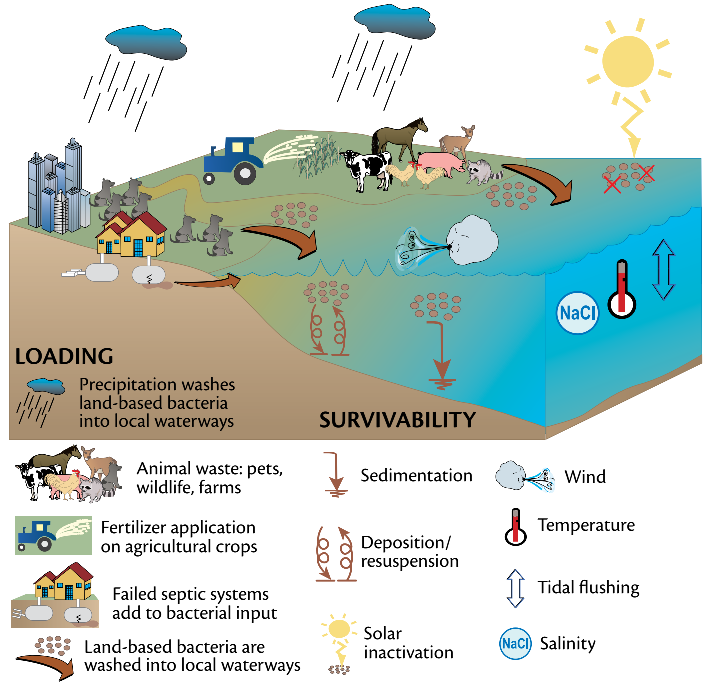
Bacteria

Bacteria occur naturally in both fresh and salt water. Bacteria are also commonly found in the intestines of humans and other warm-blooded animals. Most are harmless to humans and animals, but some are pathogenic and can cause illness in swimmers. Pathogens can come from the feces of many animals, including wildlife and pets, or from humans, through leaking septic systems and broken sewer lines. Testing for all pathogens is difficult, so we usually test for the presence of indicator bacteria. Indicator bacteria, such as enterococci and *E. coli*, are present in large numbers, so they are easy to find and relatively inexpensive to monitor. These indicators are not harmful themselves, but can come from similar sources as pathogens. The presence of these indicators suggests that harmful pathogens may also be present. During significant rainfalls, there is an increased risk for elevated and unsafe bacteria in natural waters. Enterococci are generally used as an indicator in brackish and salt water, and *E. coli* are usually used in fresh waters (USEPA 1986).

Contacting your local health departments who monitor public bathing beaches and issue advisories when EPA bacteria criteria are not met is recommended.



This conceptual diagram illustrates the sources and fates of bacteria in an ecosystem.

H. Kelsey

Local health departments monitor bacteria levels at public beaches throughout the state.

Field sampling procedures

Sampling locations should be in areas of high recreational use, such as public beaches and boating areas. There should be a minimum of 5 sites sampled, but the appropriate number will probably vary among tributaries—the number of samples should be representative of recreational use and potential exposure in the water.

For this protocol, we do not recommend calculating an overall grade for the sub-region or waterbody because bacteria data is so variable. An overall score can not be calculated for less than 5 sites for statistical reasons. Additionally, reference sites, in mid-channel locations, should also be sampled. These sites will provide a comparison to bacteria "hot spots" and provide a more random sampling design. A randomly sampled, non-targeted bacteria program is rare in the Mid-Atlantic region. If the objective of your bacteria sampling program is to determine human health risks while swimming or boating, you do not need to have a random sampling design.

In the mid-Atlantic region, bacteria should be assessed from Memorial Day to Labor Day. These months cover the period of time when people come into contact with the water via swimming, wading, boating, and fishing, among other uses. A minimum of twice monthly sampling is recommended for assessment. However, weekly sampling is preferred.

Sampling should always occur on the same day of the week, independent of weather. Including data from both dry weather and after rainfall in the analysis provides for an overall health assessment of the tributary. This will be reported as a frequency of attainment. Reporting data for only dry weather dates is also useful for identifying hot spots and for trend analysis such as comparison between rivers and between years.

Sample equipment

- Cooler with ice
- Labeled sterile sample bottles and caps
- Extendable pole
- Chest waders
- Disinfectant gel or sanitizer
- Boat (optional)

Sample procedure

Adherence to sample collection protocols is crucial to obtain accurate sample results and to ensure the integrity of the bacteria monitoring process. The following recommended steps for sample collection are taken from the EPA's 2002 National Beach Guidance and Required Performance Criteria, 1992, Standard Methods for Water and Wastewater Examination.

1) Prior to monitoring, fill bacteria sampling cooler halfway with ice. All samples must be placed at 1 to 4°C at all times until filtration.
2) Only autoclaved sterile containers must be used and all bottles must be pre-labeled before going out into the field.
3) Identify the sampling site on the data sheet and compare to the sterile bottle.

At station:

4) Record all information while at the station on the data sheet—number of waterfowl, people/swimmers, pets, etc.
5) Attach the bottle to the sampling pole, securing it tightly; open the cap without touching the inside of the lid.

S. Hornor

A researcher collects a water sample to check for bacteria.

B. Sadiowski

After a sample is taken, the water is filtered to collect bacteria cells and placed on a growing medium.

6) If sampling from a boat, make sure that the boat motor has not stirred up the water. If the water is shallow, sampling should be done through wading.
7) If sampling from shore, wear chest waders in areas where high bacterial counts are expected or are unknown. Carefully wade out to waist-deep water, making sure to minimize disturbance of the bottom sediments and the water column.
8) Extend the pole outward and dip at approximately 0.3 meter depth (1 to 1.5 feet deep).
9) Fill the bottle to shoulders, tightly cap the bottle.
10) Record the date and time of sampling on data sheet.
11) Place sample on ice.
12) After samples have been collected from a station, wipe arms/hands with disinfection gel to reduce exposure to potentially harmful bacteria or other microorganisms (EPA 2002).

Taking duplicate samples or re-sampling areas is recommended in case of unexpected results or noteworthy data, but is not necessary.

Laboratory analysis

The IDEXX method (http://www.idexx.com/view/xhtml/en_us/water/enterolert.jsf) or the membrane filtration method should be used to analyze samples. These methods are recommended by the U.S. EPA and are common methods used at government and academic laboratories in the mid-Atlantic. Field analysis of bacteria is not recommended. Organizations with properly equipped laboratories can conduct their own analysis (IDEXX only). Check with the laboratory for their Standard Operating Procedure to make sure the field collection method you use is appropriate for their lab methods.

Data analysis

The EPA threshold for enterococci in swimming and contact recreation areas is 104 MPN 100 ml^{-1} when using IDEXX (USEPA 1986) and 104 cfu 100 ml^{-1} when using membrane filtration. Based on EPA guidelines, risk for swimming-associated illness is too high when the criterion (104 MPN 100 ml^{-1}) is exceeded. The EPA threshold for *E. coli* is 235 organisms 100 ml^{-1} for any single water sample.

Data provided from the laboratory are analyzed to calculate a percent of samples below the appropriate (enterococci or *E. coli*) threshold. The percent of samples in a sampling season (Memorial Day to Labor Day) that a sample was below the appropriate threshold is the percent passing (score) for each station. A summary of steps for calculating bacteria scores is:

1) Make sure the data used for analysis are from the relevant months. For bacteria, the minimum sampling period is Memorial Day to Labor Day with twice monthly sampling.
2) Make sure the appropriate threshold for enterococci or *E. coli* is used.
3) Calculate the percent of samples that were below the threshold for a station score. Do not average the individual station values before calculating the percent. Compare each station value directly to the threshold to see if it meets the threshold value. (For example: a data value of 200 MPN 100 ml^{-1} is above the 104 threshold, therefore it scores a zero. A data value of 100 MPN 100 ml^{-1} is below the threshold and therefore it scores a one. Take the average of the ones and zeros to find the percent of samples that are below the threshold.)

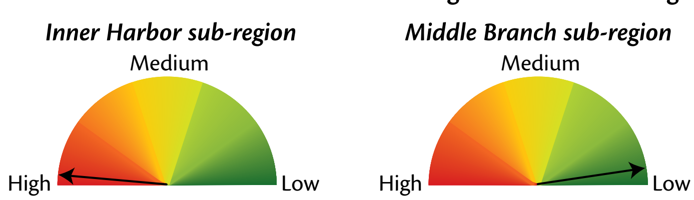
Scoring and description for bacteria indicator.

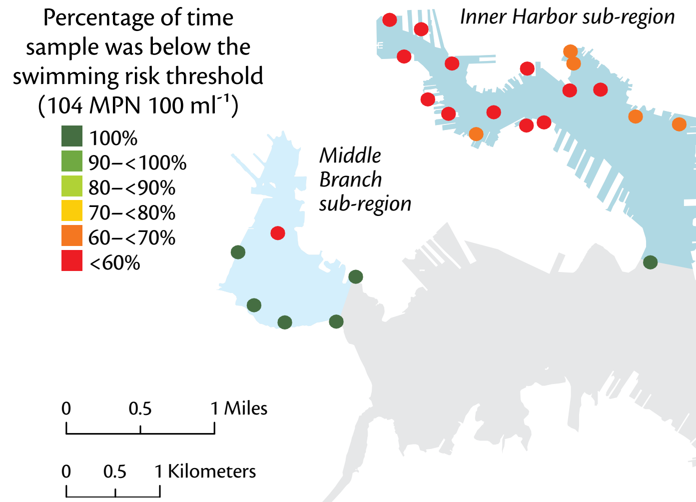
Score	Narrative
100	Excellent
90.00–<100	Good
80.00–<90	Moderate
70.00–<80	Moderately Poor
60.00–<70	Poor
<60	Very Poor

Communicating bacteria score results

Since bacteria is a human health indicator, it is communicated differently than ecological indicators. For bacteria, station average scores are calculated, then

Annual relative risk of humans becoming sick from swimming





This map of Baltimore Harbor bacteria results illustrates the variability in station scores, and therefore the need for presenting the results by station, rather than as an average sub-region score.

presented on a 10-point scale (not the 20-point scale used by ecological indicators). Furthermore, due to the variability of bacteria scores within small areas, a map of station average scores should be presented along with the overall sub-region or region information. The map and the associated text should include the specific wording presented here: Percentage of time sample was below the swimming risk threshold. This ensures the correct interpretation of the data. For an overall sub-region average, "fire danger" symbols or dials can be used to illustrate relative risk of becoming sick from swimming. This is provided by calculating an overall sub-region score, with Low = 100% passing and High = 60% passing. To calculate the sub-region score, station scores are averaged into a sub-region score.

Suggested narrative

Bacteria indicators differ from other ecosystem health indicators in that they include both targeted (samples are taken at fixed locations designed to evaluate swimming illness risk) and random (samples are taken at randomly assigned locations to represent all potential locations) sampling. Indicator bacteria are useful to evaluate how safe water is for swimming, but are not easily used to describe ecosystem health. There is no clear link between more traditional measures of ecosystem health (core indicators) and bacteria concentration. For that reason, it is not recommended that bacteria scores be integrated with other ecosystem health indicators.

There are also many factors that can affect bacteria concentration and therefore, the interpretation of results. The suggested list below describes some of these important factors and should be included in a narrative statement within the bacteria section of your report card to help provide context and interpretation of results. This narrative can also be in a separate document that's referenced in the report card.

- *Rainfall and dry weather data.* Perhaps the most important transport mechanism for fecal bacteria to coastal waters is by rainfall runoff. Bacteria are transported from animal feces by stormwater, and measurements of fecal indicator bacteria may often be high following rain events. The annual score for bacteria in a report card is designed to represent the percent of days when it is safe to swim (as recommended by EPA guidelines), so these analyses should include sampling during or immediately following significant rainfall. To help interpret the score, report the number of sampling days on which rainfall was a factor. However, when comparing among regions or time series, it is useful to remove the rainfall data from analysis so that comparisons are performed using similar conditions. With any comparison that has different *numbers* of rain dates, you will need to drop the data from rain dates, or else the results are biased toward higher values in the dataset with more rain dates. This allows direct comparison of results from other tributaries and at individual swimming areas in different years.
- *Potential sources.* Fecal bacteria (and pathogens) can come from a variety of animal sources, including humans, wildlife, pets, and even soils. It is well known that fecal pollution from human sources presents a higher risk to humans. Monitoring for fecal bacteria does not provide information about the source of the bacteria. The U.S. EPA recommends that fecal indicator bacteria thresholds be applied regardless of the likely bacteria source. It is very difficult to determine the source of bacteria found in the water. Even so, in reporting bacteria scores, it is useful to discuss the potential sources of the bacteria to provide context and interpret results.
- *Scoring.* Currently, there are single thresholds for both enterococci and *E. coli* bacteria for full contact recreational use. The use of a single threshold indicator, while helpful, does not show the resolution that a multiple threshold indicator does.
- *Limitations of indicator bacteria.* When fecal indicator bacteria are present, pathogens are more likely to be present, but they may not always be there. The likelihood of getting sick from swimming is therefore not perfectly correlated with indicator bacteria concentration. Still, these indicators are the current, best information to predict illness risk, and EPA guidelines say that the risk of illness from swimming is too high when bacteria concentrations exceed the guidelines. Due to the difficulty in assigning risk from different sources, and because rainfall is a major contributor of fecal pollution, the Maryland Department of the Environment recommends that people do not swim in the 48-hour period immediately following rainfall greater than one inch.
- *Health implications.* To improve the linkages between illness and swimming, we recommend that gastrointestinal illnesses following swimming are reported to the health department and other public health databases.
- *Homework/tips.* Including information in the report card about what citizens can do to decrease bacteria is always helpful.
- *Site specific details.* Site specific details help citizens identify locations of high bacteria concentrations and raise awareness of where bacteria concentrations are a problem in the ecosystem.

Literature cited

City of Baltimore (2010) National Pollution Discharge Elimination System Annual Report, 2009. City of Baltimore, Department of Public Works, Bureau of Water and Wastewater, Surface Water Management Division.

USEPA (1986). Ambient Water Quality Criteria for Bacteria–1986. U.S. Environmental Protection Agency, Washington, DC. EPA–440/5-84-002.

USEPA (2006) Method 1600: Enterococci in Water by Membrane Filtration, Using membrane-*Enterococcus* Indoxyl-$-D-Glucoside Agar (mEI). EPA-821-R-06-009

USEPA (2006) Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (modified mTEC). EPA 821-R-02-023.