

Assessing the Importance of Sand as a Source of Fecal Indicator Bacteria

(*Escherichia coli* and *Enterococcus*)

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PURPOSE

The purpose of this activity is for students to investigate water quality, and the factors that affect it, at a local beach. This is accomplished by (1) measuring concentrations of *Escherichia coli* and *Enterococcus*, two types of bacteria commonly used as fecal indicators, in water and sand samples from a local beach; (2) interpreting the collected data with graphs, tables, and statistical tests; and (3) investigating the relationship between concentrations of fecal indicator bacteria (FIB) and other factors such as sampling location, distance from the shoreline, and grain size of sand.

AUDIENCE

This activity was developed as an independent research project for middle and high school students. However, it is also suitable for undergraduate or beginning graduate students, especially nonmajors, and it may be adapted into a class exercise instead of an individual project. Measuring FIB concentrations using IDEXX kits is a straightforward, easily mastered procedure, making it suitable for addressing questions ranging from the very simple (Are FIB concentrations higher at one site than at another?) to the complex (How do FIB concentrations vary in response to spring-neap tidal cycles or other environmental parameters?) (Boehm and Weisberg, 2005).

BACKGROUND

Human waste is a serious threat to coastal water quality because it contains many types of pathogens, or disease-causing organisms (Table 1). Swimming at beaches polluted with human waste can cause diarrhea and eye, ear, skin and respiratory infections, as well as more serious problems such as hepatitis and meningitis (US Environmental Protection Agency, 2006; Rose et al., 1999). The Beaches Environmental Assessment and Coastal Health (BEACH) Act was passed in 2000 to protect swimmers from waterborne disease. It requires states to set water-quality standards for pathogens and/or indicator organisms, and to implement water-quality monitoring programs at recreational beaches (US Environmental Protection Agency, 2006).

Testing water for pathogens can be difficult for a number of reasons, including (1) pathogens may be difficult or impossible

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to culture in the laboratory; (2) the detection limit of laboratory methods may be much higher than the infectious dose, so water that tests “clean” could still cause disease; and (3) often, the pathogens behind swimming-related diseases are unknown, and it would be prohibitively expensive and time consuming to test for all pathogens that might be present. Thus, scientists, beach managers, and environmental policymakers typically test water for bacterial indicators, rather than actual pathogens.

FIB are species of bacteria that indicate the presence or absence of human waste and the pathogens associated with it. An ideal indicator should have the following characteristics: (1) quick, easy, affordable detection; (2) not naturally present in the environment; (3) concentrations in the environment correlated to the amount of fecal pollution present; and (4) decay rate comparable to pathogen(s) of interest (US Environmental Protection Agency, 2006). In practice, we have yet to discover the ideal indicator. The most common indicators used to set state and federal water quality standards in the United States are *Escherichia coli* and *Enterococcus*.

Escherichia coli and *Enterococcus sp.* (chiefly *E. faecium* and *E. faecalis*) are two types of bacteria that are ubiquitous in the human gut and thus present at high concentrations in human waste. Both organisms are facultative anaerobes, which

means that they can live either with or without oxygen, and most strains of both organisms are commensal with humans, although a few virulent strains of each are known. *E. coli* is rod-shaped, Gram-negative, and non-sporulating, while *Enterococcus sp.* are spherical, Gram-positive bacteria.

Present US state and federal water-quality standards are based on an epidemiological study (Cabelli et al., 1982) that showed a direct, linear relationship between concentrations of *E. coli* and *Enterococcus* in bathing waters and swimming-associated risk of gastrointestinal illness at beaches in New York, Louisiana, and Massachusetts. Some later studies (e.g., Seyfried et al., 1985; Corbett et al., 1993, and Haile et al., 1999) supported the use of these bacteria as fecal indicators; however, others have shown that FIB are not always correlated with pathogens in wastewater (Thompson et al., 2003; Van Heerden et al., 2003) or environmental waters (Lipp et al., 2001; Noble and Fuhrman, 2001; McQuaig et al., 2006). In addition, when FIB originate from nonpoint sources, they may not correlate with human health risk (Colford et al., 2007).

Recently, beach sand has been implicated as a source of FIB to coastal marine waters (Oshiro and Fujioka, 1995; Ferguson et al., 2005; Yamahara et al., 2007). Sand may become contaminated by human or animal feces, wastewater, or polluted

Table 1. Characteristics of waterborne pathogens.

Type of Pathogen	Characteristics	Examples	Size	Prevalence	Pathogenicity
Bacteria	Single-celled organisms; no nucleus; no chlorophyll. Contain a single chromosome and typically reproduce by binary fission. Found in all types of environments ¹	Coliforms including: <i>Escherichia coli</i> , <i>Enterococcus</i> , <i>Streptococcus</i> , <i>Clostridium</i>	Generally 0.5–5.0 µm; some up to 500 µm in length ²	3.2 x 10 ¹¹ bacteria per g of human feces ³	Most bacteria are harmless to humans
Protozoa	Single-celled organisms with a nucleus; reproduce by fission; found mainly in aquatic environments ¹	<i>Giardia lamblia</i> , <i>Cryptosporidium parvum</i>	Generally 10–50 µm; some up to 1 mm in length	Up to 200,000 and 40,000 cysts of <i>Cryptosporidium</i> and <i>Giardia</i> , respectively, per L of raw sewage ⁴	30% of known protozoa species are pathogens ¹
Viruses	Infectious agent composed of genetic material inside a protective protein coating (capsid); cannot reproduce without a host cell (plant, animal or bacterium) ¹	Common cold, flu, HIV, chicken pox, enterovirus, rotavirus, norovirus	10–30 nm in diameter	5000-28,000 plaque-forming units per L raw sewage ⁵	Most probably harmless to humans

Sources: ¹US EPA, 2006; ²Shulz and Jorgensen, 2001; ³Whitman et al., 1998; ⁴Robertson et al., 1999; ⁵Metcalf et al., 1995.

seawater, or it may serve as a natural environmental reservoir for FIB. The presence of sand and sediment can allow bacteria to survive and reproduce in situations where they would otherwise die off (Lee et al., 2006). It is still unclear whether the persistence of FIB in environmental sands and sediments represents an additional health risk, or if it weakens the connection between FIB and waterborne pathogens.

Despite their limitations, *E. coli* and *Enterococcus* are used to set US water-quality standards because they can be detected quickly, easily, and affordably. In this activity, students use the Enterolert and Colilert kits (IDEXX, Westbrook, ME). These kits are used by researchers and beach monitors to measure concentrations of *Enterococcus* and *E. coli*, respectively, at beaches across the country.

The FIB detection method employed in this activity uses selective media to culture, or grow, the bacteria of interest. A selective medium is a liquid or gel that favors the growth of a particular type of microorganism. The reagents in the Colilert and Enterolert kits are “food” or nutrients that can be used by only certain types of bacteria. When the bacteria metabolize (“eat”) the reagents, they change color. In the case of the Colilert test, two different reagents are packaged together, one that can be used by all coliform bacteria and that turns yellow when metabolized, and one that can only be used by *E. coli* that turns fluorescent when metabolized.

The Quanti-Trays in the kits allow easy quantification of bacteria present in the original sand or water sample (Figure 1). Each colored well represents an individual colony of bacteria, while each well that remains unchanged is free of the bacteria in question. The number of colonies that form is related to the original concentration. For example, if there were only one bacterium in the original sample, a maximum of one colony could form. For larger numbers of bacteria, this gets more complicated, because some bacteria could fail to form colonies, or more than one colony-forming bacterium could end up in the same well. The relationship between the number of colored wells and the most probable number (MPN) of bacteria in the original sample was determined experimentally and is presented in the table in Figure 1. Because it is possible for different concentrations of bacteria to result in the same number of colored wells, we can never calculate the exact number of bacteria in the original sample, only the MPN.

RESEARCH QUESTIONS

The questions addressed in this activity are:

- Are *E. coli* and *Enterococcus* present in water from a local beach? If so, do they exceed state and federal standards?
- Are *E. coli* and/or *Enterococcus* present in sand from a local beach?
- Are concentrations of *E. coli* and/or enterococci related to (1) distance of the sampling location from the shoreline, (2) distance from specific beach facilities or natural features (e.g., bathrooms, standing water, river outlet), or (3) depth of the sampling location?
- Are concentrations of *E. coli* and/or enterococci related to sand properties, such as water content or grain size?

MATERIALS

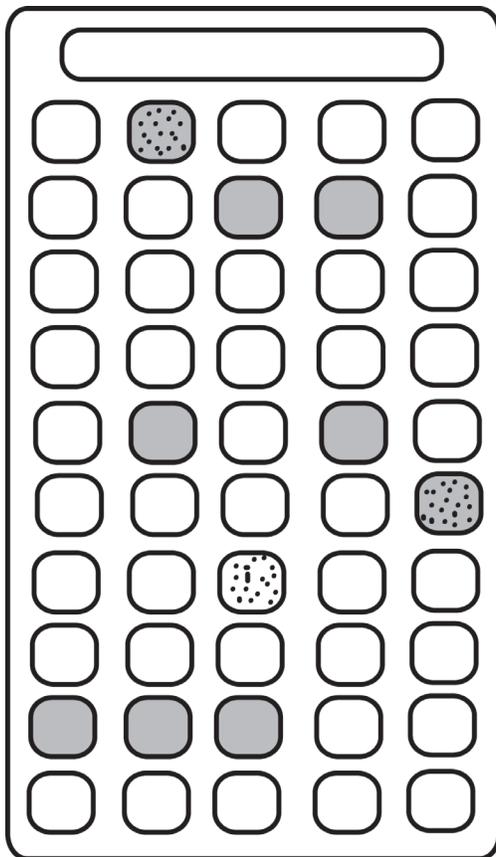
Note: Materials used in more than one step are only listed once to avoid repetition.

For sand collection:

- Latex or nitrile gloves (1 pair per person)
- Sterile collection container with a volume of about 100 mL. Either use a new container for each sample or sterilize the container by washing thoroughly with soap and water, soaking for at least one hour in a 10% solution of household bleach (making sure the solution gets inside each container), and finally rinsing thoroughly with water.
- Hand auger or small shovel
- Meter stick or tape measure
- Cooler with ice or ice packs
- Field notebook or data sheets

For FIB analysis:

- Latex or nitrile gloves (1 pair per person)
- Balance
- Sterile ~100 mL plastic bottle (1 per sample)
- Distilled or purified water
- Labeling tape
- Permanent marker
- Stopwatch or watch with second hand
- Sterile 10 mL pipette (1 per sample)
- Pipette bulb
- Colilert-18 and Enterolert reagents (IDEXX, Westbrook, ME) (1 of each per sample).*
- 90-mL bottles of Butterfield buffer (Whatman Inc., Kent, UK)



No. of wells giving positive reaction	MPN per 100 mL sample	95% confidence limits	
		Lower	Upper
0	<1.0	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
15	17.8	10.8	29.4
20	25.4	16.5	39.4
25	34.4	23.3	51.2
30	45.3	31.5	65.6
35	59.1	42.0	84.4
40	78.2	56.4	111.2
45	109.1	78.6	158.7
50	200.5	135.8	135.8

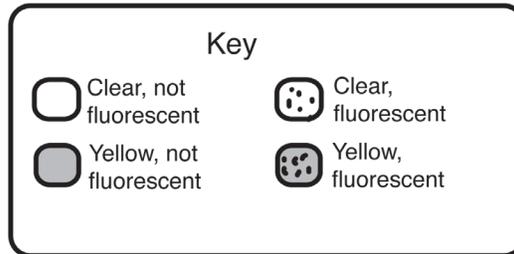


Figure 1. Reading a Quanti-Tray to determine the most probable number of bacteria in a water or sand sample. For Colilert tests, mark the yellow wells (total coliform). Then, under a black light, mark the wells that are both yellow and fluorescent (*E. coli*). Wells that are fluorescent but not yellow should not be counted. In this example, there are nine yellow wells, of which two are also fluorescent, indicating 9.9 and 1.0 most probable number (MPN) per 100 mL of total coliform and *E. coli*, respectively. It should be noted that the table in this figure is abridged; the full table included in the kit lists the MPN for every possible number of wells (1 to 51). Adapted from information enclosed with IDEXX Colilert-18, Enterolert, and Quanti-Tray products

(2 per sample) (If Butterfield buffer is unavailable, 90 mL of distilled water in a sterile container may also be used.)*

- Quanti-Tray (IDEXX, Westbrook, ME) (2 per sample)*
- Quanti-Tray sealer or clothes iron*
- Incubator (If you do not have access to an incubator, you can make one using a Styrofoam cooler and a 15-watt light bulb—see Appendix 1.)*
- Scientific thermometer
- Black light

For measuring water content:

- Small, oven-safe containers (for example, muffin cups, silicone baking cups, or small boats made out of aluminum foil)
- Oven

For measuring grain size:

- Set of nested sieves covering the general size range of 100 μm (fine sand) to 30 mm (coarse gravel)*

* Note: A local university, government agency, or nonprofit organization that monitors water quality may be able to help you by donating or letting you use these supplies and/or equipment. Check before you make an expensive purchase!

ACTIVITY

Generating Hypotheses and Tests

Once the students have decided upon their research question(s), they should generate a hypothesis for each one. Hypotheses should be based on background research (if time allows), or on logical reasoning about what factors affect bacteria in the environment. A key feature of a good hypothesis is that it is testable using the time and resources available. Some example hypotheses related to the research questions above are:

- *E. coli* and *Enterococcus* will not exceed the state standard at the local beach, because we have never observed any evidence of wastewater there.
- *E. coli* and *Enterococcus* will be present in sand from a local beach, because they have been found in sand samples at many

other locations.

- FIB concentrations in sand will be highest near bathrooms, parking lots, and roads because of greater human impact in these areas.
- FIB concentrations will be higher in wet sand than in dry sand because bacteria need water to live.

With the guidance of the instructor, students should create a sampling plan to test their hypotheses. One simple, but very useful, study design is to compare two groups of samples that should be different if the hypothesis is true. To use this design appropriately, students should make sure that (1) the parameter of interest is different between the two sample groups, (2) other parameters likely to affect the results are not different between the two sample groups, and (3) there are enough samples in each group. In general, it is a good idea to have at least 10 samples in each group.

It is important to include controls in the experiment. A negative control is a sample that will test negative for FIB unless there is a problem with contamination. Tap water or bottled water would be good negative controls. A positive control is a sample that will test positive unless there is a problem with the detection method. Untreated wastewater, a stool sample, or a known bacterial sample (available from www.idexx.com) would be good positive controls; however, due to the hazards associated with handling these materials, this activity may be conducted using only a negative control.

PROCEDURES

Sand and Water Collection

Wear gloves during sample collection to prevent contamination of samples. For sand, fill the sterile collection container by scooping sand directly into the container or using a clean spoon. For water, submerge the sterile collection container to fill it. Close the container and place on ice immediately. It is important to keep samples in a cold, dark location until analysis. Record the location, depth, distance from shore, date, and time of collection for each sample.

FIB Analysis

Conduct FIB analyses within six hours of sample collection. Gloves should be worn while performing FIB analyses. Using the balance, measure out approximately 10 g of sand into a sterile 100 mL container and record the exact mass. Add distilled (or purified) water to fill the container, close, and shake for

two minutes. Allow suspended particles to settle for at least 30 seconds, then pipette 10 mL of the water into each of two new, 90-mL bottles of Butterfield buffer or distilled water. If you are testing a water sample, simply pipette 10 mL of the sample into the buffer (or distilled water) bottle. Label each bottle with the location, depth, date, and time of collection and with either an “E” for Enterolert or a “C” for Colilert. Add one packet of Enterolert reagent to each “E” bottle and one packet of Colilert reagent to each “C” bottle. Close and shake the bottles to dissolve the reagents. Label Quanti-Trays with the same information as the buffer bottles. Carefully pour the entire contents of each buffer bottle into the corresponding Quanti-Tray and seal the tray using either a Quanti-Tray sealer, or a clothes iron set to medium heat.

Incubate Enterolert trays at 41°C (106°F) for 24–28 hours and Colilert trays at 35°C (95°F) for 18–22 hours. Remember to record the date and time when you begin and end each incubation. To read Colilert trays (Figure 1), first mark all wells that have a distinct yellow color. Record the number of large and small wells that turned yellow. Then, put the tray in a dark place, use the black light to identify the fluorescent wells, and mark these with a different symbol. Record the number of large and small wells that are both yellow and fluorescent (nonyellow, fluorescent wells are not counted). To read an Enterolert tray, use the black light to identify fluorescent wells. Use the table that comes with each Enterolert or Colilert kit (also available online at <http://www.idexx.com/water/quantitray/moreinfo.jsp>; abridged version in Figure 1) to convert well numbers to a concentration (MPN/100 mL). For water samples, this number is the final concentration. For sand samples, divide the water concentration by the mass of the original sand sample to get MPN/g of sand. Yellow Colilert wells represent total coliform, yellow and fluorescent Colilert wells represent *E. coli*, and fluorescent Enterolert wells represent *Enterococcus*.

Water Content Analysis

For each sample, tare a small, oven-safe container on the balance. Place approximately 10 mL of sand from the original collection container into the container, weigh the sample, and record this number as the original sample mass (M_o). Bake the sample in a 93°C (200°F) oven for one hour, then reweigh. Record the second number as the dry sample mass (M_d). Percent water content is calculated as $(M_o - M_d) / M_o$.

Grain-Size Analysis

For each sample, bake about one cup (250 mL) of sand at 200°F for one hour to dry it (you can do the baking step for water-content analysis at the same time, using two different containers). Use the nested sieves to divide the sample into different size fractions and record the mass of material in each sieve. To calculate the fraction of the original sample in each size category, divide the mass of material in that sieve by the sum of the masses in all sieves.

Data Analysis

This activity presents a wide range of possibilities for data analysis, depending on students' academic level. Data should first be organized into a table and grouped based on the hypotheses to be tested. For younger students, simply calculating the mean, median, and range of sample groups in order to compare them may be appropriate.

For more advanced students, this activity provides a nice segue into a discussion of the responsible use of statistics. Before using a statistical test to compare two sample groups, students should create histograms to investigate each group's distribution. This can be done by hand, or by using the histogram feature in Microsoft Excel's data analysis tools. The decision tree in Figure 2 can then be used to determine what statistical test to use and how to transform the data. The formulae used for common statistical tests are provided in Appendix 2; they can also be found in an introductory statistics text or online. Students may also be interested in looking at how one parameter they measured is related to another, for example, how sand water content is related to *E. coli* concentration. The most common way of doing this is to use simple linear regression. Before performing the linear regression, it is important to make sure that both the dependent and the independent variable have an approximately normal (i.e., unimodal and symmetrical) distribution (Figure 2). If they do not, a nonparametric method (Appendix 2) should be used.

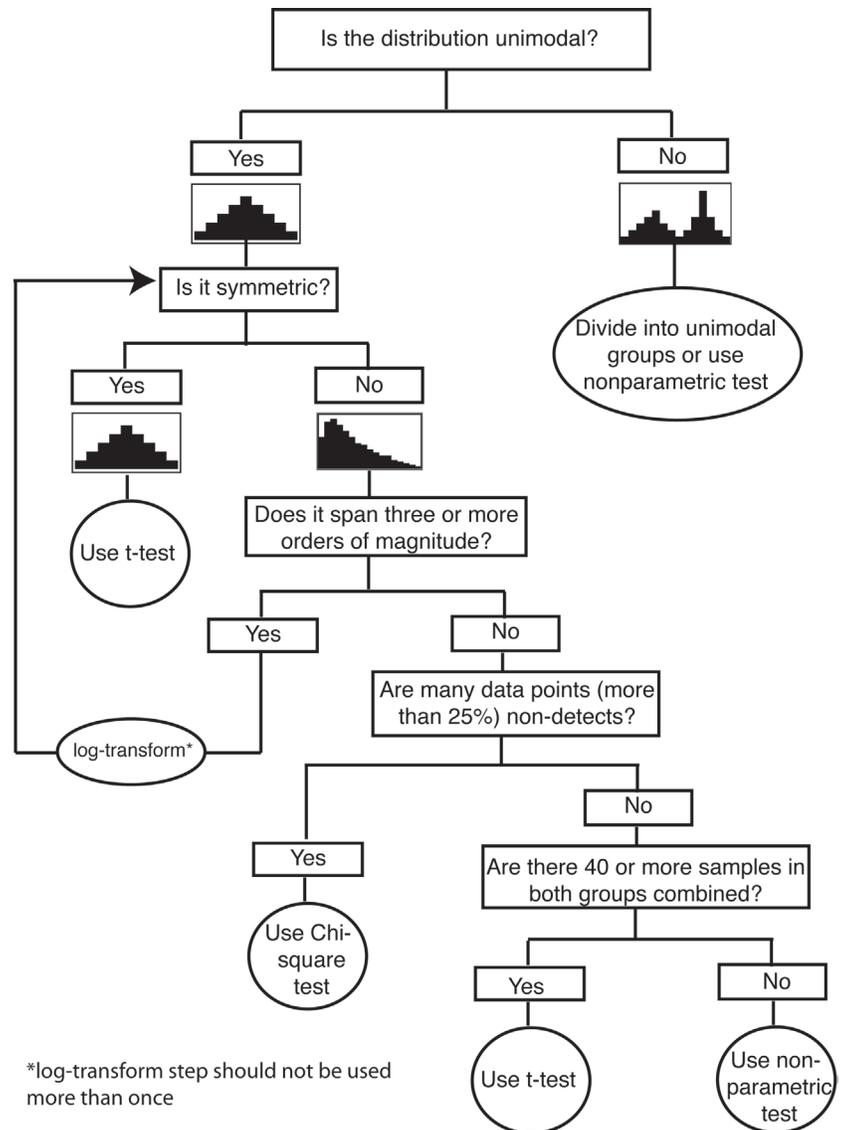


Figure 2. Decision tree for choosing an appropriate statistical test to compare two groups of samples. When the distributions of the two groups differ from each other, choose the path that requires the fewest assumptions. For example, if one distribution is symmetrical and one is not, choose "not symmetrical." Adapted from Moore and McCabe (2003)

Microsoft Excel or another statistics program can be used to create a scatter plot of the data and perform the linear regression, and the t-test for linear correlations (Appendix 2) can be used to determine whether the relationship is statistically significant. Finally, it is a good idea to make a plot of the residuals (the amount by which each measured y-coordinate deviates from the value predicted by the linear regression). The residuals should be scattered randomly about an average value of zero, and should not be related to (1) the order in which the samples were collected or (2) the magnitude of the measurements.

DISCUSSING RESULTS AND DRAWING CONCLUSIONS

Good scientific research presents not only the results, but also a discussion of how the results relate to each other and to other information available about the system being studied. Some questions to start the discussion are:

- Do the results support the hypotheses?
- If not, did you learn something unexpected about the system?
- Of the factors you measured, which (if any) is most related to the prevalence of FIB? Why do you think this is?
- Do the two FIB measured in this study behave similarly? What does this say about their use as fecal indicators?
- Does this study support the idea that sand is a source of FIB to beach water? Why or why not?
- Based upon your results, do you think FIB fit the Environmental Protection Agency's definition of an ideal indicator? Why or why not? Can you think of an indicator that might work better?
- Did this activity change how you feel about your local beaches? How?

GENERAL COMMENTS

This activity was designed by Adina Paytan and conducted by Ella Madsen and Rose Leopold during winter 2006–2007, with a follow-up project in 2008. Madsen and Leopold placed first in the environmental category of the Santa Cruz County Science Fair at the middle school level and received an honorable mention at the California State Science Fair. Before beginning this activity, check with local authorities about proper disposal of Quanti-Trays containing bacterial cultures, which are considered bacteriological biohazard materials (www.idexx.com).

POSSIBLE MODIFICATIONS

This activity can be expanded, simplified, or modified to accommodate students' interests, academic levels, local environments, and time constraints. Depending on the questions to be addressed, samples could be collected in as little as an hour during a class field trip and processed within one day. The activity can be conducted once, and then the results can be used to direct future research projects. For example, the year after completing this activity, Madsen and Leopold did a follow-up project in which they tested water samples for FIB, then ran the water through sand cores from the beach to see if the sand added or removed FIB from the water.

Before beginning sample collection, students or instructors should plan out how long each analysis will take. For example, bacterial incubations should be read during a four-hour window, so it is important to make sure in advance that someone is available to read the trays when they are done. To save time and money, students can use either the Enterolert or the Colilert-18 kit, instead of both. Some suggested variations include:

- Collecting replicate samples to assess the precision of the sampling method.
- Conducting a time series to see how FIB concentrations change over time, perhaps in response to factors such as tides, daylight, and rainfall.
- Comparing two locations with different levels of human impact (for example, a heavily used beach vs. a relatively isolated one).
- Investigating whether FIB concentrations in water and sand from the same location are related.
- Measuring FIB concentrations in soil and water samples collected from locations on campus or near students' homes.
- Investigating the relationship between FIB and other parameters, such as organic content of sand or nutrient concentrations in water, if the facilities to measure these parameters are readily available.

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REFERENCES

- Boehm, A.B., and S.B. Weisberg. 2005. Tidal forcing of enterococci at marine recreational beaches at fortnightly and semidiurnal frequencies. *Environmental Science and Technology* 39:5,575–5,583.
- Cabelli, V.J., A.P. Dufour, L.J. McCabe, and M.A. Levin. 1982. Swimming-associated gastroenteritis and water quality. *American Journal of Epidemiology* 115(4):606–616.
- Colford, J.M., T.J. Wade, K.C. Schiff, C.C. Wright, J.F. Griffith, S.K. Sandhu, S. Burns, M. Sobsey, G. Lovelace, and S.B. Weisberg. 2007. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology* 18(1):27–35.
- Corbett, S.J., G.L. Rubin, G.K. Curry, and D.G. Kleinbaum. 1993. The health effects of swimming at Sydney beaches: The Sydney Beach Users Study Advisory Group. *American Journal of Public Health* 83(12):1,701–1,706.
- Ferguson, D.M., D.F. Moore, M.A. Getrich, and M.H. Zhowandi. 2005. Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California. *Journal of Applied Microbiology* 99(3):598–608.
- Haile, R.W., J.S. Witte, M. Gold, R. Cressey, C. McGee, R.C. Millikan, A. Glasser, N.

- Harawa, C. Ervin, P. Harmon, and others. 1999. The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology* 10(4):355–363.
- Lee, C.M., T.Y. Yin, C.C. Lin, G.A. Kohbodi, A. Bhatt, R. Lee, and J.A. Jay. 2006. Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments. *Water Research* 40:2,593–2,602.
- Lipp, E.K., S.A. Farrah, and J.B. Rose. 2001. Assessment and impact of microbial fecal pollution and human enteric pathogens in a coastal community. *Marine Pollution Bulletin* 42(4):286–293.
- McQuaig, S.M., T.M. Scott, V.J. Harwood, S.R. Farrah, and J.O. Lukasik. 2006. Detection of human-derived fecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. *Applied and Environmental Microbiology* 72(12):7,567–7,574.
- Metcalf, T.G., J.L. Melnick, and M.K. Estes. 1995. Environmental virology: From detection of virus in sewage and water by isolation to identification by molecular biology—A trip of over 50 years. *Annual Review of Microbiology* 49:461–487.
- Moore, D.S., and G.P. McCabe. 2003. *Introduction to the Practice of Statistics*, 4th ed. W.H. Freeman and Company, New York, NY, 828 pp.
- Noble, R.T. and J.A. Fuhrman. 2001. Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: Low correlation to bacterial indicator levels. *Hydrobiologia* 460:175–184.
- Oshiro, R. and R. Fujioka. 1995. Sand, soil and pigeon droppings: Sources of indicator bacteria in the waters of Hanauma Bay, Hawaii. *Water Science and Technology* 31:251–254.
- Robertson, L.J., P.G. Smith, A.T. Grimason, and H.V. Smith. 1999. Removal and destruction of intestinal parasitic protozoans by sewage treatment processes. *International Journal of Environmental Health Research* 9:85–96.
- Rose, B., R.M. Atlas, C.P. Gerba, M.R. Gilchrist, M.W. LeChevallier, M.D. Sobsey, M.V. Yates, G.H. Cassell, and J.M. Tiedje. 1999. *Microbial Pollutants in Our Nation's Water: Environmental and Public Health Issues*. American Society of Microbiology, Washington, D.C., 16 pp.
- Schulz, H.N., and B.B. Jørgensen. 2001. Big bacteria. *Annual Review of Microbiology* 55:105–137.
- Seyfried, P.L., R.S. Tobin, N.E. Brown, and P.F. Ness. 1985. A prospective study of swimming-related illness. *American Journal of Public Health* 75(9):1,068–1,070.
- Thompson, S.S., J.L. Jackson, M. Suva-Castillo, W.A. Yanko, Z. El Jack, J. Kuo, C.L. Chen, F.P. Williams, and D.P. Schurr. 2003. Detection of infectious human adenoviruses in tertiary-treated and ultraviolet-disinfected wastewater. *Water Environment Research* 75(2):163–170.
- United States Environmental Protection Agency. 2006. Implementing the BEACH Act of 2000: Report to Congress. EPA-823-R-06-001, 111 pp.
- Van Heerden, J., M.M. Ehlers, W.B. Van Zyl, and W.O.K. Grabow. 2003. Incidence of adenoviruses in raw and treated water. *Water Research* 37(15):3,704–3,708.
- Whitman, W.B., D.C. Coleman, and W.J. Wiebe. 1998. Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences of the United States of America* 95:6,578–6,583.
- Yamahara, K.M., B.A. Layton, A.E. Santoro, and A.B. Boehm. 2007. Beach sands along the California coast are diffuse sources of fecal bacteria to coastal waters. *Environmental Science and Technology* 41:4,515–4,521.

ADDITIONAL ONLINE RESOURCES

This list is only a small sampling of the vast amount of information that is available online about fecal indicator bacteria and their use for water quality monitoring.

- The California Coastal Commission's Water Quality Program WebWeb site (<http://www.coastal.ca.gov/web/nps/npsndx.html>) tells what the state of California is doing to help preserve water quality.
- The Clean Beaches Council (<http://www.cleanbeaches.org>) and the Surfriider Foundation (<http://surfriider.org>) have information on water quality at beaches across the United States.
- IDEXX Laboratories, Inc. (www.idexx.com) offers information about monitoring fecal indicator bacteria using the company's products, including the Enterolert and Colilert-18 kits.
- The US Geological Survey (www.usgs.gov) collects a wealth of information about water quality nationwide.
- Wikipedia's pages on fecal coliforms, including *E. coli*, (http://en.wikipedia.org/wiki/Fecal_coliforms) and *Enterococcus* (<http://en.wikipedia.org/wiki/Enterococcus>) provide a basic overview of these organisms.

APPENDIX 1. BUILDING A SIMPLE INCUBATOR

You can build a simple incubator for your FIB samples using a Styrofoam cooler, a 15-watt light bulb, an electrical cord with a socket (the kind you would use to hang a lightbulb from the ceiling), and duct tape. First, make sure the cooler is large enough to hold both the Quanti-Trays and the lightbulb, without the lightbulb touching the trays. Make six 2.5-cm-diameter holes in the top of the cooler and one in one of the sides. The holes at the top can be covered with duct tape to keep heat in, or uncovered to let heat out. Put the lightbulb in the cooler, screw it into the socket, and run the socket's cord out through the side hole. Cut a small slit in the top of the cooler, just large enough to insert a thermometer (make sure the thermometer is accurate at temperatures from 30–50°C [86–122°F]). Turn the lightbulb

on and measure the temperature every 10 minutes, until it stops changing. The temperature inside the incubator should be 35°C (95°F) for Colilert and 41°C (106°F) for Enterolert. If the temperature is too high, open more holes at the top; if it is too low, close more holes. Make sure that you can keep the incubator within one or two degrees of the target temperature before you begin to collect samples. After putting the samples in the incubator, check the temperature every 30–60 minutes for the first hour or two and make adjustments as necessary. Depending on the number of samples you are planning to run, and whether you are planning to run both Colilert and Enterolert samples, you may need to build more than one incubator.

APPENDIX 2. FORMULAE FOR COMMON STATISTICAL TESTS

Adapted from Moore and McCabe, 2003.

1. Two-sample *t* significance test

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where \bar{x}_1 and \bar{x}_2 are the means of the two groups, s_1^2 and s_2^2 are the variances of the two groups, and n_1 and n_2 are the number of measurements in each group, respectively. To calculate the degrees of freedom, choose the lower value between n_1 and n_2 and subtract 1. A *t*-distribution table (found in most statistics textbooks) or an online *p*-value calculator can then be used to determine the *p*-value; $p < 0.05$ is a common criterion for significance in scientific work.

2. Chi-square statistic

The Chi-square statistic tests whether the incidence of a certain event (for example, detection of *E. coli*) is spread randomly over different groups. In this test, we classify samples as either positive (*E. coli* was detected) or negative (*E. coli* was below the detection limit) rather than using actual concentration.

For example:

	Wet Sand	Dry Sand	Row Total
Detected	15	8	23
Not Detected	5	24	29
Column Total	20	32	52

We calculate the expected count for each cell, if detects and nondetects were distributed randomly:

$$\text{expected count} = (\text{row total} \times \text{column total}) / (\text{table total})$$

Next, we calculate the contribution to the Chi-square value for each cell:

$$[(\text{observed count} - \text{expected count})^2] / \text{expected count}$$

And, finally, we sum the individual contributions to calculate Chi-square. There are $(r - 1) \times (c - 1)$ degrees of freedom, where r is the number of rows and c is the number of columns. A Chi-square table can be used to find the *p*-value.

3. Wilcoxon rank-sum test: A nonparametric way to compare two groups

First, rank all the samples without regard for which group they are in. Then, sum the ranks for each group. For each group, calculate the *U* statistic:

$$U_i = R_i - [n_i(n_i + 1)] / 2$$

where i is the group number (either 1 or 2), R_i is the sum of ranks for group i , and n_i is the number of measurements in group i . Select the smaller of the two *U* statistics and use a table to find the *p*-value.

4. Testing the significance of a linear correlation

The significance of a linear correlation can be tested by computing a *t* statistic:

$$t = \frac{r \sqrt{n - 2}}{\sqrt{1 - r^2}}$$

where r is the correlation between the two variables (or, the square root of the r^2 statistic from simple linear regression) and n is the number of measurements. The *p*-value can be found using the same table as in the two-sample *t*-test.