**OWML: NAWQA (Cycle 3)**

**PROTOCOL FOR SAMPLING FOR INDICATOR BACTERIA, COLIPHAGE, AND AEROBIC ENDOSPORES, FY2017**(Updated 4/5/2017)

**Overview**

Samples are collected by study-unit personnel and sent to the Ohio Water Microbiology Laboratory (OWML) for analysis of bacterial indicators (total coliforms, *E. coli,* and enterococci), somatic and F-specific coliphage, and aerobic endospores on a subset of samples.  Samples must be stored in the dark on ice or refrigerated at all times.

* For each well, fill (to the shoulder) one 1-L bottle and one 3-L bottle.  Sample bottles will be cleaned and sterilized by the OWML and shipped to WSCs.
* Holding times are 30 hours for bacterial indicators and aerobic endospores and 48 hours for coliphage.  Because of the shorter holding time for bacterial indicators, ship samples to the OWML for overnight delivery on the day of sample collection.

The sample in the 1-L bottle is analyzed for total coliforms and *E. coli* by the OWML using the Colilert Quanti-Tray method (Idexx, Westbrook, Maine), for enterococci using the mEI agar method, and for aerobic endospores using the nutrient agar plus trypan blue method.  The sample in the 3-L bottle is analyzed for coliphage by the OWML using the two-step, enrichment method.  This method determines the presence or absence of somatic and F-specific coliphage.

Refer to the [*USGS National Field Manual, Biological Indicators*](http://water.usgs.gov/owq/FieldManual/Chapter7/index.html) [https://water.usgs.gov/owq/FieldManual/Chapter7/index.html] for detailed instructions on sample collection, parts of which are included below.

**Preparations for the field**

Equipment and supplies.  Refer to the list of [*equipment and supplies*](http://130.11.184.63/internal/Equipment%20and%20supplies_2014.xlsx) [Equipment and supplies\_2017.xlsx].  Every site will need a sterile 3-L bottle, a sterile 1-L bottle, sterile deionized water, and alcohol reagent in a spray bottle (for sterilizing spigots). Every network will need at least one bottle of sterile buffered water for the field blank. The OWML will clean and sterilize all sample bottles and prepare sterile deionized water and sterile buffered water for you. Contact the OWML (GS-W-OHCLB\_OWML@usgs.gov) to schedule this.

Clean, sterile sample bottles from the OWML will have autoclave tape (with dark diagonal lines) on them and will be bagged separately. Leave bottles in the bags until sample collection at the site.

Sterile DI water will come in 500-mL bottles and will have autoclave tape, a label with the lot number and expiration date, and parafilm around the lid. Once opened, the bottle of sterile DI water must be stored in the refrigerator and used within 24 hours. *Note: Please return empty bottles to the OWML at the end of sampling.*

Sterile buffered water for the field blank will come in a 3-L bottle. It will have autoclave tape, a label with lot number and expiration date, and parafilm around the lid. The OWML will send a separate blank chain-of-custody form with each blank.

Forms and scheduling. Obtain the OWML [*Analytical Service Request Form*](http://oh.water.usgs.gov/micro/appendices/Appendix%20B3_service%20request%20form.docx) [Appendix B3\_analytical services request form.docx] and check the following analyses:

* Coliphage: enrichment, presence/absence, Method 1601
* *E. coli* and total coliforms: Colilert QuantiTray 2000
* Enterococci by mEI
* Aerobic endospores (NA + TBl) – only on a subset of samples determined by PAA

Be sure to contact the OWML initially with an estimated schedule and then provide the laboratory at least 2-days advance notice when you are sending samples (a one week notice is even better).

GS-W-OHCLB\_OWML@usgs.gov

**Sample collection**

Well purging. Most of the wells are existing systems with dedicated pumps.  Purge the well as required for NAWQA sampling.  If wells do not have a dedicated pump, follow the instructions for disinfection of equipment in the [USGS National Field Manual, Chapter 7, Section 7.1.2B](http://water.usgs.gov/owq/FieldManual/Chapter7/7.1.html) [https://water.usgs.gov/owq/FieldManual/Chapter7/index.html].

Sample collection. Collect the bacteria and coliphage samples last. Spray the spigot inside and out with ethanol.  Allow the spigot to dry and rinse with STERILE deionized water.  Let the tap run for about 10 seconds.  For indicator bacteria and aerobic endospores, fill one 1-L sterile sample bottle with water directly from the tap leaving at least 2 inches of headspace in the bottle.   For coliphage, fill a 3-L sterile sample bottle with water directly from the tap leaving at least 2 inches of headspace. Do not place the bottle lid down while filling the bottle; instead, hold the lid or have a second person, with clean gloves, hold the lid.

If you are attaching tubing to the spigot to collect the sample, make sure that the tubing and connector are sterile.

After collection, keep samples in the dark and immediately chill the samples in a cooler with ice or in a refrigerator at 1 to 4ºC.

**Sample shipping**

Samples should be shipped on ice on the day of sample collection, using “Priority Overnight shipping”. Ship samples preferably on Monday, Tuesday, or Wednesday. Samples shipped on Thursday and arriving at the OWML on Friday will receive a 20% surcharge on analysis costs due to the need for weekend work on the analyses. Do not ship samples on Friday.

Each sample bottle should be double bagged in heavy-duty zip-lock plastic bags. Ice in coolers also should be double bagged in heavy-duty zip-lock plastic bags. Place the bags of ice on the bottom of the cooler, then put the sample bags on top of the ice, not in the ice. Add packing material to the sides and top of the cooler. Under no circumstances should sample bottles be in direct contact with shipping ice, because shipping ice, when melted, can be a source of microbiological contamination.

**Reporting results**

Results from bacteria and coliphage samples analyzed at the OWML are available within 2–4 weeks of sample arrival.  All data supported by NWIS will be sent to the Water Quality Data Transfer System (QWDX) and available for download at [https://qwdx.cr.usgs.gov](https://qwdx.cr.usgs.gov/).  An email will be sent to all clients when the data is available for download. If a sample is positive for a microorganism, the WSC contact and the PAA will be notified by the OWML immediately.

Parameter and method codes for the Quanti-Tray tests for bacterial indicators are reported in most probable number per 100 mL (MPN/100 mL):

* 50569, BAC47, Total coliforms, defined substrate technology (24-28 hrs)
* 50468, BAC14, *E. coli*, defined substrate technology (24-28 hrs)

Parameter and method codes for the membrane-filtration tests for bacterial indicators and aerobic endospores are reported in colony-forming units per 100 mL (CFU/100 mL):

* 90909, BAC58, Enterococci, mEI membrane-filtration method
* 31732, BAC76, Aerobic endospores, nutrient agar plus trypan blue membrane filtration method

Parameter and method codes for coliphage tests are reported as presence or absence per L:

* 99332, VIR14, Coliphage, somatic, *E. coli* CN-13 host, 2-step enrichment
* 99335, VIR08, Coliphage, F-specific, *E. coli* F-amp host, 2-step enrichment

For coliphage results, presence is indicated by an “M” in the remark field, and an absence is indicated by a “U” in the remark field (the value field is left blank).

**Quality control**

The primary quality-control samples are field blanks. Field blanks should be collected at the rate of either (a) 1 per 10 samples or (b) 1 per network and WSC, whichever is more frequent. If contamination is found in the initial field blank, a trip blank and equipment blank (described below) may be required. You will be notified if these need to be collected. All blanks are analyzed for coliphage, total coliforms, *E. coli*, enterococci, and aerobic endospores at the OWML. **The initial field blank should be collected during the first week of sampling**. Matrix spike samples also will be collected for coliphage analyses.

*Note: During Cycle 2, bacteria controls were required because samples were being analyzed for bacteria in the field. These are no longer required because the samples and controls are being analyzed at the OWML.*

Results from quality-control samples processed at the OWML are available within 2–4 weeks of sample arrival, sent to the Water Quality Data Transfer System (QWDX) and available for download at [https://qwdx.cr.usgs.gov](https://qwdx.cr.usgs.gov/). If a blank sample has a positive detection of any constituent, the WSC and PAA will be notified immediately and the OWML will immediately send out blank water for another blank sample to be collected. If you don’t hear back from the OWML about your blank sample, it had no detections.

Quality-control laboratory samples are described below:

* Field blanks —A field blank is collected by pouring about ½ L of sterile buffered water into a clean and sterile 1-L sample bottle and about 2 ½ L of sterile buffered water into a clean and sterile 3-L sample bottle at a well sampling location under actual field conditions. If you are using tubing to collect the sample, the sterile buffered water must be poured through the tubing and collected into the sample bottles. Sterile buffered water should be obtained from the OWML by contacting:

GS-W-OHCLB\_OWML@usgs.gov

Field blank data are stored in NWIS with the following codes:

* + Sample-medium code is “OAQ” for artificial quality-control sample
	+ Sample-type code is “2” for blank
	+ Parameter code 99100 (blank-solution type), is “70” for sterile buffered water PO4/MgCl2
	+ Parameter code 99101 (source of blank water) is “200” for other
	+ Parameter code 99102 (blank-sample type) is “100” for field blank

For the regular sample associated with the field blank, use the following code:

* + Parameter code 99111 (QC sample associated with this environmental sample) is “10” for blank or “100” for more than one type of QA sample.
* Trip blanks —A trip blank is a 3-L bottle of sterile buffered water from the OWML that has been brought into the field but not opened. The PAA will let you know if a trip blank is needed. The trip blank should be obtained from the OWML by contacting:

GS-W-OHCLB\_OWML@usgs.gov

Trip blank data are stored in NWIS with the following codes:

* + Sample-medium code is “OAQ” for artificial quality-control sample
	+ Sample-type code is “2” for blank
	+ Parameter code 99100 (blank-solution type), is “70” for sterile buffered water PO4/MgCl2
	+ Parameter code 99101 (source of blank water) is “200” for other
	+ Parameter code 99102 (blank-sample type) is “30” for other

For the regular sample associated with the trip blank, use the following code:

* + Parameter code 99111 (QC sample associated with this environmental sample) is “10” for blank or “100” for more than one type of QA sample.
* Equipment blanks — An equipment blank is collected by passing about ½ L of sterile buffered water through sterile equipment, such as a sampling pump or tubing, into a clean, sterile 1-L sample bottle, and about 2 ½ L of sterile buffered water into a clean, sterile 3-L sample bottle. This will be done in a controlled environment, such as the WSC office. The PAA will let you know if an equipment blank is needed. Sterile buffered water should be obtained from the OWML by contacting:

GS-W-OHCLB\_OWML@usgs.gov

Equipment blank data are stored in NWIS with the following codes:

* + Sample-medium code is “OAQ” for artificial quality-control sample
	+ Sample-type code is “2” for blank
	+ Parameter code 99100 (blank-solution type), is “70” for sterile buffered water PO4/MgCl2
	+ Parameter code 99101 (source of blank water) is “200” for other
	+ Parameter code 99102 (blank-sample type) is “80” for equipment blank

For the regular sample associated with the field blank, use the following code:

* + Parameter code 99111 (QC sample associated with this environmental sample) is “10” for blank or “100” for more than one type of QA sample.

For coliphage analysis, the following QC is also required:

* Laboratory matrix spikes—Matrix spikes for coliphage are required when a new water source is sampled.  Collect one matrix spike near the beginning of the sampling period on either a Monday or Tuesday.

To collect a matrix spike along with your regular sample, **collect a total of 10 L of ground water** from the same well. This includes your regular 3-L and 1-L samples, a 3-L bottle for the matrix spike for F-specific coliphage, and a 3-L bottle for the matrix spike for somatic coliphage. Send the four bottles to the OWML for laboratory spiking and analysis.

Laboratory matrix spikes are used to assess the effect of matrix interferences (from a GW type) on coliphage analysis.  Known concentrations of each type of coliphage are spiked into 3 L of GW and then split into three replicate bottles. Each bottle is tested for the presence or absence of coliphage.  For the test to be acceptable, at least one out of three bottles must be positive for coliphage.

Coliphage matrix spike data are stored in NWIS with the following codes:

* + Sample-medium code is “WGQ” for GW QC sample
	+ Sample-type code is “1” for spike
	+ Parameter code 99106 (type of spike), is “20” for laboratory
	+ Parameter code 99107 (spike source) is “25” for OWML

For the regular sample associated with the matrix spike, use the following code:

* + Parameter code 99111 (QC sample associated with this environmental sample) is “40” for spike or “100” for more than one type of qa sample.

**WARNING: Be sure that you offset the sample time for the coliphage matrix spike to avoid confounding data for any other qc spike, replicate, or blank samples associated with chemical constituents.**

**Autoclave Operation and QC**

Following the 2017 updated microbiological sampling and QC procedures, WSCs should no longer need to autoclave sample bottles or DI water. However, any equipment other than sampling pumps that comes into contact with the microbiological sample, such as sample tubing, should be autoclaved. Be sure that the material is autoclavable. The instructions below describe required/best practices for autoclave operation and use.

The autoclaves are operated at 15 lb/in2 steam pressure, producing an inside temperature of 121 to 124oC. Autoclave time depends on the type and amount of equipment or liquid. For dry glassware, tubing, and plastic, the autoclave time is 15 minutes.

Be sure to start the timer after the autoclave has reached temperature and pressure. Do not overload the autoclave. Heat-sterilizing tape should be used with each run to identify supplies that have been autoclaved. Heat-sterilizing tape only indicates that the autoclave reached the correct temperature, but it doesn’t indicate the length of time at that temperature. To confirm effective sterilization, biological indicator (or spore indicator) tests should be done at the beginning of sampling and then at least quarterly. More information about biological indicator tests, as well as ordering information can be found at Fisher Scientific (800-766-7000; catalog number: 14-490-25).