 nm wavelength UV light turned on. Blue fluorescence indicate the presence of Enterococci.
7.11 Count the number of fluorescent Quanti-Tray wells and record on the sample invoice form. Refer to the 51-Well Quanti-Tray MPN table to find the Most Probable Number (MPN)/100ml.

7.12 Calculate the Most Probable Number (MPN)/100ml by referring to the 51-well Quanti-Tray MPN table (See Appendix 1). Multiply the MPN value on the table by the dilution factor (10).

7.13 Record on sample invoice form.

8.0 REPORTING:

8.1 Report results in computer by worklist.

8.2 Report results as MPN/100ml.

8.3 Call results of every Enterococci result over the value of 104/100ml to the submitter.

8.4 Review results for accuracy before filing.

9.0 REFERENCES:


9.2 Idexx, Inc., Enterolert® product insert

9.3 Connecticut Department of Public Health, Division of Laboratory Services QA Manual

9.4 U.S. Environmental Protection Agency (USEPA), 40 CFR Part 136

9.5 IDEXX 51-Well Quanti-Tray MPN Table

# IDEXX 51-Well Quanti-Tray® MPN Table

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<th>No. of wells giving positive reaction</th>
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Appendix 1.C.

Colilert Test Kit Procedure for Determination of Total Coliforms and E. coli in freshwater

(DPH does not have an SOP for fecal coliform and will instead follow the Colilert test kit procedure)

Colilert Test Kit Procedure

Introduction and Product Use

Colilert® is used for the simultaneous detection, specific identification, and confirmation of total coliforms and E. coli in water. DO NOT USE FOR MARINE WATER. For Marine Water use Colilert Cat No. MW 200.

Principle

Colilert is based on the Defined Substrate Technology® (DST™). DST utilizes indicator nutrients which cause target microbes contained in the sample and incubated in the DST reagent system to produce a color change (or another signal, i.e., fluorescence), both indicating and confirming their presence. The indicator-nutrient is cleaved by the target microbe which metabolizes the nutrient and frees the indicator to express a specific color. The growth and reproduction process of the target microbe is fueled by the nutrient.

Colilert is a specially designed reagent formulation of salts, nitrogen, and carbon sources that are specific to total coliforms. It provides specific indicator nutrients: ONPG (O-Nitrophenyl-β-D-galactopyranoside) and MUG (4-Methylumbelliferyl-β-D-glucuronic acid) for the target microbes, total coliforms and Escherichia coli. As these nutrients are metabolized, yellow color (from ONPG) and fluorescence (from MUG) are released confirming the presence of total coliforms and E. coli, respectively. Non-coliform bacteria are suppressed and cannot metabolize the indicator nutrients. Consequently, they do not interfere with the specific identification of the target microbes during the test incubation period.

Performance Characteristics

Total coliforms and E. coli are specifically and simultaneously detected and identified at 1 CFU/100 ml of sample, in 24 hours or less, by inoculating the reagent with the water sample and incubating it. No further sample manipulation or testing is necessary. Field and in-house data show Colilert to be sensitive and specific for the detection of total coliforms and E. coli at the 1 CFU/100 ml level in water samples with as many as 20,000 heterotrophic bacteria present per ml.

Materials

W100 - 100 tubes each containing Colilert reagent
W200 - 200 tubes each containing Colilert reagent.

Materials required but not provided:
1. Sterile 10 ml or 20 ml transfer pipets
2. 35°±0.5°C Incubator Cat. No. W1300
3. Long wavelength (365nm) ultraviolet lamp, i.e., Cat. No. WL160
4. Color and fluorescence comparator - Cat. No. W102

(All items listed above are available from IDEXX Laboratories, Inc. Please refer to back cover.)

Storage and Shelf Life

Store at 4°-30°C, away from light. Colilert is stable under these conditions through the expiration date provided on the label.

Sample Collection

Aseptically collect water samples as described in the 17th Edition, Standard Methods for the Examination of Water and Wastewater.

Procedural Notes

1. Adhere to good laboratory practice throughout the test procedure. Avoid touching the reagent or the inside of the tubes or caps.
2. Colilert is for analytical testing only.
3. Do not pipet by mouth.
4. Thoroughly mix all samples immediately before inoculating.
5. Never autoclave Colilert prior to use. This will destroy the reagent which is heat labile.
6. Avoid prolonged exposure of the inoculated Colilert system to...
direct sunlight. The indicator compounds may be hydrolyzed, creating a false positive (yellow) result.

7. After incubation, Colilert should be incubated for 24 hours at 35° ± 0.5°C. Avoid incubation at this temperature beyond 24 hours because non-colliform heterotrophic bacteria present may now possibly overcome the suppressant systems if left longer, yielding a false positive result. A 24-hour incubation period should be verified or the sample repeated.

8. Colilert is a primary water test. Colilert performance characteristics do not apply to samples altered by any form of pre-enrichment or concentration. This includes any method such as growth on a membrane filter or growth in lactose broth containing non-specific growth enhancing step, or any pre-enrichment method such as filtering the sample through a membrane filter and then using the filter to inoculate Colilert.

a. Do not transfer colonies or cultures pre-grown in any enrichment media to Colilert. Colonies grown in such non-specific media may or may not be coliforms. Colilert’s suppressant reagents may be overloaded by transferring such heavy inocula of certain very weak β-galactosidase containing non-colliforms (e.g., some Aeromonas and Pseudomonas), causing a false positive total coliform (yellow) result. Similarly, transfer of high numbers of other heterotrophs (for example, Flavobacterium) can cause a false positive β-galactosidase fluorescence and an inaccurate indication that E. coli is present. While one would not normally expect to encounter such extremely high levels of heterotrophs in a water sample, pre-enrichment could produce them.

b. Do not pre-filter a sample and then place that filter in Colilert. The filtration step can concentrate coliforms but also non-colliform heterotrophs, particulates, and certain chemicals (divalent cations, heavy metals, etc.) which can overgrow and suppress coliforms adversely affecting the sensitivity of the test. Furthermore, coliform bacteria can become trapped in the filter, restricting their access to the indicator-nutrients in the Colilert reagent and their subsequent growth and detection.

9. Do not dilute the sample in buffered water for addition to Colilert. Colilert is already buffered and additional buffer compounds can adversely affect the growth of the target microbes and test performance.

10. If additional confirmation is desired after incubating 24 to 26 hours and reading results, transfer 0.1 ml with a pipet to EC + MUG or other confirmation media.

11. Upon mixing of Colilert reagent with the sample, a transient blue color may appear in samples containing an excessive amount of free chlorine. The sample should be considered invalid and testing discontinued.

12. As with any coliform test method, if large numbers of refrigerated samples are prepared for incubation simultaneously, they should be warmed to room temperature before being placed in the incubator to avoid chilling of the incubator contents, especially when using smaller low wattage incubators.

Test Procedure

1. Select the appropriate number of tubes per sample for your MPN test (5, 10, etc.).
2. Aseptically fill each Colilert tube with 10 ml of a well mixed water sample.
3. Cap the tubes tightly.
4. Mix vigorously to dissolve the reagent by repeated inversion. Some particles may remain undissolved. Dissolution will continue during incubation.
5. Incubate inoculated reagent tubes at 35° ± 0.5°C for 24 hours.
6. Read tubes at 24 hours. If yellow color is seen, check for fluorescence. Color should be uniform throughout the tube. If not, mix by inversion before reading.
Test Results and Interpretation

At 24 hours, compare each tube against the color comparator. If no yellow is observed, the test is negative for total coliforms and E. coli. If any tube has a yellow color greater or equal to the comparator, the presence of total coliforms is confirmed.

If yellow is observed at 24 hours, check each tube for fluorescence by placing the U.V. lamp three-five inches in front of the tube and making sure it is facing away from your eyes and toward the tube. **Observe for fluorescence in a dark environment. If fluorescence of tube(s) is greater or equal to fluorescence of the comparator, the presence of E. coli is specifically confirmed.**

The comparator is the lowest level of yellow and fluorescence which can be considered positive. A typical positive test is much more intense than the comparator.

If a sample is yellow after 24 hours of incubation, but slightly less than the positive comparator or indeterminate, it may be incubated up to an additional 4 hours (but no more than 28 hours total). If the sample is coliform positive, the color will intensify. If it decreases in intensity, consider the sample negative. If the sample color remains indeterminate, the laboratory should consider the sample invalid and request another sample from the same site. Some water samples containing humic material may have an innate color. If a water sample has background color, compare inoculated Colilert tubes to a control blank of the same water sample.

If an inoculated Colilert tube is inadvertently incubated over 28 hours, the following guidelines apply: No yellow color is a valid NEGATIVE TEST. A yellow color after this incubation period should be verified or test repeated.

To find the concentration of total coliforms or E. coli per 100 ml, compare the number of positive tubes per sample set to the standard MPN (Most Probable Number) probability chart as shown.

MPN Index and 95% Confidence Limits for Various Combinations of Positive and Negative Results When Five-10 ml Portions are Used

<table>
<thead>
<tr>
<th>No. of Tubes Giving Positive Reaction Out of 5 of 10 ml Each</th>
<th>MPN Index/100 ml</th>
<th>95% Confidence Limits (Approximate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt; 2.2</td>
<td>0 - 6.0</td>
</tr>
<tr>
<td>1</td>
<td>2.2</td>
<td>0.1 - 12.6</td>
</tr>
<tr>
<td>2</td>
<td>5.1</td>
<td>0.5 - 19.2</td>
</tr>
<tr>
<td>3</td>
<td>9.2</td>
<td>1.6 - 28.4</td>
</tr>
<tr>
<td>4</td>
<td>16.0</td>
<td>3.3 - 52.9</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 16.0</td>
<td>8.0 - Infinite</td>
</tr>
</tbody>
</table>

MPN Index and 95% Confidence Limits for Various Combinations of Positive and Negative Results When Ten-10 ml Portions are Used

<table>
<thead>
<tr>
<th>No. of Tubes Giving Positive Reaction Out of 10 of 10 ml Each</th>
<th>MPN Index/100 ml</th>
<th>95% Confidence Limits (Approximate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt; 1.1</td>
<td>0 - 3.0</td>
</tr>
<tr>
<td>1</td>
<td>1.1</td>
<td>0.03 - 5.9</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>0.26 - 6.1</td>
</tr>
<tr>
<td>3</td>
<td>3.6</td>
<td>0.69 - 16.6</td>
</tr>
<tr>
<td>4</td>
<td>5.1</td>
<td>1.3 - 16.4</td>
</tr>
<tr>
<td>5</td>
<td>6.9</td>
<td>2.1 - 16.8</td>
</tr>
<tr>
<td>6</td>
<td>9.2</td>
<td>3.1 - 21.1</td>
</tr>
<tr>
<td>7</td>
<td>12.0</td>
<td>4.3 - 27.1</td>
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<tr>
<td>8</td>
<td>16.1</td>
<td>5.9 - 36.8</td>
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<td>9</td>
<td>23.0</td>
<td>8.1 - 59.5</td>
</tr>
<tr>
<td>10</td>
<td>&gt; 23.0</td>
<td>13.5 - Infinite</td>
</tr>
</tbody>
</table>
Quality Control Procedures

Routine quality control should be conducted on each lot of Colliert received to ensure integrity and proper product performance.

Recommended Procedure

1. Prepare one set of Quanti-Cult™ cultures (Lot # WKU1 1001) according to the Quanti-Cult directional insert. Each set contains the following organisms which are pre-quantitated and ready to use:
   - *Pseudomonas aeruginosa*
   - *Klebsiella pneumoniae*
   - *Escherichia coli*

2. Reconstitute the contents of each of three Colliert tubes with 10 ml of sterile water (distilled or deionized). Mix thoroughly to aid dissolution.

3. Add the entire contents of each Quanti-Cult vial to separate tubes of reconstituted Colliert.

4. Incubate the inoculated Colliert vessels at 35°C ± 0.5°C for 24 hours. Results should be observed within 24 hours as follows:
   - *Pseudomonas aeruginosa* - no color, no fluorescence
   - *Klebsiella pneumoniae* - yellow, no fluorescence
   - *Escherichia coli* - yellow, fluorescent

Alternatively

1. Reconstitute each of three Colliert tubes with 10 ml of sterile water (distilled or deionized). Mix thoroughly to aid dissolution.

2. Label the tubes: "*Escherichia coli*," "*Klebsiella pneumoniae*," and "*Pseudomonas aeruginosa*" respectively.

3. Touch a sterile inoculating loop or needle to an 18-24 hr. pure culture slant of one of the bacteria listed. (Alternatively, ATCC strains or equivalent may be used as the source of the inoculum.)

4. Transfer the inoculum to the appropriately labeled Colliert tube.

5. Repeat steps 3 and 4 for the two remaining control organisms.

6. Incubate the inoculated Colliert tubes at 35°C ± 0.5°C for 24 hours.

Results should be observed within 24 hours as follows:

- *E. coli* - Yellow and fluorescent (ATCC #2924, 11775 or equivalent)
- *K. pneumoniae* - Yellow, no fluorescence (ATCC #15683 or equivalent)
- *Pseudomonas aeruginosa* - No color, no fluorescence (ATCC #13145, 27853) or equivalent

ATCC (American Type Culture Collection, 1-800-638-6597)

If the results listed above are not obtained, repeat the test on additional aliquots from the same lot. If again the proper results are not obtained, please call IEXX Laboratories, Inc.
Appendix 1.D.

Anguilla Brook Bacteria Trackdown and Watershed-Based Plan
Bacteria Field Sampling Manual
Anguilla Brook Bacteria Trackdown and Watershed-Based Plan

Bacteria Field Sampling Manual

Funded in part through CT DEEP by an US EPA Clean Water Act §319 NPS program grant.
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About This Manual

Thank you for volunteering to help with this project! This effort would not be possible without you.

These pages are meant to help you in collecting your water samples. They give you some background on the project, detailed instructions for collecting water samples, an explanation of the water quality indicators we'll be measuring this summer, and a field sheet. Please try to review this package before your first sampling date.

If you have questions about the sampling schedule or sites, or if conflicts arise and you can't collect on a date you volunteered for, or if you'd like to pass along information, please contact the program staff:

**Judy Rondeau, Assistant Director**
Eastern Connecticut Conservation District
(860) 774-9600 ext. 13
[judy.rondeau@comcast.net](mailto:judy.rondeau@comcast.net)

or

**Jean Pillo, Watershed Conservation Project Manager**
Eastern Connecticut Conservation District
(860)928-4948 ext 605
[Jean.Pillo@comcast.net](mailto:Jean.Pillo@comcast.net)

The Eastern Connecticut Conservation District would like to specially acknowledge The Connecticut River Coastal Conservation District for providing their Quality Assurance Project Plan and Volunteer Handbook as models for this project.
I. Your Work as a Volunteer

Your contribution of time to this project is highly valuable. We want you to know that we deeply appreciate your time, effort and commitment. The success of this project ultimately depends on you. Please help us by doing the following:

- Please try to assist with the collection of water samples on the dates you agreed to. If for some reason you can't, contact the program director as soon as possible. Also, if possible, find a substitute who can take your place.
- Please follow the sampling instructions carefully. They have been designed the way they are to assure that all samples are collected the same way.
- Please be on time on a sampling day. It is very important that the water samples be collected early in the day in order to get them to the transfer location on time. There are other people depending on it!
- Please review the following instructions carefully, before your first sampling. If you have questions, please contact Eastern Connecticut Conservation District Anguilla Brook Bacteria Trackdown Project Manager Judy Rondeau at 860-774-9600 ext. 13.

II. A Brief Look at What Happens on a Sampling Date

You have volunteered to assist with the collection of water samples on one or more of sampling dates. In the morning of each of these dates, you will visit each of the sites and assist with the collection of water samples in the provided containers, and fill out a simple field sheet. All sampling must be completed before noon.

After all samples are collected, the samples must be brought to a designated courier location as identified on the monitoring plan and site route map. The samples will be analyzed at the Connecticut State Laboratory for *E. coli* concentrations (see explanation of the water quality indicators on page 8).

The following pages explain in detail what to do on one of these sample collection dates.

III. Preparation

- Meet ECCD staff at the Norwich office or other predetermined time and location. Sample containers and other supplies will be provided at this time.
- Receive a cooler with frozen ice-packs to keep samples below 10°C.
- Use route/site map to locate sampling locations. A trial route run will be conducted prior to the first sampling date.
Checklist:

___ Sterile single-use 120-mL screw-cap bottles to collect water sample for bacteria analysis. (One for each site, one for each duplicate and blank, plus a few extras in case a bottle is lost or becomes contaminated)
___ Labels, if bottles are not pre-labeled by the lab
___ Sampling pole, with extra rubber bands
___ Cooler with ice-packs (frozen)
___ Nitrile gloves
___ Field sheet
___ Clipboard and Pencil
___ Waterproof marker
___ Thermometer
___ First Aid Kit

Volunteers may also want to bring:
___ Waders or waterproof knee-high boots
___ Walking stick
___ Clippers or pruners

IV. What to Do on a Sample Collection Day

What to Do at Each Sampling Site

Step 1. Carefully make your way to the stream.
Step 2. Record notes about weather conditions on the field sheet.
Step 3. Record observations on the field sheet using the codes at the bottom of the sheet.
Step 4. Record any other observations or comments.
Step 5. Collect stream water temperature following the instructions below.
Step 6. Label the sample bottles with site #, date, and time with a waterproof marker before you collect your sample.
Step 7. Collect water sample following the instructions below.
Step 8. Fill out the field sheet following the instructions below.

Repeat all steps for each site.

- Once you have collected all your samples, fill out the Chain of Custody (COC) form. On the entry for the sample collected at Anguilla Brook 01 (AB-01) ONLY, indicate in the additional info line “Analyze sample for E. coli, Enterococci and fecal coliform.”
- Return the samples, field sheet and COC form to the pre-arranged collection spot.
- The ECCD project manager will collect the samples from volunteers and review the field sheets and COC forms.
The ECCD project manager will deliver the samples to Ledge Light Health District for pick-up and delivery to the Department of Public Health Microbiology Lab in Rocky Hill.

How to Collect Water Samples

In General: Sample away from the riverbank in the main current. In any case, avoid sampling stagnant water! The outside curve of the river is often a good place to sample since the main current tends to hug this bank. In shallow stretches, wade into the center current carefully to collect the sample. **Always approach your sample site from downstream.**

Collecting Water Samples in 120-ml bottles for Bacteria Analysis

- **Wading:** Try to disturb as little bottom sediment as possible. In any case, be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Allow the current to carry disturbed sediments away before collecting the sample. Collect the water sample on your upstream side.
  - Before entering the stream, write the site number (e.g. AB01), date and time on the bottle on the label attached to the bottle, while the bottle is still dry.
  - **Leave the cap on the bottle.**
    - Enter the stream to the area of main flow (known as the thalweg).
    - Facing upstream, submerge the closed bottle under the surface to a depth of 6” to 10” beneath the surface, or mid-way between the surface and bottom, if shallow.
    - Uncap the bottle under water with the mouth pointing upstream/into the current. **Avoid touching the inside of the bottle or the cap.**
    - Recap the bottle underwater.
    - Once above the water, uncap and pour off excess water to the 100 ml line imprinted on the bottle. Then recap the bottle.
    - Place the sample in the cooler under ice packs.

- **Sample Collection Using a Pole:** Use the sampling pole if the stream can’t be accessed due to steep banks, vegetation or other impediments. Twist the segments of the pole to extend the length to reach the stream.
  - Before attaching the bottle to the pole, write the site number (e.g. AB01), date and time on the bottle on the label attached to the bottle, while the bottle is still dry.
  - Remove the cap from the bottle just before sampling. **Avoid touching the inside of the bottle or the cap.**
  - Turn the pole so the bottle is upside down and dip it into the area of main flow to a depth of 6” to 10” beneath the surface, or mid-way between the surface and bottom, if shallow.
  - Turn the bottle upright into the current and allow it to fill.
  - Carefully retract the pole to retrieve the bottle.
  - Pour off excess water to the 100 ml line imprinted on the bottle. Recap the bottle carefully, and remember, don’t touch the inside!
Collecting Duplicate Samples

Duplicate samples are collected at some sampling locations to measure precision of both field and laboratory procedures. Two samples are collected simultaneously at a site by the same person, and sent for analysis at the lab. Results are compared to determine precision.

- At the site assigned for duplicate sampling, you will follow the same sampling procedures as above but collect two side-by-side samples instead of one single sample:
  - For a **wading sample**, remove the caps before you enter the stream, taking care not to touch the inside of the caps or bottles.
  - Holding the bottles side-by-side in one hand, follow the procedure above to collect the water samples.
  - Pour off excess water to the 100 ml line imprinted on the bottle. Recap the bottles carefully, remembering not to touch the inside!
  - Place the samples in the cooler under ice packs.
  - For a **collection pole sample**, affix two bottles to the pole side-by-side using rubber bands.
  - Remove the caps collecting the samples, taking care not to touch the inside of the caps or bottles.
  - Follow the procedure above to collect the water samples.
  - Pour off excess water to the 100 ml line imprinted on the bottles. Recap the bottles carefully, remembering not to touch the inside!
  - Place the samples in the cooler under ice packs.

- To document the duplicate sample on the field form, start a new row on the field sheet.
  - In the first column under site #, record the site number for one bottle as you normally would, for example, site # AB01. Record the rest of the information the same way as for your other water samples.
  - For the duplicate sample, start a new row on the field sheet. In the first column under site #, record the site number followed by a "D," for example, site # AB01D.

Collecting Blank Samples

Blank samples are collected at some sampling locations to measure precision of both field and laboratory procedures. The blank sample consists of a sterile buffer solution provided by the DPH laboratory. At the site assigned for the collection of the blank sample:

- Write the site number, date and time on the collection bottle, adding a “B” after the site number (eg. DB02B).
- Remove the plastic safety wrap and open the bottle of buffer solution.
- Transfer the buffer solution to the bacteria sampling bottle, taking care not to touch the inside of either bottle or cap. Recap the bottle.
- Place the blank sample in the cooler under ice packs.
• Document both the stream sample and the blank sample on the field sheet, as described above, adding “B” after the site number on the blank sample.

How to Measure Water Temperature
You may want to secure the thermometer to your wrist with a string to avoid losing it in case it falls in the water. Measure the temperature from the same area that your water samples were taken.

1. If there are bubbles within the liquid column, hold the top end of the thermometer (opposite the bulb) and shake it vigorously several times to remove any air in the enclosed liquid. When you are sure there are no bubbles in the column, take the temperature reading.
2. Immerse the thermometer 6” to 10” (completely submerging the thermometer) beneath the surface or, if the water is shallow, measure mid-way between the surface and the bottom.
3. After one minute, raise the thermometer only as much as is necessary to read the temperature. Do not hold the thermometer from the base or your hands will warm the bulb and artificially raise the temperature. Read the thermometer at eye level.
4. Quickly document the temperature reading on the field sheet. If the air temperature is significantly different from the water temperature or it is a windy day, the thermometer reading may change rapidly after it is removed from the water; try to take the reading while the bulb of the thermometer is still in the water.
5. Submerge the thermometer in a different but nearby location.
6. Repeat Steps 2 and 3 to get two more readings.
7. Record the average of the three readings on your data sheet.

Safety Tips for Water Sampling
While every effort has been made to assure that water-sampling locations are safely accessible, collecting water samples requires certain precautions and safety measures. Following are tips for people collecting water samples:

If you are in doubt as to your ability to safely collect a sample, don't do it! Be aware of your own physical limitations and the difficulty collecting water at certain locations under certain conditions. If a sample site is too difficult under any conditions, let your project director know.

• High flows can turn even the most placid water into a raging torrent. Don't attempt to collect a sample if you feel the least bit of risk. Avoid dangerous situations.
• Let someone know where you are going and when you expect to return.
• Always collect samples with a partner.
• Some sample sites require wading. In any case, assume that you may get wet and wear appropriate clothing, e.g. footwear that can get wet (hip waders, old sneakers, "river runner" sandals, etc.) and will not slip on wet rocks. Bring a towel and a change of clothes as a precaution.
• Bring a stick or pole along for balance climbing down steep banks or if you're wading.
• Be careful when pulling off to the side of the road and leaving your car, so as not to endanger yourself or create a traffic hazard.
• Wear the provided safety vest so you are visible to traffic.
• Consider leaving your wallet, cell phone and keys in or around your car so they don't wind up downstream, or place them in a waterproof bag.
• Watch out for poison ivy and multiflora rose - they like stream banks!
• If sampling from a bridge, be wary of passing traffic! Don't lean over bridge rails while sampling unless you are firmly anchored to the ground or bridge structure with good hand and foot holds.

V. What Are We Trying to Accomplish?

We're trying to learn several things about the local waterways. Our objectives include:

• To document the impact of nonpoint pollution sources on bacterial water quality indicators in Anguilla Brook and Wequetequock Cove.
• To determine whether Anguilla Brook and its tributary streams meet Connecticut Water Quality Standards for bacteria (E. coli).

VI. How Will We Carry Out the Study?

Rivers, streams and estuaries are complex systems of physical, chemical and biological characteristics. It would be impossible to measure all of these. We can, however, look at certain "water quality indicators" that will answer our questions. Measuring these indicators will give us a number - a level or a concentration in the river - that we can compare to the Connecticut Water Quality Standards, which list the safe or desirable levels of these indicators. We can also look for other physical signs of stream health that give us clues about potential sources of water quality problems.

Water Sampling and Analysis: Volunteers will assist with the collection of water samples at weekly intervals at selected locations along in your project area. One sample will be collected at each site unless otherwise instructed. Each sample will be collected in a sterilized screw-top bottle to be analyzed for fecal indicator bacteria at the Connecticut State Laboratory. Water samples must be delivered to the team leader for delivery to Ledge Light Health District at a pre-arranged time. This time will be determined when field assignments are made. Fecal bacteria samples have a maximum hold time of 6 hours. Any sample delivered to the lab after that 6-hour hold time may be discarded. If processed, the results may not be representative of stream bacterial levels at the time the sample was collected.

VII. Explanation of the Water Quality Indicators

Following is a brief description of the water quality indicator this testing method is designed to measure.

E. coli Bacteria: The presence of certain bacteria, known as fecal indicator bacteria (or FIBs) in water means that there is human or animal fecal material in the water. While not necessarily harmful themselves, their presence means that other disease-causing organisms may be present and that recreational contact with the water may pose a health risk. Bacteria are typically
measured by filtering a water sample and counting how many bacteria grow in a 24-hour period under favorable conditions. The results are reported in terms of the number of bacteria colonies per 100 milliliters of water sample. Numbers over a certain level mean that there may be a health risk from water contact. We will be looking at one type of bacteria – *E. coli* bacteria. *E. coli* is the fecal indicator bacteria used by the State of Connecticut for freshwater, both to evaluate general sanitary conditions and to determine whether established bathing areas are safe for swimming. Sources of fecal bacteria may include wastewater treatment plants, on-site septic systems, combined sewer overflows, domestic and wild animal manure, urban runoff, boat discharges and others.
Appendix 1: State of Connecticut Water Quality Standards

The State of Connecticut Department of Energy and Environmental Protection (DEEP) is required under Section 303(c) of the federal Clean Water Act to develop Water Quality Standards that outline State policies regarding the uses and related classifications of Connecticut’s water resources, addressing both surface and ground waters, and the standards and criteria necessary to support such designated uses. The State of Connecticut water quality standards for bacteria are as follows:

### Table 1. Indicator Bacteria - Freshwater

<table>
<thead>
<tr>
<th>Designated Use</th>
<th>Indicator</th>
<th>Criteria by classification</th>
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<tbody>
<tr>
<td>Drinking Water Supply (1)</td>
<td>Total coliform</td>
<td>Monthly Moving Average less than 100/100ml</td>
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<td>Single Sample Maximum 500/100ml</td>
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<td>Recreation (2)(3) – Designated Swimming (4)</td>
<td>Escherichia coli</td>
<td>Geometric Mean less than 126/100ml</td>
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<td></td>
<td></td>
<td>Single Sample Maximum 235/100ml</td>
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<td>Recreation (2)(3) – Non-designated Swimming (5)</td>
<td>Escherichia coli</td>
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<td></td>
<td>Single Sample Maximum 410/100ml</td>
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<td></td>
<td>Single Sample Maximum 576/100ml</td>
</tr>
</tbody>
</table>

### Table 2. Indicator Bacteria – Saltwater

<table>
<thead>
<tr>
<th>Designated Use</th>
<th>Indicator</th>
<th>Criteria by classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell fishing (6) – Direct Consumption</td>
<td>Fecal coliform</td>
<td>Geometric mean less than 14/100 ml 90% of sample less than 31/100 ml</td>
</tr>
<tr>
<td>Shell fishing (6) – Indirect consumption</td>
<td>Fecal coliform</td>
<td>Geometric mean less than 88/100 ml 90% of sample less than 260/100 ml</td>
</tr>
<tr>
<td>Recreation - Designated swimming (4)</td>
<td>Enterococci</td>
<td>Geometric mean less than 35/100 ml Single sample maximum 104/100ml</td>
</tr>
<tr>
<td>Recreation - All Other Uses</td>
<td>Enterococci</td>
<td>Geometric mean less than 35/100 ml Single sample maximum 500/100ml</td>
</tr>
</tbody>
</table>
Notes for Tables 2A and 2B:

(1) Criteria applies only at the drinking water supply intake structure.

(2) Criteria for the protection of recreational uses in Class B waters do not apply when disinfection of sewage treatment plant effluents is not required consistent with section 22a-426-4(a)(9)(E) of the Regulations of Connecticut State Agencies.

(3) See section 22a-426-9(a)(2) of the Regulations of Connecticut State Agencies.


(5) Includes areas otherwise suitable for swimming but which have not been designated by state or local authorities as bathing areas, waters which support tubing, water skiing, or other recreational activities where full body contact is likely.

(6) Criteria are based on utilizing the mTec method as specified in the U.S. Food and Drug Administration National Shellfish Sanitation Program-Model Ordinance (NSSP-MO) document Guide for the Control of Molluscan Shellfish 2007.
Appendix 2

Sample Data Forms

Appendix 2.A. Environmental Microbiology Fresh Surface Water Examination

Appendix 2.B. ECCD Field Sheet for Water Samples
Appendix 2.A.

### FRESH SURFACE WATER SUBMISSION FORM

**Environmental Microbiology**  
Connecticut Department of Public Health  
Katherine A. Kelley State Public Health Laboratory  
195 West St. Rocky Hill, CT 06067  
PH (860) 920-6699 FAX (860) 920-6703

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>REGULAR</th>
<th>RESAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Type</td>
<td>(Circle One)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PROFILE NO.</th>
<th>NAME AND ADDRESS</th>
<th>COLLECTED BY:</th>
<th>TOWN:</th>
<th>DATE COLLECTED:</th>
<th>CONTACT INFORMATION:</th>
<th>PHONE #: ( )</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>FRESH SURFACE WATER</th>
<th>Date and Time Received</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test A-Code: EC-SW</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>For Lab Use Only:</th>
<th>Time Collected:</th>
<th>Collector’s No.:</th>
<th>Location:</th>
<th>Additional Info:</th>
<th>LW _____ SW _____</th>
<th>E. coli Count/100ml:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accession #</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>POSITIVE WELLS.</td>
<td>E. coli Count/100ml:</td>
</tr>
<tr>
<td>Test:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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<td></td>
<td></td>
<td></td>
<td>POSITIVE WELLS.</td>
<td>E. coli Count/100ml:</td>
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<tr>
<td>Test:</td>
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<td></td>
<td></td>
<td></td>
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<td>E. coli Count/100ml:</td>
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<td>Test:</td>
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<th>Location:</th>
<th>Additional Info:</th>
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</thead>
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<td>Accession #</td>
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<td></td>
<td></td>
<td></td>
<td>POSITIVE WELLS.</td>
<td>E. coli Count/100ml:</td>
</tr>
<tr>
<td>Test:</td>
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</tr>
</tbody>
</table>

For Lab Use Only: DATE AND TIME ANALYZED  
ANALYZED BY: METHOD (Circle test performed): COLILERT-30 COLILERT-24 COLISURE

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**Eastern Connecticut Conservation District**

**Field Sheet for Water Samples**

Note: This sheet must be included with all water samples

Samplers: ___________________________________________

Previous 3 Days Weather: ______________________________

Current Weather: _________________________________

Note: Label all bottles with site #, date and time

<table>
<thead>
<tr>
<th>Site #</th>
<th>PP Bottle (125 mL) (number of bottles/site)</th>
<th>Time Sample Taken</th>
<th>Air Temperature</th>
<th>Water Temperature</th>
<th>Observation Codes (Use codes at bottom of page)</th>
<th>Other observations/Comments</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

Indicate Duplicates (D) and Blanks (B) after each site number

Team Leader Signed: __________________________________ Date: __________________ Health Dept. Signed: ___________________________ Date: __________________

Team Leader Phone #: __________________________________________________________

Observational Codes:

**Water Level**

A. Very low
B. Low
C. Average
D. High
E. Very high

**Water Odor**

F. Rotten Egg
G. Chlorine
H. Musky
I. Gas or Oil
J. None
K. Other: ______________

**Observed Use**

L. Swimming
M. Fishing
N. Boating
O. Other: ______________

---

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