

Virginia Department of Health
Provisional Freshwater Monitoring Guidance for Issuing and Lifting Advisories
Revised September 2016

This set of provisional recommendations may be used by operators of freshwater recreational water areas to monitor *E. coli* levels and to issue and lift advisories.

1. Recreational water monitoring should be conducted at least weekly during the swimming season, which is usually from mid-May through September. The particular day of the week should be determined in cooperation with the laboratory conducting the analysis. Other sampling intervals, such as monthly sampling, may be used when appropriate.
2. The number of sampling locations per recreational water area should be determined by the organization conducting the sampling. Sampling locations should represent the area used by swimmers and/or recreation water access points. Recreational water access locations and sampling sites should be identified by latitude and longitude and should remain uniform over the years if interested in analyzing long term water quality trends.
3. Samples should be collected on the regular monitoring day, rain or shine, unless conditions are dangerous to sampling staff. If a decision is made not to sample because an exceedance of the water quality standard is expected, then the recreational waters should be posted with an advisory.
4. Water samplers should use hand sanitizer before collection and wear latex-type gloves to avoid contaminating the sample during collection.
5. Water samplers should wade out to knee-deep water (at least 1.5 feet deep) and collect the samples approximately 1 foot below the surface. Care should be taken to avoid stirring up bottom sediments while wading to collect the sample. Samples should be as free of sediments as possible. The use of a "sampling pole" can help a sampler avoid sediments.
6. Samples should be stored in a sealable plastic bag in a cooler with ice or ice-packs to maintain a temperature of <math><8^{\circ}\text{C}</math>. Sample hold time should not exceed six hours from when samples are collected to delivery to the lab. Samples at the lab should be refrigerated and analyzed within two hours of arrival.
7. The indicator organism used for freshwater recreational use is *E. coli*. Laboratory analysis of *E. coli* levels in water samples need to be conducted using an EPA-approved method. For *E. coli*, these methods are typically membrane filtration or Colilert®.
8. If the results of sampling show the site to be below the water quality standard, the next water sample should be taken the next week (or next scheduled sampling date) on the regularly scheduled sampling day.
9. Health advisories should be issued at recreational areas that exceed the water quality standard for bacteria. Signs should be posted in plain view near monitoring and recreational access sites.

Interpretation of Results and Data Averaging

10. The single sample maximum water quality standard for *E. coli* is 235 colony forming units per 100 ml (235 cfu/ 100 ml) for membrane filtration or 235MPN per 100 ml for Colilert quantitray. This is the appropriate threshold for issuing and lifting recreational use advisories in freshwater. Samples above this level are an exceedance of the Virginia Water Quality Standards for primary contact recreational use. *The geometric mean water quality standard for E. coli is 126; application of the geometric mean should not be used for advisories, and is discussed in #20, below.*
11. Laboratories typically dilute environmental water samples with sterile water and then analyze the dilution. This is because environmental water samples may be heavily loaded with bacteria. By diluting the sample, it is possible to count colonies on a Petri dish, whereas an undiluted sample would result in bacteria that cover the dish and colonies would not be readily countable. When laboratory results show that the value is greater than some upper threshold value, for example: >800 cfu/100 ml, the result is considered “too numerous to count” or “TNTC”. Similarly, if few or no bacteria are present, labs may report either a number, such as “6 cfu/100 ml,” or may indicate a result less than 10 as “<10.”
12. Duplicate results from the same sample, e.g., 2 duplicates analyzed at 10 ml from the sample bottle, can be averaged and the average result can be used to determine if an advisory is necessary. Duplicate sample analysis is typically conducted by the laboratory to evaluate quality control of analysis. Duplicate results should be similar, but may not be.

Example 1: Averaging Duplicate samples			
Beach Site	Sample Time	* <i>E. coli</i> (cfu/100 ml)	Duplicate average
Sunset Beach	8:50	250/175	213
Crystal Beach 1	9:15	84/86	85
Crystal Beach 2	9:16	31/52	42
Crystal Beach 3	9:17	31/20	26
Strawberry Lake	10:00	30/30	30
Pineapple Stream	11:35	20/20	20
Emerald Beach 1	13:00	30/<10	16
Emerald Beach 2	13:01	<10/<10	1

**E. coli* recreational use standard is 235 colony forming units (cfu) per 100 milliliters (ml) or 235MPN per 100 ml.

Example 1 shows that the duplicate average is a simple arithmetic average of duplicate results. Even though the result for Sunset Beach comes close to 235, the result is not greater than 235 and therefore meets the water quality standard.

13. Multiple samples taken on the same date from the same recreational water site should also be averaged, and the average result should be used to determine whether the recreational waters are in compliance or should be posted with an advisory and re-sampled.

In Example 2, the results for Crystal Beach: 85, 22, and 201 are averaged for the entire site, resulting in a site average of 103. Note that averages are rounded to the nearest whole number.

Example 2: Determining Recreational Waters Site Average			
Beach Site	Sample Time	*E. coli (cfu/100 ml)	Site Average
Sunset Beach	8:50	133	133
Crystal Beach 1	9:15	85	
Crystal Beach 2	9:16	22	
Crystal Beach 3	9:17	201	103
Strawberry Lake	10:00	29	29

*E.coli recreational use standard is 235 colony forming units (cfu) per 100 milliliters (ml).

If the results show that one of three samples at one recreational water site exceeds the water quality standard, use the average value to determine if an advisory should be issued for the site and be re-sampled. In Example 3, data show that one out of three samples taken from Crystal Beach exceed the water quality standard. The site average is within the water quality standard. As a general rule, if only one value out of three for the same site exceed the standard, consider the site to be in compliance if the site average meets the standard.

Example 3: Determining Recreational Waters Status from Site Averages			
Beach Site	Sample Time	*E. coli (cfu/100 ml)	Site Average
Sunset Beach	8:50	90	90
Crystal Beach 1	9:15	275	
Crystal Beach 2	9:16	80	
Crystal Beach 3	9:17	15	123
Strawberry Lake	10:00	15	15

*E.coli recreational use standard is 235 colony forming units (cfu) per 100 milliliters (ml).

There are times when one sample exceeds the standard and the other samples comply but are close to an exceedance. In Example 4, there is one sample that exceeds the standard. The other two sample results, even though they are below the standard, are elevated and result in a site average that exceeds the standard. In this instance, VDH recommends posting an advisory and re-sampling.

Example 4: Determining Recreational Water Status from Site Averages			
Beach Site	Sample Time	*E. coli (cfu/100 ml)	Site Average
Sunset Beach	8:50	85/86	85
Crystal Beach 1	9:15	450	
Crystal Beach 2	9:15	230	
Crystal Beach 3	9:15	200	293
Strawberry Lake	10:00	30/30	30

*E.coli recreational use standard is 235 colony forming units (cfu) per 100 milliliters (ml).

If data are collected that show multiple sites at a single recreation area exceed the water quality standard, consider issuing a swimming advisory and resampling. Recreational site averages can be calculated for comparison to the water quality standard, but issuance of an advisory may also be appropriate to be protective of human health. In Example 5 below, two of three samples from Crystal Beach exceed the water quality standard, however, the site average does not exceed the standard. Posting an advisory and resampling as a precaution, is recommended in such a case.

Example 5: Determining Recreational Water Status from Site Averages			
Beach Site	Sample Time	*E. coli (cfu/100 ml)	Site Average
Sunset Beach	8:50	57/63	60
Crystal Beach 1	9:15	310	
Crystal Beach 2	9:15	280	
Crystal Beach 3	9:15	94	228
Strawberry Lake	10:00	30/30	30

*E.coli recreational use standard is 235 colony forming units (cfu) per 100 milliliters (ml).

14. For results that are reported at two different dilutions (e.g., a membrane filter test conducted at a 25 ml dilution and at a 50 ml dilution), you should use the more accurate result for determining whether an advisory is necessary. The larger sample volume of 50 ml means multiplying a colony count by 2 (to report in terms of 100ml). To report a colony count from a 25 ml dilution means multiplying the count by 4. The result from the larger-sample volume of 50 ml and the fact that results are multiplied by 2 instead of 4 are considered more accurate than the 25 ml dilution result. For a membrane filter test, we're looking for a certain concentration of bacteria on the filter. We're looking for a result that shows more than 20 and less than 80 colonies of bacteria on the test plate. Using two different dilutions for a membrane filter test can provide you with a usable result in instances when there is a high amount of bacteria in the water. If the number of colonies is too high the result may read "TNTC," or, too numerous to count. This is not a usual result for recreational monitoring. For recreational monitoring results, we look for the number of colonies on the plate and the dilution. Use the result from the higher-volume dilution unless the number of colonies would be >80 or the result is TNTC. Example 6 below demonstrates this recommendation.

Example 6: Determining Reportable Results from Membrane Filter Test*					
Beach Site	Sample Time	25 ml dilution E. coli (cfu/100 ml)	50 ml dilution E. coli (cfu/100 ml)	# of Colonies/plate 25ml / 50ml	Reported Result
Bird Beach 1	10:00	76	88	19 / 44	88
Bird Beach 2	10:15	320	150	80 / 75	150
Bird Beach 3	10:30	100	120	25 / 60	120
Bird Beach 4	10:45	96	74	24 / 37	74

*E.coli recreational use standard is 235 colony forming units (cfu) per 100 milliliters (ml).

In Example 6, all the "Reported Results" are from the 50 ml dilution, however, they weren't always the highest value measured. Knowing that you are looking for a plate count between 20 and 80 colonies and that using the 50 ml dilution because it is more accurate can help you determine which lab-reported result to use.

Issuing and Lifting Recreational Water Advisories

15. If there is an exceedance of the water quality standard, the site should be posted with an appropriate sign advising recreational users that water quality does not meet state water quality standards and that recreational water use is not recommended until further notice. A follow-up sample should be collected as soon as possible and delivered to the lab.

16. No additional sampling should be conducted until the results of the last test are received, either by fax, phone or e-mail.
17. If the follow-up sample shows the recreational water site results are below water quality standards, lift the advisory by removing the posted sign.
18. If the follow-up sample exceeds the water quality standard, the advisory should continue and another water sample should be collected as soon as possible. The single sample maximum is used to issue and lift advisories.
19. An alternative to additional sampling is to leave an advisory in place if conditions appear not to have improved after the last sample. For example, if rainfall is expected to have caused an exceedance of the standard and it is likely that rain or runoff will lead to another sample exceedance, you may delay resampling until after the rain stops and water levels and water flow rates return to normal, as long as the advisory stays in place. If a decision to resample is made, the advisory can be lifted when bacteria levels are in compliance.
20. The geometric mean state standard is used for assessment purposes by the Department of Environmental Quality to determine if the water on their bi-annual report to the federal government. The geometric mean is not for issuing and lifting advisories. The geometric mean is calculated on samples from the same site. Duplicate or replicate samples from the same day are not used to calculate a geometric mean; duplicate and replicate samples are averaged arithmetically and the result is used in the calculation of the geometric mean. State Water Quality Standards require the geometric mean to be used for the assessment when a site is sampled four or more times in a calendar month.

Please contact your local health department to make them aware of your water monitoring program.

Local health departments often receive questions from the public when advisories are issued. Local health departments can also help interpret sample results and assist with public notification and other recommendations. Find contact information for your local health department at the following website: <http://www.vdh.virginia.gov/local-health-districts/>.

References:

- Virginia Water Quality Standards. 9VAC25-260-170. Bacteria; other Recreational Waters: <http://law.lis.virginia.gov/admincode/title9/agency25/chapter260/section170/>
- Virginia Department of Health, Coastal Beach Monitoring Website: <http://www.vdh.virginia.gov/environmental-epidemiology/beach-monitoring/>
- EPA Beach Monitoring Website: <http://www2.epa.gov/beaches>
- Standard Methods for the Examination of Water and Wastewater, 22nd Edition.