Catoctin Watershed Project

Stewardship for the Catoctin Creek Watershed

Bacteriological Monitoring Program Sample Collection and Analytical Protocols

1. **Sample Packet Preparation** (Estimated time: 1 hour; Responsibility: Collection Teams)
   a. **Preparation Location** – Materials for bacteriological sample collection are stored in the storage area in the laboratory at the Leesburg Water Pollution Control Facility (LWPCF) at 25 West Market Street (Rt. 7), Leesburg. Mr. Charlie Elgin is the Laboratory Supervisor, 703-771-2786, celgin@leesburgva.gov. The sample packets for the next collection cycle are to be prepared at the laboratory when completed samples for the current cycle are being dropped off.
   b. **Sample Number** – The sample number for each station is preset in the Sample Log, according to the collection date.
   c. **Sample Collection Form –** A "Bacteriological Monitoring – Sample Collection Reporting Form" is to be filled out for each monitoring station and included in the packet:
      i. Go to the "Sample Log" to determine the next series of samples. Write the station location and sample number on the sample collection form.
   d. **Sample Collection Bottle** – An empty water sample bottle is to be used, one for each station. Write the sample number on the empty sample bottle with a permanent marker.
   e. **Sample Collection Packet –** Prepare a sample collection packet for each monitoring station. Write the station location on a plastic z-loc bag with permanent marker. Fill the plastic bag with the Sample Data Collection Form, the empty sample bottle, and one eyedropper.

2. **Field Sample Collection** (Estimated time: 1 hour; Responsibility: Collection Teams)
   a. **Sample Materials Needed**
      i. Receive the sample packets as arranged.
      ii. Ballpoint pen
      iii. Water shoes/waders if collecting the sample in the stream.
      iv. Clean plastic bucket with rope or other sampling device if sampling from bridge or shore
      v. Thermometer
      vi. Small ice cooler with wet ice
   b. **Sample Collection Time** – A routine sampling schedule is arranged to help ensure samples will be collected under a variety of environmental conditions. Sampling during the morning of the sampling day is preferred. However, the water sample maybe collected in the evening prior to the sampling day, if necessary.
   c. **Sample Collection --** Visit the monitoring site to determine where you can park in safe location, and whether the sample will be taken by wading in the water or from a bridge.
      i. Collect one sample using the empty water sample bottle. This should be marked with the sample number. Return the sample bottle to the plastic bag.
      ii. Fill out the Sample Collection Reporting Form, and return the form to the plastic bag.
      iii. Put the plastic bag containing the samples and form in the cooler on wet ice.
   d. **Sample Delivery –** Deliver the iced sample to the laboratory at the Leesburg Water Pollution Control Facility (LWPCF) at 25 West Market Street (Rt. 7), Leesburg. (Or deliver the sample to another person per any arrangement.)

i. Take the Sample Collection Reporting Form out of the plastic bag and fill in the blanks pertaining to Sample Handling and Storage. Return the form to the plastic bag.

ii. Place the plastic bags with the samples into a designated container in the refrigerator

e. **New Sample Packet** – Prepare and take the sample packets for the next collection cycle. (Or, sample packets can be picked up by another team member, as arranged)

3. **Laboratory Preparation of Sample** (Estimated time: 1 hour; Responsibility: Lab Team)

a. **Thaw media bottles** – Remove the correct number of media bottles from freezer (2 x number of water samples). This can be done the day before, and placed in the refrigerator. If done the day of sample collection, media bottles can be placed in tray of warm water for approximately 20 minute until thawed.

b. **Review Field Sample Collection** – Review the Sample Collection Reporting Form for each sample to make sure it is complete and the sample was handled properly.

c. **Label Petri Dish** – Mark a Petri dish with a permanent marker for each subsample with the sample number, sample station, and date. Write in small letters along the side of the lower dish so as not to hinder the counting of cells.

d. **Add Sample to Media** – Add the proper volume of sample from the sample bottle to a media bottle (typically twice with 2ml in pipette each time for 4 ml total). Tighten the cap on the media bottle. Gently swirl the media sample bottle to mix contents <u>without</u> forming air bubbles.

e. **Prepare Petri Dish** --

i. Remove the top of the Petri dish and replace it as quickly as possible to avoid airborne bacteria from getting on the media. Slowly pour the sample/media mix into the Petri dish to avoid getting bubbles.

ii. Try not to pick-up the filled Petri dish, to avoid getting the media on the sides of the dish. Instead, gently slide the dish along the smooth top of the lab bench to a spot where the media in the dish can solidify.

f. **Incubation** – Samples are to be placed in an air incubator set at 35^{o}C.

i. Allow 60-90 minutes for the media to set/solidify. Gently jiggle the dish after 60 minutes to determine the degree of solidification.

ii. Check the air temperature in the incubator and determine that it is approximately 35^{o}C. (The temperature may be slightly less if the door had been recently opened.)

iii. If arrangements are made with the laboratory personnel to put the Petri dishes in the incubator when they solidify, the lab preparation volunteer can leave. Tell the lab personnel when to put the dishes in the incubator, and fill in the time in the front page of the red notebook.

iv. After the media has solidified, **turn the dish upside down** (to avoid condensation dripping on the media and interfering with bacteria growth) and place it in the incubator. The dishes can be stacked one on top of another.

g. **Complete Form** – Fill in the required information under "Sample Incubation and Handling" on the Sample Collection Data Form. Put the form in the "Samples Pending" file folder.

4. Reading the Sample and Data Recording (Estimated time: 1 hour; Responsibility: Lab Team)

a. **Confirmation Analyses** – Preparing two Petri dishes for the confirmation analyses takes an hour for the media to solidify, so this should be done first. Look in the "Sample Log" and determine whether any sample being run has been randomly chosen and highlighted,

and needs to be confirmed. If so, follow the procedure under "Confirmation Analysis" below.

b. **Incubation Time** –Take the Petri dishes out of the incubator after **24 hours**, and arrange the dishes with the appropriate Sample Collection Reporting Form on the lab bench.

c. **Data Sheets** -- Fill in the information about sample incubation on the Sample Collection Data Form. Fill in the pertinent information about the sample on the Lab Data Reporting Form (on reverse side of Sample Collection Reporting Form). Some of this information is duplicative, but it is intended to help ensure that the Petri dish and the data sheets are for the same sample.

d. **Counting Colonies** – Do one sample at a time –

   i. Count the *E. coli* colonies separately for each subsample. These colonies are dark blue and purple. Do not count pink, light blue, and teal green colonies. If there are many colonies on the plate, their size may be reduced because of more limited available food. A permanent marker can be used to circle the *E. coli* colonies to help with counting. Enter results on the lab data form.

   ii. Use the proper multiplier to convert the counts to per 100 ml., and enter the average *E. coli* results on the data sheet. Put the form in the "Completed Samples" file folder.

   iii. Enter the appropriate data in the "Sample Log" for the sample.

e. **Confirmation** -- Confirmation analyses will be done on a random basis as part of the QA/QC Plan. Do a confirmation analysis if this sample has been selected.

f. **Disposal of Petri Dishes** – For the first few sample collection cycles, the used petri dishes will be stored in the freezer for later review should questions arise. At a later date to be determined, the petri dishes will be disposed of as follows:

   i. Once all analyses have been completed and the *E. coli* colonies counted, pour a small amount of alcohol or bleach onto the Petri dish, and let sit for 5 minutes to kill any bacteria (there is a remote chance that there could be a pathogenic bacteria strain growing on the plate). After 5 minutes, mark the dish with a large "X" with a permanent marker, and place in the waste receptacle.

5. **Confirmation Analyses** (Estimated time: 20 minutes; Responsibility: Lab Team)

a. When a sample number in the "Sample Log" has been randomly chosen and highlighted, a confirmation analysis needs to be performed as part of the QA/QC Plan. The purpose of confirmation is to verify that colonies counted as *E. coli* will confirm as *E. coli*, and that colonies not counted as *E. coli* will not confirm as *E. coli*. There are two analyses to perform. These analyses are done after all the regular samples have been read and their data have been entered into the proper forms and logs.

b. **Prepare Petri Dishes** – Pour a dish of the #1 medium into one Petri dish and a dish of #2 medium in a second Petri dish. Slide them to an area on the bench where they can solidify for an hour.

c. **Prepare Sample** – Retrieve the confirmation dishes and the appropriate sample dishes (both subs).

   i. Turn each of the confirmation Petri dishes over and mark the bottom with two pie shaped sections with a permanent marker. Label one area with a small "+" for *E. coli* confirmed and the other with a "-" for non-*E. coli*. Write the sample number on the edge of each Petri dish where the pie sections are located. (These dishes can be reused so only use a portion of the dish.)

   ii. Select **two** colonies from each of the two subsample dishes (**four total**) that have been marked and counted as *E. coli* colonies. Use a clean toothpick (don't touch

the working end), and "pick" a small piece of the colony. Transfer this to the "+" pie segment on each of the two confirmation dishes.

iii. Select **two** of the teal green or light blue colonies from each of the two subsample dishes (**four total**) that have not been counted as **E. coli**. Use a clean toothpick (don't touch the working end), and "pick" a small piece of the colony. Transfer this to the "-" pie segment on each of the two confirmation dishes.

iv. Confirm that the incubator temperature is approximately 35°C. Turn these two confirmation dishes upside down and place in the incubator.

v. Place the two subsample dishes in a plastic Ziploc bag that is marked with the sample number and date, and "Confirmation Sample." Place the "Sample Collection/ Lab Data Reporting Form" for this sample in the bag, as well. Place the bag in the refrigerator in the sample holding container. This will be reviewed again the next day if any of the confirmation analyses do not confirm as positive or negative as expected.

d. **Reading Confirmation Dishes** – *E. coli* and non-*E. coli* colonies will react differently on the two confirmation Petri dishes after 24 hours of incubation at 35°C.

i. Remove the confirmation Petri dishes from the incubator after 24 hours.

ii. *E. coli* positive colonies will be colored pink on dish #1 and teal green on dish #2.

iii. Non-*E. coli* colonies will appear clear on each dish. Colonies that appear clear on dish #1 and teal/green on dish #2 are non-*E. coli* as well.

iv. Record the results on the Sample Collection/Lab Data Reporting Form for the sample, and in the "Sample Log." If the colonies are not positive and negative as expected, look at the original dishes and make a note on the Lab Data Reporting Form about the color and size of the colonies that might explain why the colonies where not correctly counted.

v. Put the Sample Collection/ Lab Data Reporting Form in the "Completed Samples" file.

vi. Sanitize the two dishes with the original sample for 5 minutes, and dispose in the appropriate waste container.

vii. Put the two confirmation dishes back into the "Confirmation Sample" plastic bag, and return it to the refrigerator for future use.

Catoctin Watershed Project
Bacteriological Monitoring Timeline

Day	Time	Activity	Responsiblity
Tuesday	evening	Field sample collection, if necessary[1]	Collection Team
Wednesday	8 – 11 am	Field sample collection, delivery to lab (LWTP)[2]	Collection Team
	11 am - noon	Lab preparation of samples	Lab Team
	1 pm	Place Petri dishes in incubator	Lab Team (or LWPCF employee, by arrangement)
Thursday	1 pm	Lab preparation of confirmation sample, if required	Lab Team
	1 -2 pm	Reading the sample and data recording	Lab Team
	2 pm	Place confirmation Petri dish in incubator	Lab Team
Friday	2 pm	Read confirmation dishes	Lab Team

[1] If sample cannot be collected Wednesday morning, it can be collected Tuesday evening and delivered to the lab on Wednesday morning before 11:00 am. Sample must be kept in the refrigerator during this period.

[2] For convenience, sample packets can be prepared and taken for the next collection cycle after delivery of samples for the current cycle on Wednesday, or after counting the colonies on Thursday. However, since the LWTP is open 24/7, sample packets can be prepared any time according to each team's preference.

Catoctin Watershed Project
Stewardship for the Catoctin Creek Watershed

Catoctin Watershed Project
Bacteriological Monitoring Sample Log

Sample No	Station No	Sample Station	Date	Sampling Procedure Followed (Yes/No)	Sample Results CU/100 ml					Lab Tech (Pre/Read)
					Sub A	Sub B	Average	Confirmation	Comparison	
0001	5	CAXO04.57								/
0002	6	CAXO06.5								/
0003	2	MIHO02.5								/
0004	1	MIHO04.5								/
0005	3	XCATO02.1								/
0006	4	NOCO00.42								/
0007	9	NOCO04.38								/
0008	8	NOCO09.37								/
0009	7	SOCO01.66								/
0010	10	SOCO07.06								/
0011	12	SOCO11.30								/
0012	11	SOCO14.10								/