



Indicator Bacteria in Fresh Water

Table 1: Quality Control: Indicator Bacteria in Fresh Water

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective	Data Quality Indicator or Reasoning
Sterility Checks²	Per new lot of dehydrated culture media as instructed in SM 9020B.4.i.5 ¹ and SM 9222D.1.a	No growth	Evaluation of bias from contamination
	For non-sterile filters and pads per lot as instructed in SM 9020B.4.h.1.1	No growth	Evaluation of bias from contamination
	<p style="text-align: center;"><u>Membrane Filter</u></p> Media, filters, buffered dilution water, rinse water, and all equipment per series of samples as instructed in SM 9020B.8.a.5 ¹	No growth	Evaluation of bias from contamination
	<p style="text-align: center;"><u>Multiple Tube Fermentation</u></p> Media, dilution water, and glassware as instructed in SM 9020B.8.a.5 ¹	No growth	Evaluation of bias from contamination

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective	Data Quality Indicator or Reasoning
Laboratory Positive Control	<p>Per new lot of dehydrated culture media for the following methods: Colilert, Colilert-18, Colisure, Enterolert, or other chromogenic/fluorogenic methods.</p>	Positive response	Confirmation of adequate target organism recovery/detection
Laboratory Negative Control	<p>Per new lot of commercially prepared culture media ampules for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g., SM 9222, m-ColiBlue24, EPA 1603)</p> <p>Per batch for laboratory-prepared culture media for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g., SM 9222)</p>	Negative response	Evaluation of bias from contamination and off target reactions
Laboratory Duplicate	Per 10 samples or per analytical batch, whichever is more frequent	<p>For MTF and tray-based methods (i.e. 9221 and 9223B), duplicate sample MPN must be within 95% Confidence Interval.</p> <p>For plate-based methods (i.e. SM 9222)³:</p> $R_{log} \leq 3.27\bar{R}$ <p>(Computation of R_{log} from duplicate laboratory sample analyses)</p>	Evaluation of analytical precision and variability
Laboratory Blank⁴	Per 10 samples or per analytical batch, whichever is more frequent	No growth	Evaluation of bias from lab processing contamination

Field Quality Control ⁵	Frequency of Analysis	Measurement Quality Objective	Data Quality Indicator or Reasoning
Field Blank, Equipment Blank	Per method or SOP	Negative response	Evaluation of bias from contamination at sampling site or from sampling equipment

¹ Citations from *Standard Methods for the Examination of Water and Wastewater*, 23rd edition (3,4)

² Sterility Checks

The specific type and number of sterility checks are method dependent. For example, membrane filter tests require the testing of filters for sterility, while multiple-tube or pour plate procedures do not.

³ Method for Determining Precision for plate-based methods (i.e. SM 9222):

To determine precision for bacterial analysis, the following procedure (adapted from Standard Methods 9020 Section 8.b) will be used. Note: When determining the precision of bacterial analyses, it is important to distinguish between different matrices (drinking water, wastewater, ambient water). Duplicate results from different matrices must be kept separate when calculating precision.

To calculate the laboratory precision for bacterial analyses, the results from the preceding 15 positive samples of a specific type (matrix) are used to calculate a running mean. The results used to calculate the running mean must all correspond to the same quality control parameter, in this instance laboratory duplicates (as opposed to field duplicates). The results of different quality control parameters such as laboratory and field duplicates must not both be used to calculate a single running mean. Note: Field duplicates are not a current SWAMP requirement (see footnote 5).

Step 1: Record the results from duplicate analyses (these results are here designated as D₁ and D₂).

Step 2: Calculate the logarithm (here designated as L₁ and L₂) of each duplicate result. Note: If either of the values D₁ or D₂ are less than 1, add 1 to both values before calculating the logarithms.

$$L_1 = \text{Log}D_1$$

$$L_2 = \text{Log}D_2$$

Step 3: Calculate the range of logarithms (R_{log}) for each pair of duplicates (i.e L₁ & L₂).

$$R_{log} = |L_1 - L_2|$$

R_{log} is equal to the absolute value of the difference between the two numbers.

Step 4: Calculate the mean of R_{log} (\bar{R}) or the duplicates analyzed

$$\bar{R} = \sum \frac{R_{log}}{n}$$

The mean of the range of logarithms is equal to the sum of the ranges of logarithms divided by the number of pairs of duplicates.

$\sum R_{log}$ = the sum of the ranges of logarithms calculated for each pair of duplicates

n = the number of pairs of duplicates (in this case, n = 15)

Step 5: Assess the precision of the duplicate analyses. For the laboratory to demonstrate an acceptable level of precision, the range of logarithms for a particular duplicate must be less than the mean of the range of logarithms multiplied by 3.27.

$$R_{log} \leq 3.27\bar{R}$$

The range of logarithms for the current set of duplicates is less than or equal to 3.27 multiplied by the mean of the preceding ranges of logarithms.

⁴ Laboratory Blanks

Analysis and reporting of laboratory blanks are required only when samples are diluted prior to analysis. If samples are not diluted in the sample batch, no laboratory blanks are required for that specific sample batch.

⁵ Field Duplicates

While SWAMP recommends that field duplicates be collected and analyzed, they are not a current SWAMP requirement. Projects are encouraged to require field duplicates in their QA project plan (QAPP) if it supports their specific quality objectives.

Table 2: Sample Handling: Indicator Bacteria in Fresh Water

Recommended Container	Recommended Preservation	Required Holding Time
Factory-sealed, pre-sterilized, disposable whirlpak® bags or 125-mL sterile plastic (high density polyethylene, polystyrene, or polypropylene) or glass container	Cool to ≤10 °C; for samples containing chlorine, sodium thiosulfate is pre-added to the containers in the laboratory	8 hours <i>for compliance monitoring</i>
		24 hours <i>for routine ambient monitoring</i>

Each “Required Holding Time” is based on the assumption that the “Recommended Preservation” (or a method-mandated alternative) has been employed. All samples analyzed past the 8-hour compliance holding time will be flagged for user notification, however, will still be considered SWAMP compliant for routine ambient use. If the 24-hour holding time for analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified, and the data must be flagged accordingly.

Sample analysis should begin as soon as possible after receipt, a holding time of no more than 8 hours is highly recommended. For purposes of compliance monitoring, sample incubation must be started no later than 8 hours from time of collection and no later than 24 hours for routine ambient monitoring. ^(1,2,3)

Table 3: Corrective Action: Indicator Bacteria in Fresh Water

Laboratory Quality Control	Corrective Action
Sterility Checks	Identify contamination source and take appropriate action; discard membrane filter/pad or prepared media lot; discard sample results if checks made during analysis
Laboratory Positive Control	Identify cause and take appropriate action; discard prepared media and remake from start or purchase new lot
Laboratory Negative Control	Identify cause and take appropriate action; discard prepared media and remake from start or purchase new lot
Diluent Control	Identify contamination source and take appropriate action; qualify data as needed
Laboratory Duplicate	Verify results; qualify data as appropriate
Laboratory Blank	Identify contamination source and take appropriate action; qualify data as needed

Field Quality Control	Corrective Action
Field Blank, Equipment Blank	Examine field log; identify potential contamination source; qualify data as needed

References:

- (1) Meyers, D.N., et. al. 2014. U.S. Geological Survey TWRI Book 9. Fecal Indicator Bacteria. Ch. 7, V. 2.
- (2) Pope, M.L., et. al. 2003. Assessment of the Effects of Holding Time and Temperature on *Escherichia coli* Densities in Surface Water Samples. Applied and Environmental Microbiology, Vol. 69, No. 10, p. 6201-6207.
- (3) [Standard Methods Committee](#). SM Section 9060 B. Standard Methods for the Examination of Water and Wastewater. 23rd edition.
- (4) [Standard Methods Committee](#). SM Section 9020. Standard Methods for the Examination of Water and Wastewater. 23rd edition.

Terms appearing in the tables are defined in the [Surface Water Ambient Monitoring Program Quality Assurance Program Plan](#).