THE Volunteer Monitor

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volunteers conduct Bacteria Methods Comparison study

by Eric O'Brien

An interesting fact came to light at a 2002 strategic planning meeting for the Great Lakes region: out of the six states attending (Iowa, Indiana, Michigan, Minnesota, Ohio, and Wisconsin), only two had volunteer monitoring programs that included testing for bacteria. These were Iowa's IOWATER program, run by Iowa Department of Natural Resources (DNR), and Indiana's Hoosier Riverwatch, sponsored by Indiana DNR.

This discovery was the beginning of what would become the Citizens Monitoring Bacteria Project, a multiyear, multistate undertaking.

Soon after the meeting, representatives from Iowa DNR, Indiana DNR, Purdue University, Michigan State University, the University of Minnesota, the Ohio State University, and the University of Wisconsin formed a workgroup to encourage more bacteria monitoring by volunteer programs in the region. We decided that our first step should be to conduct a study to compare several different bacteria testing methods. Recognizing the potential value of our efforts, not only in our region but around the country, we applied for and received a grant from USDA Cooperative State Research, Education, and Extension Service (CSREES).

Iowa and Indiana took the lead in designing and carrying out the first year of the study while researchers in Wisconsin worked on creating survey questionnaires to determine the volunteers' opinions of the different methods. Michigan, Minnesota, and Ohio were charged with developing training and outreach materials.

We began the comparison study in 2004, expecting that at the end of a year we would have a clearcut "winner"—but it didn't quite work out that way, as we shall see.

"Real world" conditions

It's important to emphasize that our project was not a pure method-comparison study in which other variables besides the methods themselves are strictly controlled. To the contrary, we intentionally kept the "messiness" in. Our goal was to compare the performance of the different methods in the hands of actual volunteer monitors, sampling at their own monitoring sites and performing the analyses in their own homes. The volunteers' opinions and perceptions were also taken into account in evaluating the different methods.

Choosing methods for the study

All the methods we studied were for enumerating the indicator *E. coli*, which is, or soon will be, the indicator of choice for all the states in our region for ambient freshwater monitoring. In selecting the methods, we kept in mind the different needs and resources of different volunteer monitoring programs. Pro-

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Indiana volunteers at training workshop review protocols for 3M Petrifilm, Coliscan Easygel, and Coliscan MF.

Bacteria Monitoring

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THE Volunteer Monitor

The Volunteer Monitor is a national newsletter, published twice yearly, that facilitates the exchange of ideas, monitoring methods, and practical advice among volunteer monitoring groups.

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Next Issue

The theme for the next issue of *The Volunteer Monitor* is observational monitoring. This includes stream walks, habitat assessments, wildlife inventories, erosion surveys, visual monitoring for invasive species, and many other activities. Articles could focus on any aspect of observational monitoring, from survey design and volunteer training to data management and interpretation to actions that have resulted from the monitoring. Please send suggestions for article topics to the editor (contact information at left).

National Monitoring Conference

San Jose, May 7-11, 2006

Volunteer monitoring representatives will find plenty to keep them busy at the National Water Quality Monitoring Council (NWQMC) conference coming up this May 7-11 in San Jose, California. There will be formal and informal meetings and gatherings specifically for discussing volunteer monitoring-related issues, and volunteer monitoring colleagues will be describing their work and sharing their experiences in conference sessions such as:

- Monitoring strategies: Study design for volunteers
- Agency-volunteer partnerships
- Training volunteer monitors
- "Raising the Bar": Quality assurance for volunteer data
- Volunteer monitoring databases
- Data interpretation
- Taking action

Many conference sessions not specifically tailored to volunteer monitoring will also be of great interest—for example, sessions on turbidity testing, macroinvertebrate monitoring, rapid bacteria testing methods, watershed assessments, evaluating the effects of urbanization on water quality, stormwater monitoring, and numerous others. Workshops of particular relevance to volunteer monitoring organizations include "Data to Action," "Words and Water Quality," and "Wetlands Bioassessment."

Please visit the conference website, www.nwqmc.org, to view the entire agenda including field trips, find out about conference logistics, and register online. (On-site registration will also be available.) The website also features "bulletin boards" to facilitate room sharing and ride sharing.

River Rally

New Hampshire, May 5-9, 2006

River Network's National River Rally will be held May 5-9 at the historic Mount Washington Hotel in New Hampshire's White Mountains. The event brings together hundreds of river conservationists, keepers, monitors, and others involved in watershed protection and restoration.

River Rally helps grassroots groups harness the power of citizen involvement to protect rivers. Sessions will focus on such topics as assessing watershed health, links between human health and water pollution, using the Clean Water Act, restoration methods, and fundraising. River Rally is primarily attended by River Network Partners, but everyone is welcome. For more information visit www.rivernetwork.org/rally.

In Memoriam We are very sad to report the recent passing of *The Volunteer Monitor*'s Distribution Manager, Susan Vigil. Many newsletter subscribers experienced firsthand Susan's willingness to go the extra mile in responding to special requests, often at the last minute. She will be very much missed.

grams like IOWATER and Hoosier Riverwatch, in which volunteers conduct testing in their homes, require simple, low-tech methods, while programs in which samples are analyzed at a central location (such as the program office or a high school lab) are in a better position to purchase equipment and carry out more sophisticated tests.

On the low-tech end, we selected three methods: Coliscan Easygel incubated at 35˚C, Coliscan Easygel incubated at room temperature, and the 3M Petrifilm *E. coli*/Coliform Count Plate. The two Easygel methods were already being used by volunteers in our region, with IOWATER using the 35˚C incubation temperature and Hoosier Riverwatch incubating at room temperature.

Easygel was the only method tested that was incubated at a temperature other than 35˚C. Information from Micrology Laboratories, the maker of Coliscan Easygel, states that either incubation temperature can be used but adds that 35˚C incubation has several advantages, including quicker results, less batch-to-batch variation, and better inhibition of non-coliform bacteria.

Both Easygel and Petrifilm are pour-plate methods in which the water sample is added directly (i.e., without filtration) to the medium. After incubation, *E. coli* colonies are recognized by their distinctive appearance—dark blue to purple color on Coliscan Easygel, and a combination of blue color plus gas bubbles on 3M Petrifilm.

The more technically demanding methods we selected were Coliscan-MF, also from Micrology Laboratories, and IDEXX Laboratories' Colisure with Quanti-Tray/2000. After the first year of the study we added IDEXX Colilert with Quanti-Tray/2000.

Coliscan-MF is a membrane filtration method in which the desired volume of sample is drawn through a 45-micrometer membrane filter, which is placed in a petri dish on top of an absorbent pad soaked with Coliscan medium. Colonies show the same color reactions as on Coliscan Easygel.

IDEXX Quanti-Tray methods are multiple-well methods based on the classic multiple tube fermentation approach. The water sample is mixed with dehydrated medium and poured into the Quanti-Tray, which is passed through a special sealer. Colisure and Colilert are different media formulations that give different color reactions indicating *E. coli* growth: red or magenta with Colisure and yellow with Colilert.

All the methods we chose except 3M Petrifilm had an established history of use by volunteer monitoring programs in the U.S. The Petrifilm method was developed for testing food and dairy products and had not, to our knowledge, been used before in the volunteer monitoring world. However, we deemed it to be very simple, and we knew of some research projects that had used it for testing ambient water.

[*Note:* For more detailed descriptions of all the methods, please see the article on page 8.]

Study design

Seven experienced volunteers from the IOWATER program and five from Hoosier Riverwatch were recruited for the study and carefully trained in sampling and analysis protocols. All the volunteers tested all three simple methods (Easygel-35˚C, Easygel-room temperature, and Petrifilm). To reduce costs for supplies and equipment, and save time for the volunteers, the Coliscan MF method was tested only in Indiana and the IDEXX methods only in Iowa.

Volunteers selected their own sampling sites, often at locations where bacteria data were of particular interest to the local watershed association. Each volunteer sampled between two and five sites, approximately 10 times per year.

Samples were collected in sterile 500-milliliter (ml) bottles. A 100-ml aliquot was removed and shipped overnight, on ice, to the University of Iowa Hygienic Laboratory for analysis by EPA method 1603 (membrane filtration with modified mTEC medium). Volunteers brought the remainder of the sample to their homes for analysis by the different study methods. For all methods except Petrifilm (which requires exactly 1 ml of sample water), volunteers adjusted the sample volume based on the expected density of bacteria.

For 35˚C incubation, the Indiana volunteers used Hovabator chick incubators (about $45), which are recommended by Micrology Laboratories. Because the Iowa volunteers were testing the IDEXX methods, they used incubators purchased through IDEXX (about $500; but comparable products are available for less).

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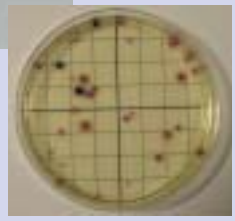
The Simple Methods

Coliscan Easygel

Water sample mixed with liquid Coliscan medium is poured into the Coliscan plate, which is coated with ingredients that cause the mixture to gel.



To make colony counting easier, the volunteers placed Easygel plates on a paper marked with a grid pattern.

3M Petrifilm

The sample is added directly to dehydrated medium on the film. The top layer of film traps gas bubbles produced by coliform bacteria (including *E. coli*).

Volunteers read results at both 24 and 48 hours, except for Colilert which was read only at 24 hours. This protocol covered all the manufacturers' recommended incubation times, and allowed us to see if there were major differences between the suggested incubation time and alternative times.

Results after one year

At the end of a year we compared the volunteers' results with the reference laboratory results, using linear regression analysis. The two methods showing the best agreement with the laboratory results were IDEXX Colisure/Quanti-Tray and Coliscan Easygel incubated at 35˚C. Petrifilm results showed good agreement except for high counts, which were underestimated by the Petrifilm method. The problem was greatest for counts over 5,000 *E. coli*/100 ml.

The volunteers' results were not as good with Easygel-room temperature and Coliscan MF. We felt that the relatively poor results with Coliscan MF were partly due to volunteers' initial technical difficulties in performing the membrane filtration procedure.

The volunteer preferences mirrored the accuracy results fairly closely. Volunteers had generally positive things to say about Easygel-35˚C, Petrifilm, and Colisure, but were less enthusiastic about the Easygel-room temperature and Coliscan MF methods.

Year 2

So here we were at the end of a year with three good candidates. We decided to continue the comparison study in Iowa and Indiana for another year before making final recommendations. At the same time, volunteers in the other four states (Michigan, Ohio, Minnesota, and Wisconsin) began testing water samples with 3M Petrifilm and Coliscan Easygel-35˚C, sending split samples to certified labs to collect more data on the reliability of these methods. We chose these two methods for use in the other states for a combination of reasons: the first-year results indicated they were reliable, the volunteers liked them, and they were less expensive than the Colisure method.

For the second year of the study (2005), the Iowa volunteers added the IDEXX Colilert/Quanti-Tray 2000 to their suite of methods. The big advantage of Colilert is that it, unlike the other methods in our study, is approved by the Environmental Protection Agency (EPA) for testing ambient waters. (The "ambient" category includes recreational waters.) We had initially chosen Colisure over Colilert because at the time we expected IDEXX to obtain EPA approval for both methods, and we thought that with turbid or muddy samples Colisure's red color reaction would be easier to see than Colilert's yellow reaction.

The graphs on the next page show the 2005 Indiana and Iowa results for all six volunteer methods compared to the laboratory reference method. In constructing the graphs we used data from the incubation time that gave the best results, which was 24 hours for all the methods except Easygel-room temperature. The Petrifilm and Coliscan-35˚C results from the other four states were quite similar (data not shown).

We also analyzed the 2005 results by looking at how well each method matched the reference method in determining exceedance of a regulatory cutoff value, EPA's recommended single-sample maximum of 235 *E. coli*/100 ml for primary-contact recreation (see table at right). This is a "yes-no" type of analysis—that is, if a given method came to the same conclusion as the reference method, this was counted as agreement, regardless of how close the actual counts were. This approach allowed us to incorporate some results that had been excluded from the linear regression analysis because of differences in detection levels (i.e., we could not directly compare non-detects from some of the volunteer methods with numerical counts obtained with the reference method). For this reason, the "N" values in the table are somewhat higher than those in the graphs.

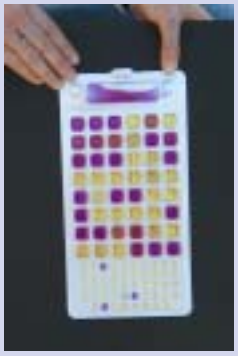
Both 3M Petrifilm and Coliscan MF showed improved results in 2005 compared to 2004. For the Petrifilm, this

The More Sophisticated Methods

Coliscan MF

The water sample is filtered, then the filter is placed on Coliscan medium.

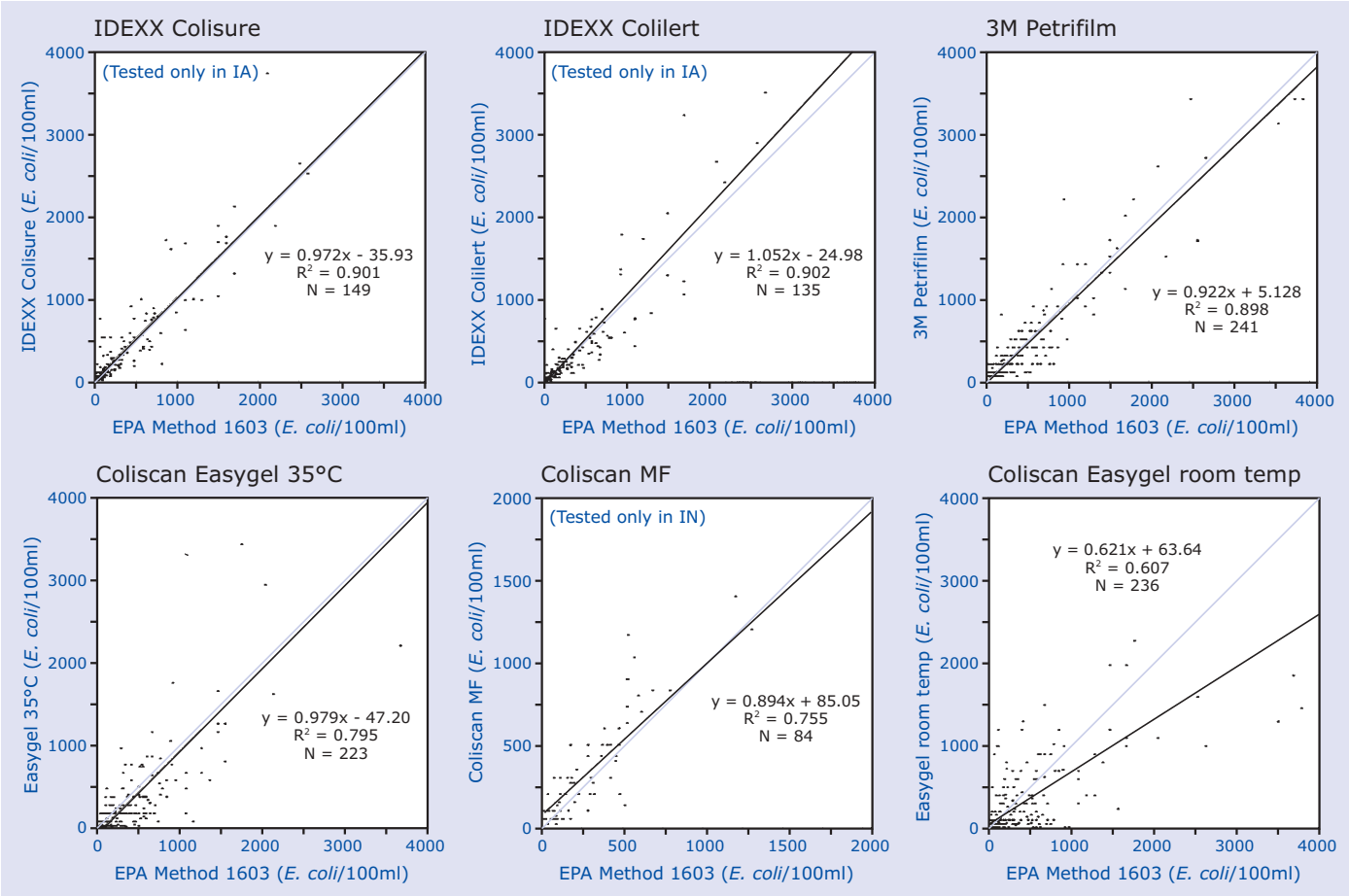
IDEXX Colisure and Colilert



A special sealer must be used to seal off the individual wells in the IDEXX Quanti-Tray.

IDEXX Colisure. Wells with a red or magenta color plus fluorescence are positive for *E. coli.*

Volunteer results for six methods plotted against University of Iowa Hygienic Laboratory results using EPA Method 1603, modified mTEC (2005 data). The light blue line with slope 1.0 (y = x) is provided for comparison.



improvement may have been partly due to the fact that in the second year fewer samples had high bacteria counts. In the case of Coliscan MF, the volunteers probably became more adept with the filtration process.

As in the first year, Easygel-room temperature came in last. This method performed poorly with regard to both precision (i.e., a lot of scatter is seen on the graphs) and accuracy (counts were underestimated compared to the reference method).

Final decisions

In the end we decided that four of the methods could work very well for volunteers: IDEXX Colilert Quanti-Tray, IDEXX Colisure Quanti-Tray, Coliscan Easygel incubated at 35˚C, and 3M Petrifilm. We were pleased with how well the volunteers' results from all these methods compared with laboratory results. Although the Coliscan MF matched the reference laboratory results almost as closely as the four selected methods during the second year of the study, this method was the least popular with volunteers, who found it messy and time-consuming and felt that it offered many opportunities for error.

The volunteers found the four selected methods easy to use, and they expressed a high level of confidence in all of them. For all four methods, there were some volunteer complaints about difficulties in distinguishing color reactions and interpreting results, but there was no clear consensus on which method was easiest for volunteers to interpret.

The two IDEXX methods were the most accurate in the hands of our volunteers. These methods also have the lowest detection limit (1 *E. coli*/100 ml). In addition, Colilert is EPA-approved, making it a good choice for programs that want state agencies to accept and use their data. The major obstacle for volunteer programs is cost—especially the cost of the Quanti-Tray sealer ($4,000). The per-sample cost is also higher than for the other two methods, although this is offset by the fact that Easygel and Petrifilm are often run in triplicate to

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Percent agreement on criterion exceedance (2005 results)

Method	Number of results (N)	Agreement (%)
IDEXX Colisure	171	88.3
3M Petrifilm	268	85.4
IDEXX Colilert	161	84.5
Coliscan Easygel-35˚C	245	80.0
Coliscan MF	94	79.8
Coliscan Easygel-room temp	261	69.3

Agreement of study methods with reference method for exceedance of criterion of 235 *E.* coli/100 ml (Indiana and Iowa data).

improve sensitivity and accuracy and check precision. The IDEXX methods are most feasible in areas with high volunteer density, allowing many volunteers to share the equipment.

3M Petrifilm was the least expensive method and the volunteers found it the easiest to use. It also takes up the least space—a stack of 20 Petrifilm plates is about the size of a deck of cards. Probably the biggest disadvantage is the detection limit. Because only 1 ml of water sample is used, 100 bacteria/100 ml is the lowest density that can be detected. (By running the test in triplicate you can treat the results as a single 3-ml sample, thereby lowering the detection limit to 33/100 ml.) The other drawback is that 3M has done comprehensive validation studies of Petrifilm only for use in the food industry, not for water testing. However, we did find one published study (Vail et al., 2003) comparing Petrifilm to several other methods for testing *E. coli* in water.

Unlike the Petrifilm method, Coliscan Easygel was specifically developed for testing water, and it has a considerable track record with volunteer water monitoring programs (see for example the articles in this issue from Alabama Water Watch and Texas Watch). Costs are almost as low as for Petrifilm, and up to 5 ml of water sample can be used, resulting in a detection limit of 20/100 ml. One disadvantage is that Easygel takes 40 minutes to an hour to solidify, and you have to wait for the plates to solidify before inverting them and placing them in the incubator.

The individual situation of each volunteer group will probably be the determining factor in deciding which method they choose. In some cases, it may be advantageous to use a combination of methods—for example, Petrifilm or Coliscan Easygel for quick screening, and Colilert for situations requiring a widely accepted, EPA-approved method. In our region, most volunteer programs don't have the funding to use the IDEXX methods on a large scale, so we feel that the majority of volunteers in the Midwest will use either Coliscan Easygel incubated at 35˚C or 3M Petrifilm.

We are continuing to collect comparison data in 2006, with the Iowa and Indiana volunteers using all four of the selected methods and volunteers in the other four states using 3M Petrifilm and Coliscan Easygel-35˚C. Stay tuned to our project website, www.usawaterquality.org/volunteer/EColi/, for study updates, as well as copies of our training and outreach materials, which will be available later this year.

Comparison of the four selected methods

	Coliscan Easygel-35˚C	3M Petrifilm	IDEXX Colilert and Colisure
Supplies cost per test*	$1.85	$1.06	$5.05
Equipment requirements	Incubator	Incubator	Quanti-Tray sealer, UV light, incubator
Agreement with reference lab	Good	Good	Best
Lower detection limit	20/100 ml	100/100 ml	1/100 ml
EPA approved for ambient water	No	No	Colilert - Yes Colisure - No

*Prices based on quotes given to the IOWATER Program in Fall 2005.

Eric O'Brien is a Research Biologist with Iowa DNR's Iowa Geological Survey. He also assists the IOWATER Program with bacterial monitoring projects. He may be reached at eobrien@igsb.uiowa.edu; 319-353-2835.

Reference

Vail, J.H., et al. 2003. Enumeration of Waterborne *Escherichia coli* with Petrifilm Plates: Comparison to Standard Methods. *Journal of Environmental Quality* 32:368-373.

Connecticut Uses Volunteer Bacteria Data for TMDLs

The Connecticut Department of Environmental Protection (DEP) has used volunteer-collected data to calculate total maximum daily loads (TMDLs) for bacteria in several watersheds. DEP can use the volunteers' existing long-term monitoring data going back as far as five years, and volunteer groups have also assisted DEP by collecting samples at specific locations where the agency needs data for a TMDL.

In 2004, the Connecticut River Watch Program recruited extra volunteers for a sampling blitz to gather the remaining data that DEP needed for a bacteria TMDL for the Mattabassett River watershed. The volunteers collected over 200 samples from June through September, which were analyzed for *E. coli* at the state health department lab. According to Kelly Streich, an environmental analyst for DEP's Bureau of Water Management, most of the *E. coli* data used to develop the TMDL was collected by volunteers.

DEP has also used data collected by volunteers with the Harbor Watch/River Watch program in Westport for bacteria TMDLs. In this case, the volunteers perform both sample collection and analysis, testing for *E. coli* at the program's certified lab. Both volunteer groups whose data have been used for TMDLs have quality assurance project plans approved by DEP and EPA.

After TMDL plans are written, volunteers also help with implementation by looking for likely sources of contamination. According to Streich, the two biggest fecal contamination problems for municipalities working under municipal stormwater discharge (MS4) permits are usually illicit connections and stormwater runoff. Volunteers conduct "track-down surveys" to look for both types of problems. Stormwater pipes that are discharging during dry weather could indicate an illicit connection or broken sanitary sewer line. If there is excessive sediment in a stream, or the water looks very silty after a rain, it's likely the area is receiving high stormwater loads. The sediment carried in the stormwater may contain a lot of bacteria, especially if it is carrying fecal waste from pets or wildlife.

Evidence from the surveys helps remediate bacteria problems. Even in situations where not much can be done to reduce the amount of bacteria in stormwater, the amount of bacteria in streams can still be reduced by controlling stormwater and preventing it from reaching streams.

Volunteer vs. agency comparison: *E. coli* monitoring

by Steve Hanson

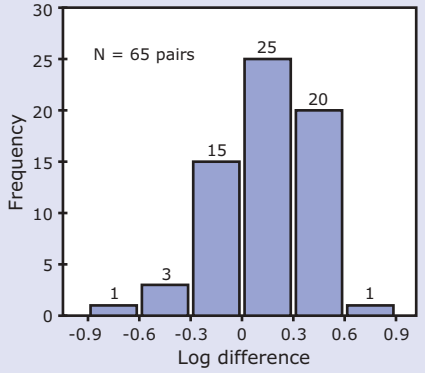
The Oregon Department of Environmental Quality Laboratory Division (ODEQ) Volunteer Monitoring Program supports community-based organizations in conducting various water monitoring activities. Our goal is to help improve the quantity and quality of data collected at the local level.

A number of the watershed groups we work with test water samples for *E. coli*, Oregon's fecal bacteria indicator organism for freshwater. Because volunteer bacteria results may be used for such purposes as 303(d) listing and watershed characterization for TMDL plans, it's important for ODEQ to feel confident about the data quality—especially since some private landowners and other stakeholders involved with the 303(d) and TMDL processes have expressed concern over the quality of volunteer-generated data. So in 2003 I performed an assessment to determine the comparability of volunteer-collected bacteria data to the data we at ODEQ collect.

I conducted my assessment by analyzing quality control data from 1997 through 2002. This data consisted of results from side-by-side samples collected simultaneously by a volunteer organization and an ODEQ staff member. At the time of my assessment, ODEQ had conducted 13 such side-by-side sampling events with nine different volunteer groups. The results had been used as a quality check for the individual groups, but prior to the work described here ODEQ had not done an analysis of data from multiple organizations.

The ODEQ samples from the side-by-side sampling were analyzed at the Oregon Health Department Laboratory using membrane filtration with mTEC (Standard Methods 9213D), except for the 2002 samples, which were analyzed with IDEXX Colilert Quanti-Tray/2000. Five of the volunteer groups sent their samples to outside laboratories, of which one used multiple tube fermentation and the rest used the Colilert Quanti-Tray method. The other four volunteer groups performed bacteria analysis themselves using Colilert Quanti-Tray. ODEQ loaned these groups the necessary equipment, including incubators, UV lights, and Quanti-Tray sealers. It has been ODEQ's policy to lend high-quality water monitoring equipment, such as meters for chemical parameters, to volunteer monitoring organizations, to help standardize data collection and improve data quality. Because we are able to purchase in quantity, the state often saves money compared to providing funding to individual organizations to purchase their own equipment.

Log differences, volunteer vs. ODEQ *E. coli* samples



My approach was to compare the variability observed in our agency's own internal duplicate bacteria samples to the variability observed in the volunteer-vs.-agency side-by-side samples. In other words, how closely could we at ODEQ match our own results, and how closely could the volunteers match the ODEQ results? ODEQ routinely collects duplicate field samples at a minimum of 10% of sampling sites. The duplicate samples are collected and analyzed by the same methods as the regular samples.

To compare duplicate bacteria samples, we first take the logarithm of the raw counts. For example, if the counts were 406/100 ml and 126/100 ml, we would take the log of the results to get 2.6 and 2.1, respectively. The log difference is 0.5. This log transformation is done to compensate for the nonnormal distribution of bacteria results. Bacteria are unevenly distributed in a water body, rather like chocolate chips in a cookie. When you "sample" a cookie you may have one bite with no chocolate chips, some bites with a few chips, and one or two bites with lots of chips. (Of course, you may want to repeat the sampling multiple times to convince yourself. I recommend starting with a nice large glass of milk.) Because of this uneven distribution, we expect to see larger differences between duplicate bacteria samples than we would expect for a parameter like nitrate or dissolved oxygen. Taking the logarithm of the values helps "normalize" the data.

For our agency's own duplicate samples, we find that 95% fall within 0.6 log units of each other. Applying the same analysis to the volunteer-vs.-agency side-by-side samples, I found that 97% fell within 0.6 log units of each other. Out of 65 pairs of results, only two pairs differed from each other by more than 0.6 log units (see histogram). The results from volunteer programs who did their own testing were of the same quality as those from volunteer programs that sent samples to outside labs for analysis. In short, that is pretty darn good. Volunteer data matched ODEQ's "professional grade" data as closely as our own duplicates. This close match is even more impressive considering that the side-by-side samples were analyzed in different labs, and in most cases by different methods. In contrast, the agency's internal duplicates are analyzed by the same method in the same lab.

The histogram is slightly skewed to the right because 60% of the time the volunteer result was higher than the ODEQ result. I have not had the chance to investigate the significance or cause of the skew. It may be related to differences in holding time—ODEQ was using a 30-hour maximum holding time (which was changed to 24 hours in 2004), whereas the volunteer organiza-

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Bacteria Methods for Recreational Waters

A Short History and Guide

by Eleanor Ely

Once upon a time, everybody used the fecal coliform indicator for testing recreational waters, whether fresh or marine. This indicator served as the basis for federal water quality criteria as far back as 1968—predating the Clean Water Act and the creation of the Environmental Protection Agency (EPA). In 1976 EPA continued the recommendation to use the fecal coliform indicator.

Things changed, or at least began to change, in 1986 when EPA published new recommendations for testing recreational waters (*Ambient Water Quality Criteria for Bacteria -1986*). The fecal coliform indicator was out; *E. coli* and enterococci were in. For freshwater, either enterococci or *E. coli* were recommended; for marine water, only enterococci. These recommendations were based on EPA epidemiological surveys showing that *E. coli* and enterococci correlated strongly with swimming-associated illness, whereas fecal coliforms showed no correlation.

EPA's recommended criteria for *E. coli* and enterococci are shown in the box at right. (Note that individual states set their own criteria, which are not necessarily the recommended levels.) The "single sample maximum" criterion is typically used for decisions about posting swimming advisories or closing beaches. The geometric mean, which combines results from two or more samples collected over a period of time, is more appropriate for such purposes as assessing whether waters are meeting designated uses, placing waters on a state's impaired water body list (303(d) list), writing TMDL plans, or setting discharge limits for permitted dischargers. (For an excellent discussion about calculating geometric means, see www.buzzardsbay.org/geomean.htm.)

State agencies were slow to adopt EPA's 1986 recommendations. Eight years ago, this newsletter (Fall 1998 issue) reported that most volunteer monitoring groups were still using methods based on the fecal coliform indicator, mainly because they wanted their data to be comparable to state agency data. But now the scales have finally tipped. Most states have adopted the new indicators or are gearing up to do so, and volunteer monitoring programs are following suit (in fact, quite a few volunteer programs began testing for *E. coli* or enterococci while their state agencies were still using the fecal coliform indicator).

Several factors encouraged the switch to the new indicators. The 1989 amendments to the Safe Drinking Water Act required that positive total coliform tests be followed up with testing for *E. coli*, providing an impetus for the development of simplified *E. coli* methods. Then in 2000 Congress passed the BEACH Act, an amendment to the Clean Water Act, which required all 30 coastal and

Bacterial Indicators

Total coliforms: A group of closely related genera of rod-shaped bacteria, some mainly of fecal origin and others that are widespread in the environment. Used as an indicator for testing drinking water.

Fecal coliforms: A subgroup of the total coliforms (mostly *E. coli* and *Klebsiella*) that can grow at 44.5°C. More fecal-specific than the total coliforms, but some *Klebsiella* can be of non-fecal origin. No longer EPA-recommended as an indicator for ambient waters but still used to determine shellfish bed closures.

***Escherichia coli (E. coli)* and enterococci:** The two current EPA-recommended indicators for ambient waters (which includes recreational waters). Either may be used for freshwater, while only the enterococci indicator is recommended for marine waters. *E. coli* is a single species within the fecal coliform group. The enterococci are not related to the coliforms. Enterococci are several related species of spherical bacteria commonly found in the intestines of warm-blooded animals.

Both *E. coli* and enterococci are more fecal-specific than the fecal coliform indicator, and therefore less likely to give a false positive result (that is, be present in water when fecal contamination is absent). Enterococci survive better than *E. coli* in salt water.

Do the indicators cause illness?

The indicator bacteria do not themselves cause swimming-associated illness. The most common swimming-associated illnesses are relatively mild infections such as gastroenteritis and respiratory infections that are caused by viruses. More serious, but less common, waterborne pathogenic (disease-causing) organisms include the hepatitis virus, bacteria such as *Salmonella* and *Shigella*, and protozoan parasites such as *Giardia* and *Cryptosporidium*.

(*Note:* Within a bacterial species there are numerous different strains. *E. coli* O157:H7 is a pathogenic strain that can cause illness, for example from eating undercooked hamburger. However, this is an atypical strain that actually is *not* detected by methods commonly used to test for the *E. coli* indicator.)

Why use indicators?

It isn't practical to test directly for the pathogens. Typically they are rare in a water sample compared to the indicator species. Also, there are many different pathogenic organisms, requiring different laboratory test procedures, and some of the pathogens are very difficult to detect. By determining the density of the indicators we can obtain an "indication" of the level of fecal contamination and the risk of illness.

EPA-Recommended Criteria

	Geometric mean	Single sample maximum
Freshwater:		
E. coli	126/100 ml	235/100 ml
enterococci	33/100 ml	61/100 ml
Marine waters:		
enterococci	35/100 ml	104/100 ml

The above criteria, originally recommended in 1986, still stand. EPA reaffirmed their continued usefulness in its 2002 document *Implementation Guidance for Ambient Water Quality Criteria for Bacteria (Draft).* The criteria shown here apply to heavily used bathing beaches. Less-strict standards may be applied in other situations—for example, waters used mainly for boating (U.S. EPA, 2002).

Values in the table are expressed as number of bacteria per 100 ml, which can be either CFU (colony-forming units) per 100 ml or MPN (most probable number) per 100 ml, depending on the analytical method used.

Great Lakes states as well as five U.S. territories to adopt EPA's recommended criteria (or criteria "equally protective of human health"). And in July 2003, EPA's official list of approved methods for detecting *E. coli* and enterococci in ambient waters was published in the *Federal Register*. This list included some simplified methods developed by private companies, which made it easier for states to test for these indicators.

[*Note:* Prior to July 2003, the question of whether a method was "EPA-approved" for testing ambient waters was something of a gray area. Although no such methods had been formally "promulgated"—i.e., published in the *Federal Register* as a final rule—EPA microbiologists have told me that certain EPA-developed methods (mTEC and modified mTEC for *E. coli*, and mE-EIA and mEI for enterococci) were considered EPA-approved, although they were not required. Now that a list of EPA-approved methods for ambient waters has been officially promulgated, states are required to use methods on the list for compliance monitoring.]

E. coli methods

Although *E. coli* and enterococci are both recommended for monitoring freshwater systems, almost all states have elected to use *E. coli*, in part because testing procedures are simpler and more choices are available. For a volunteer program coordinator setting out to choose an *E. coli* test method, the abundance of options can seem as much a curse as a blessing—and the fact that so many include "Coli" in their names just adds to the confusion. One tip: Those who may have been puzzling over the difference between Coliscan and ColiQuant can stop worrying, because they are the same. The Micrology Laboratories products Coliscan Easygel and Coliscan MF are also marketed by LaMotte Company, under the names ColiQuant EZ and ColiQuant MF, respectively.

The following discussion offers descriptions and guidance on some *E. coli* methods of particular interest to volunteer monitoring programs. (See also the front-page article for results of a comparison study of several of these methods.) All of them except mTEC and modified mTEC detect both total coliforms and *E. coli*. However, the total coliform count is mainly of interest for monitoring drinking water.

The various *E. coli* methods differ both in procedure (membrane filtration vs. pour plate vs. multiple-well) and in medium formulation. The media typically contain various ingredients that encourage the growth of the target organisms, inhibit unwanted types of bacteria, and cause target organisms to have a distinctive appearance.

With the exception of classic mTEC, all the methods we will discuss are based on detecting specific bacterial enzymes by incorporating synthetic enzyme substrates into the medium. The substrates are compounds that are cleaved by the target enzyme to produce either a colored product or a product that fluoresces under UV light. Substrates that produce a colored product are called chromogenic substrates, and those that produce a fluorescent product are called fluorogenic substrates.

When I began researching this article, my head was spinning at the large number of different enzyme-substrate reactions I was reading about. It was an "Aha!" moment when I realized that it all boiled down to detecting just two enzymes, one for *E. coli* (ß-D-glucuronidase) and one for the total coliforms (ß-D-galactosidase). What makes it seem so confusing is that the different media use a number of different substrates to detect these enzymes, sometimes in order to avoid patent infringement.

The table on the next page shows the specific substrates used in each medium as well as the observed reactions (color or fluorescence). Remember that since *E. coli* is a member of the total coliform group, it will give the same reaction as the other total coliforms on any substrate that is acted upon by the total coliform enzyme ß-galactosidase. It is the substrate for ß-glucuronidase that allows us to detect *E. coli*, since the enzyme ß-glucuronidase is specific (or at least fairly specific) to *E. coli*.

1. Membrane filtration methods

In membrane filtration, the desired volume of sample is drawn through a membrane filter. Bacteria in the sample are

continued on next page

After filtration, the membrane filter is removed and placed on an appropriate medium for the indicator organism.

Comparison of several *E. coli* testing methods

Method	Incubation Temp, °C	EPA-approved, ambient water	Substrate for ß-galactosidase* (total coliforms)	Substrate for ß-glucuronidase** (*E. coli*)	Observed reactions (TC = total coliforms; EC = *E. coli*)	Lower detection limit (bact/100 ml)	Comments
Membrane filtration methods:							
mTEC (EPA 1103.1)	35 then 44.5	Yes	None	None	EC no pink color on urea (i.e., colonies remain yellowish)	1	For all membrane filtration methods, lower detection limit = 1 per volume of sample filtered (typically up to 100 ml). A larger volume may be filtered for a lower detection limit.
modified mTEC (EPA 1603)	35 then 44.5	Yes	None	Magenta-Gluc	EC red/magenta	1	
MI (EPA 1604)	35	Yes	MUGal	IBDG	TC fluorescence EC fluorescence + blue	1	
m-ColiBlue24 (Hach Co.)	35	Yes	None (nonspecific dye stains TC red)	X-Gluc	TC red EC blue	1	
Coliscan MF (Micrology Labs)	35	No	Red Gal	X-Gluc	TC red/pink EC purple/blue	1	
Simple pour-plate methods:							
Coliscan Easygel (Micrology Labs)	35	No	Red Gal	X-Gluc	TC red/pink EC purple/blue	20	Min. sample volume 0.25 ml; max. 5 ml
3M Petrifilm *E. coli*/Coliform Count Plate	35	No	None (nonspecific dye stains TC red)	X-Gluc	TC red + gas EC blue + gas	100	Sample volume 1 ml (invariable)
Multiple-well methods:							
IDEXX Colilert Quanti-Tray	35	Yes	ONPG	MUG	TC yellow EC yellow + fluorescence	1	
IDEXX Colisure Quanti-Tray	35	No	CPRG	MUG	TC red EC red + fluorescence	1	

*Substrates for total coliform enzyme ß-D-galactosidase:
Chromogenic: Red Gal = 6-chloro-3-indolyl-ß-D-galactopyranoside; ONPG = o-nitrophenyl-ß-D-galactopyranoside; CPRG = chlorophenyl-red-ß-D-galactopyranoside. Fluorogenic: MUGal = 4-methylumbelliferyl-ß-D-galactopyranoside.

**Substrates for *E. coli* enzyme ß-D-glucuronidase:
Chromogenic: Magenta Gluc = 5-bromo-6-chloro-3-indolyl-ß-D-glucuronide; IBDG = indoxyl-ß-D-glucuronide; X-Gluc = 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide. Fluorogenic: MUG = 4-methylumbelliferyl-ß-D-glucuronide.

captured on the filter, which is then placed on a nutrient medium in a petri plate. The medium can be in solid form, or it can be a liquid medium soaked into an absorbent pad. The plates are incubated to allow the bacteria to grow into visible colonies that can be counted. Traditionally the count is reported in terms of colony-forming units (CFU) per 100 ml. Membrane filtration is a good technique for detecting low counts, because you can filter a large volume of sample—typically 100 ml, but you can use larger volumes if the water is not turbid.

Let's start with three *E. coli* membrane filtration methods developed by EPA microbiologists: mTEC (EPA Method 1103.1; Standard Methods 9213D), modified mTEC (EPA Method 1603), and MI (EPA Method 1604).

The mTEC method ("TEC" stands for "thermotolerant *Escherichia coli*") was developed first, and was the method used in the EPA epidemiological studies that led to the 1986 recommendation of the *E. coli* and enterococci criteria. Thus it has a certain venerability and cachet, and is often used as the "gold standard" in method-validation studies. However, it is relatively inconvenient because it requires (a) an initial 2-hour incubation at 35˚C followed by 22 hours' incubation at 44.5˚C and (b) transfer of the filter to urea medium as a final step to identify *E. coli* colonies. Moreover, the 44.5˚C temperature is very critical and must be maintained within 0.2˚C—any higher, and *E. coli* are inhibited; any lower, and unwanted bacteria can grow. So you need a high quality water bath or incubator that can hold a precise temperature.

The modified mTEC method is an improved version that eliminates the urea step. Instead, the medium utilizes an enzyme-substrate reaction to detect *E. coli*. However, modified mTEC still requires the two-step incubation process and the strictly maintained 44.5˚C temperature.

The MI method is the most convenient of the three EPA methods because it's much less demanding with regard to incubation temperature. Plates are incubated at 35˚C, and fluctuations within a degree or two are not critical. The medium incorporates a fluorogenic substrate for detecting total coliforms and a chromogenic substrate for detecting *E. coli*. Although the MI method has been little used by volunteer monitoring programs, it is included here because its relative simplicity makes it feasible for any vol-

unteer group that is willing to go to the effort of performing membrane filtration.

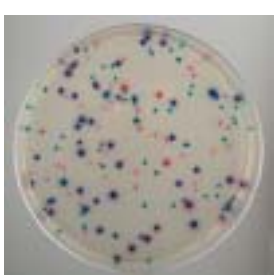
Hach Company's m-ColiBlue24 and Micrology Laboratories' Coliscan MF are membrane filtration methods that are incubated at 35˚C, utilize enzyme substrate reactions, and have been used by volunteer monitoring programs. The m-ColiBlue24 method is EPA-approved for ambient waters while Coliscan MF is not. The m-ColiBlue24 medium contains a chromogenic substrate for *E. coli* (blue color) and a nonspecific dye that stains total coliforms red. The Coliscan medium contains two chromogenic substrates, one for *E. coli*, which appear purple/blue, and one for total coliforms, which appear red/pink. (A brand-new Micrology Labs medium, "Coliscan Plus," contains two different substrates for *E. coli*, one fluorogenic and one chromogenic.)

2. Simple pour-plate methods

Pour-plate methods in which the water sample is added directly to the medium without a prior filtration step are cheap and convenient, and can be very useful for screening purposes. The biggest drawbacks are the lack of EPA approval and the inability to detect low counts (since there is no filtration step to concentrate the sample).

The Coliscan Easygel method has been widely used by volunteer monitoring programs. It uses the same chromogenic substrates as the Coliscan MF method. The water sample is added to a small bottle of liquid medium, which is then poured into a petri dish coated with ingredients that cause the medium to solidify. Anywhere from 0.25 to 5 ml of water sample can be used. The method is not recommended for very low counts because even with the largest sample volume the detection limit is 20 *E. coli*/100 ml.

Another pour-plate method, which until recently was virtually unknown among volunteer monitoring programs, is the 3M Petrifilm *E. coli*/Coliform Count Plate. This method was developed for use in the food industry and has not been marketed for water testing. However, 3M Petrifilm was one of the methods tested in a recent study by volunteer groups in the Midwest, who found the results very encouraging (see front-page article).



Colonies growing on Coliscan Easygel plate.

3M Petrifilm. The top film creates a seal.

The Petrifilm plate is not a traditional petri dish but rather a thin layer of culture medium in powder form that is sandwiched between two films. You lift the top film, place 1 ml of water sample on the circular area containing the medium, and then lower the film, creating a seal. Gas produced by coliforms from the fermentation of lactose in the medium is trapped under the seal, appearing as bubbles. The medium contains an enzyme substrate that gives *E. coli* colonies a blue color, and a nonspecific dye that stains total coliforms red.

3. IDEXX multiple-well methods

In all the methods discussed so far, you end up with a plate of colonies to count. Methods developed by IDEXX Laboratories take a different approach, based on the classic multiple tube fermentation method. In the classic method, you inoculate different dilutions of the water sample into a series of test tubes containing liquid medium. Instead of counting colonies, you count the number of tubes showing a positive reaction. This number is converted to a "most probable number" or MPN, which is an estimate of the mean density of target organism in the sample.

The IDEXX products simplify and streamline the classic method, which is very labor-intensive and also can take several days to yield results, since positive tubes must be confirmed with additional tests. IDEXX replaces the tubes with a single tray (Quanti-Tray) containing multiple wells. You mix the water sample with powdered medium, pour the mixture into the tray, and pass the tray through a sealer to seal off the individual wells. Any sample volume up to 100 ml may be used (sterile water is added as needed to adjust the total volume to 100 ml). The media contain enzyme substrates to identify total coliforms (positive wells show color) and *E. coli* (color plus fluorescence).

IDEXX currently manufactures two Quanti-Tray methods, Colilert and Colisure, for *E. coli* testing. Because Colilert is EPA-approved for ambient waters while Colisure is not, Colilert is much more widely used.

The required sealer for the Quanti-Trays costs about $4,000. While this might seem to put the IDEXX methods beyond the reach of volunteer monitoring groups, a surprising number do use either Colilert or Enterolert (a method for enterococci; see below). In some cases state agencies donate or lend a sealer to a volunteer program. At least one program, Orange County Coastkeeper, offsets the cost of the sealer by performing bacteria testing for smaller volunteer groups at a cost well below what private laboratories charge.

Enterococci methods

Currently, no quick screening method analogous to Coliscan Easygel or 3M Petrifilm is available for detecting and enumerating enterococci. The three most widely used methods are two EPA membrane filtration methods and IDEXX Enterolert Quanti-Tray. All three are EPA-approved for testing ambient water. They all require incubation at 41˚C, so if you are testing for both enterococci and *E. coli* you need a separate incubator for enterococci.

continued on next page

BACTERIA METHODS, continued

EPA's mE-EIA (EPA Method 1106.1; Standard Methods 9230C) is a membrane filtration method that requires two different media and takes 48 hours for results. mEI (EPA Method 1600) is an improved one-step, 24-hour version. The mEI medium contains the enzyme substrate indoxyl-ß-D-glucoside, which is cleaved by the enterococci enzyme ß-D-glucosidase to yield a blue color.

IDEXX Enterolert Quanti-Tray is a multiple-well method similar to the Colilert and Colisure Quanti-Tray methods discussed above. Like mEI, Enterolert detects the enterococci enzyme ß-D-glucosidase, but in this case the substrate is 4-methylumbelliferyl-ß-D-glucoside (MUD), which yields a fluorescent compound. Results are read after 24 hours.

Mixture of water sample and medium is poured into IDEXX Quanti-Tray.

Sealed Quanti-Tray, before incubation.

The lower detection limit for both EPA membrane filtration methods is 1/100 ml. Enterolert likewise has a lower detection limit of 1/100 ml when testing non-marine waters, but marine waters (estuarine or ocean) must be diluted tenfold to avoid the risk of false positives, thus raising the detection limit to 10/100 ml.

In a nutshell

So how does a volunteer monitoring program choose the best method for their needs? Obviously many factors enter into the decision, especially (a) where the tests will be performed (e.g., in volunteers' homes, in a high school lab, at the program office, at a university or agency lab) and (b) how the data will be used.

For in-home use, Coliscan Easygel and 3M Petrifilm are the most practical. Both can be used with simple homemade incubators or chick-egg incubators. However, these methods are suitable mainly for basic screening, and neither is EPA-approved for ambient water testing.

If you're looking for an EPA-approved method and/or you want to use the same method as a state agency, you'll need to choose one of the approved membrane filtration or IDEXX methods. Now you will be considering such questions as whether 44.5˚C incubation (required for mTEC and modified mTEC) is feasible for you, and whether you can afford an IDEXX sealer. If the answer is no to both, you may want to think about MI or m-ColiBlue24. On the other hand, you may have particular reasons for wanting to use modified mTEC or Colilert (for example, perhaps your state agency uses one of those methods). In that case, it might be worth going the extra mile to obtain the necessary equipment.

The future

Even though *E. coli* and enterococci have been shown to be better indicators for recreational waters than the fecal coliform indicator they replaced, they are far from ideal. Researchers are busy looking for new indicator species that would be more human-specific and have survival rates in the environment more similar to those of pathogenic organisms (especially viruses), as well as for new methodologies that would give quicker results. One day, the indicators and methods discussed above will most likely be supplemented, or even nudged aside, by new approaches, probably involving rapid genetic analysis techniques. But if the experience with switching from the fecal coliform indicator to *E. coli* and enterococci is any guide, that day may not be very soon.

Resources

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U.S. EPA. 2003. Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Ambient Water; Final Rule. *U.S. Federal Register* 40 CFR Part 136, July 21, 2003. www.gpoaccess.gov/fr/browse.html.

U.S. EPA microbiology homepage, www.epa.gov/microbes/. Links to PDF files with detailed descriptions of EPA *E. coli* and enterococci methods (Methods 1103.1, 1106.1, 1600, 1603, and 1604).

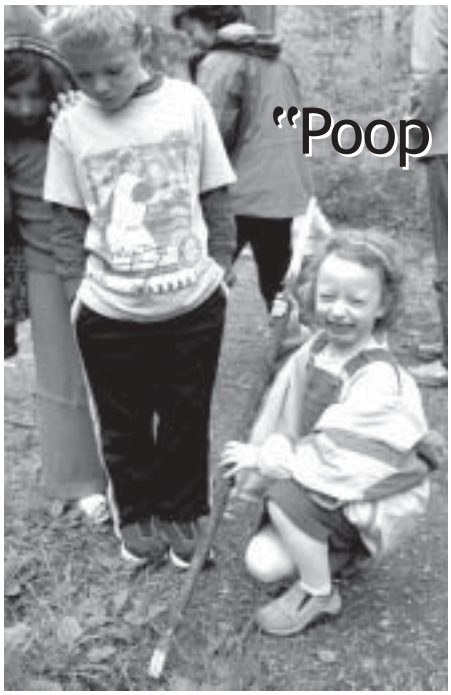
Websites for product manufacturers:
3M Corporation: www.3m.com/microbiology
Hach Company: www.hach.com
IDEXX Laboratories: www.idexx.com
Micrology Laboratories: www.micrologylabs.com

VALIDATION STUDY, continued from page 7

tions use an 8-hour holding time. It could also be related to different labs using different methods. The skew does not compromise how the data can be used, but it is a pattern that I hope to investigate in the future with additional side-by-side sampling.

The comparison study bolstered my confidence in defending the quality of bacteria results generated by volunteers. The results also motivated me to spend a chunk of my limited budget to purchase another set of IDEXX equipment to lend to volunteer groups. Keep up the good work, volunteers!

Steve Hanson is the Volunteer Monitoring Coordinator for ODEQ's Volunteer Monitoring Program. He may be reached at hanson.steve@deq.state.or.us; 503-229-5449.



"Poop Study" Engages Primary Students

"Yuck!" was a common reaction when students found dog poop along a trail in Seattle's Fauntleroy Creek watershed.

by Judy Pickens

Christopher was a bit nervous about reporting to the watershed council. He and his colleagues had prepared carefully, though, and the faces around the table were friendly. He knew their study report would go well—and that the ice cream afterwards would be *deee-licious*!

Christopher and his fellow second-graders addressing the Fauntleroy Watershed Council that evening were among 36 primary students who had surveyed pet waste in an urban creek during the previous year. Their study provided worthwhile data and demonstrated that even young children can play a role in water quality research.

Genesis of the study

In 2002, the council learned that our creek was on the Washington Department of Ecology's (DOE) list of candidates for water-cleanup attention. To improve our chances of attracting state money and expertise, I wanted to show local interest in water quality.

At the same time, teachers at KapKa Cooperative Primary School asked me to design a project that would engage their kindergarten through second-grade students over several months. I had hosted the school for years on Fauntleroy Creek and was confident that the students were up to a challenge.

Because of consistently high fecal coliform counts at the mouth of the creek, I expected bacterial pollution to be a focus of any water quality effort by the state. Earlier research had pointed to pet waste as a major source, but this assumption had never been tested. Adult researchers weren't likely to count dog poop—but I thought young students would.

With advice from DOE and school staff, I devised a year-long study to document the prevalence of pet waste near the creek and see if pet-waste stations would improve scoop compliance.

What we did

I selected a 600-foot segment of trail popular with dog walkers in Fauntleroy Park, a natural area at the headwaters of Fauntleroy Creek. Given little evidence of ground animals in the park, we felt safe in assuming that waste deposits were from dogs. Students and parent volunteers agreed to:

- count waste on the trail and within 7 feet to either side
- count on the outbound trip only
- count every other month to allow already-counted deposits to decompose
- not touch the poop!

Each team included five or six students of mixed ages, along with me and another adult volunteer or two. The children spotted deposits and an adult marked locations on a field map. Back at school, teachers helped the children use colored dots to transfer locations to a large map.

After two surveys, we made bag dispensers by mounting gallon milk jugs upside down on a post and filling them with used plastic grocery bags. These dispensers, placed next to garbage cans provided by Seattle Parks, served as pet-waste stations at major park entrances.

The children proved to be adept researchers, completing seven surveys from March 2003 through April 2004. They knew dog poop when they saw it and were eagle-eyed, finding many deposits that we adults would have missed.

Happy endings

On the trail and during their report to the council, the students evidenced a rudimentary understanding of how pollutants on land get into creeks and cause harm. They also demonstrated confidence in their ability to make a positive difference in the environment.

The study was most helpful to our watershed in three respects:

1. The children's large map showed where pet waste was most plentiful and where we needed more pet-waste stations.
2. We learned that dog walkers would keep the dispensers stocked.
3. The data established a baseline against which to compare results from follow-up research.

As a direct result of the community interest demonstrated by the study, DOE staff have become more involved with the watershed. Most notably, they chose Fauntleroy Creek for a year of sampling, which provided the most detailed water chemistry data ever collected here.

Lessons learned

We learned that one year's worth of data was not enough to establish a trend, given seasonal variation in the number of dogs on the trail. With data from two or three years, students would be able to compare counts taken the same month, year to year, and reach supportable conclusions.

We also learned that brief discussions on the trail were not enough for the students to gain a solid understanding of

continued on page 21

High-Tech Source Tracking in Maine

by Eleanor Ely

Maine has a strong heritage of shellfishing, and Maine volunteer monitors have a long tradition of helping reopen clam flats that have been closed to harvest due to fecal contamination. Thanks in part to volunteers who collected thousands of water samples for bacteria testing and conducted shoreline surveys to look for contamination sources, Maine's Department of Marine Resources was able to open 100,000 acres to shellfishing during the 1990s.

In recent years, though, few additional acres have been opened, because most of the obvious sources of fecal contamination have been dealt with. Yet some shellfishing areas remain closed due to high levels of bacteria from unknown sources. For communities in this frustrating situation, high-tech genetic source tracking methods offer the prospect of tracing the bacteria to specific host species—information that could lead to more successful control measures. However, these techniques are expensive and, until recently, they had not been tried in Maine.

In 2001, a group of researchers from Maine Sea Grant, Wells National Estuarine Research Reserve, and the Jackson Estuarine Laboratory at the University of New Hampshire undertook a study to investigate the usefulness and feasibility of ribotyping, one well-established genetic source tracking method. They selected two watersheds in southern Maine for the Microbial Source Tracking Project—the Webhannet River watershed, and the Merriland-Branch-Little River (MBLR) watershed. In both watersheds, persistent elevated levels of bacteria had for years kept shellfish beds closed to harvest.

The project's goals were twofold: First, to provide managers in the two watersheds with useful information to guide remediation efforts, possibly leading to eventual reopening of shellfishing areas; and second, to serve as a model that would generate specific guidelines for other watersheds considering similar source tracking approaches.

Ribotyping, a technique that identifies different strains of a bacterial species based on small differences in the regions of DNA that code for ribosomal RNA, has been used in tracking sources of fecal bacteria since the mid-1990s. The technique is most often performed with *E. coli* (as was done in the Maine project), and sometimes with enterococci. The ribotype profiles or "fingerprints" of strains found in the water are compared to a reference database, or library, of ribotype profiles produced by strains of *E. coli* from known host species, including humans, domestic animals, and wildlife.

Professional trapper Dana Johnson wades through icy water in a vain search for beaver scat.

Something old, something new

A large, comprehensive reference database is crucial to the success of ribotyping. Because of geographic differences in strains of bacteria, the chances of finding a match are increased if the library includes ribotype profiles from local sources. Jackson Estuarine Laboratory already had a regional database of nearly 1,000 ribotypes derived mainly from source species in New Hampshire, Vermont, and Massachusetts, but for the Maine study, the researchers wanted to supplement this database with samples collected specifically from the two study watersheds.

A technician at the Jackson Estuarine Laboratory prepares gels for electrophoresis.

Project staff managed to collect fecal material from local pet, livestock, and human sources, but when it came to wild animals they turned to local trapper Dana Johnson for assistance. Ironically, for all the high-tech equipment and procedures involved in ribotyping, the critical step of assembling a representative host species library depends on skills as old as humanity.

Johnson, who volunteered his services, says that when he looks around a stream site "it's like opening a book." He knows different animals' sign and habits—like which ones hide their droppings and which leave them in the open. Johnson succeeded in obtaining viable scat samples from red fox, muskrat, coyote, squirrel, and wild turkey. Despite his best efforts, though, he was unable to collect any beaver droppings, which are deposited directly into the water where they quickly disintegrate.

Volunteers pitch in

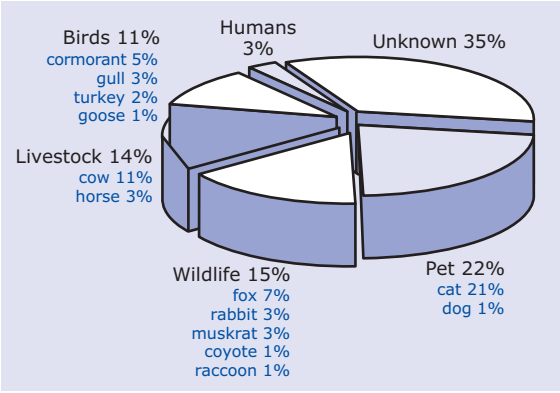
Community volunteers played a major role in collecting water samples for the study, even though the job required going out in winter weather (sometimes the volunteers' sampling equipment included a hammer for breaking through

the ice). About 50 trained volunteers from the two watersheds collected stream samples between December and May—i.e., before, during, and after the clam-harvesting season.

Lab procedures

At the Wells Reserve lab, project staff members, with some help from volunteers, used membrane filtration to obtain *E. coli* counts on the water samples. Individual *E. coli* colonies ("isolates") were sent to the Jackson Lab for ribotyping.

Because of the expense of ribotyping—$100 per isolate—the project could only afford to ribotype 100 isolates from each of the two watersheds. The researchers focused on isolates from sites that consistently yielded the highest counts, reasoning that information from those sites would be of most practical use to watershed managers.



Source species identification for 98 *E. coli* isolates from the MBLR watershed, using Jackson Estuarine Laboratory's regional library.

At the Jackson Estuarine Laboratory, skilled technicians extracted the DNA from each isolate, then performed a series of procedures that included cutting the DNA into fragments using restriction enzymes, subjecting the fragments to gel electrophoresis to separate them into a series of bands, transferring the bands from the gel to a nylon membrane, and using a labeled probe to locate and visualize the portions of the DNA that code for ribosomal RNA. For the first year of the project, these steps were performed manually. By the second year, the Jackson Laboratory had acquired a DuPont Qualicon RiboPrinter, which automates many of the steps.

What ribotyping revealed

From the point of view of watershed managers, it's nice when a source tracking investigation turns up a "smoking gun"—one or two predominant sources toward which mitigation measures can be targeted. The real world, though, is seldom so accommodating. The pie chart for the MBLR results contains several moderate-sized "slices" representing a variety of contaminant sources. The Webhannet results showed a similar variety of source species, although the distribution among categories was somewhat different.

For both watersheds, the largest single category was "unknown." Ribotype profiles in this category either did not match any profile in the reference database, or else matched profiles of "garden-variety" *E. coli* that are found in multiple species. The inability to match all bacteria strains found in a water sample to particular host species is one of the inherent limitations of all currently available genetic source tracking methods.

For management purposes the most relevant finding was that approximately 40 percent of sources in both watersheds were either human or "human-associated" (pets or livestock). There are usually actions that can be taken to reduce contamination from human-associated sources. For example, the high contribution from pet sources in the MBLR watershed prompted Maine Sea Grant to produce and distribute a flyer, "Pet Waste and Water Quality," that explains the harmful effects of pet waste on shellfishing and swimming areas and advises pet owners on proper methods for disposing of pet waste. Wildlife sources, by contrast, are generally more difficult to control, especially since some potential management options, like hunting or trapping, tend to be controversial.

Intrepid volunteers didn't let winter weather keep them from collecting samples.

Recommended: A targeted approach

Based on their experience with the Microbial Source Tracking Project, the researchers came up with guidance recommendations for future source tracking studies. The central theme of these recommendations is "targeting." Given the high cost of genetic source tracking methods, they should be reserved for carefully selected situations in which they are most likely to produce useful results. To get the best bang for the buck, the Maine researchers advise:

1. Prioritize shellfish-harvesting areas that have (a) high economic value, (b) strong community support for mitigation efforts, and (c) persistent bacterial contamination that has not been resolved by less-expensive approaches.
2. In the areas identified in step 1, conduct targeted bacteria testing to identify the locations with the highest counts, and do shoreline surveys to determine likely sources and pathways of contamination.
3. Establish a targeted local library, focusing on those species suspected to be the most important sources of contamination.
4. Collect samples for genetic analysis by conducting intensive, short-term water sampling at the targeted locations, during the time period of interest (i.e., shellfish-harvesting season), under environmental conditions that historically have produced the highest counts.
5. If the analysis identifies significant non-wildlife sources, there is hope for reducing the contamination and a reason for continuing the investigation.

For more information on the Microbial Source Tracking Project, visit www.seagrant.umaine.edu/mst.htm, or contact Kristen Whiting-Grant at Maine Sea Grant, 207-646-1555, kristen.whiting-grant@maine.edu.

What Is MST and What Can It Do?

by Eleanor Ely

"Microbial source tracking" (MST) is the name given to methods that examine the bacteria (or in some cases viruses) found in a water sample for clues that will trace them back to their host animal species. The basic idea is to find some characteristic of the microbe that will tell you whose gut it came from.

MST is a young science. Currently available techniques are expensive, and the results are not always conclusive. Therefore communities should always begin with low-tech, commonsense source tracking approaches (see sidebar on opposite page). But when these methods fail to reveal the sources of fecal contamination, some communities have turned to MST approaches such as phage typing, antibiotic resistance testing, and genetic profiling.

Phage typing

Phage typing is an MST method based on identifying F+ coliphages, which are viruses that infect *E. coli*. These phages belong to four groups, with Groups II and III predominantly associated with human *E. coli* strains and Group IV with animal strains. Group I is associated with both humans and animals. A limitation of phage typing is that it can only discriminate between human and nonhuman sources; it doesn't distinguish among different animal host species (e.g., cows, ducks, cats, raccoons).

Antibiotic resistance testing

Bacteria from a given host species tend to show resistance to those antibiotics to which the host has been exposed. Many of the antibiotics used in farm animal feed are different from those given to humans. Bacteria from wildlife species have had little exposure to antibiotics and therefore usually have low resistance.

In order to use antibiotic resistance testing for MST, it's necessary to assemble a reference database (library) of antibiotic resistance patterns for bacteria from known human and animal sources. Antibiotic resistance approaches have been quite widely used for MST because it is relatively quick and inexpensive to determine antibiotic resistance patterns of bacteria. However, assembling the library is an expensive and labor-intensive proposition.

Genetic profiling

The basic concept behind genetic MST methods is that the strains of, say, *E. coli* living in the intestinal tracts of dogs will have genetic differences compared to the *E. coli* strains inhabiting the intestines of humans or deer or geese. Various techniques developed for the science of molecular genetics are used to detect these differences in bacterial DNA.

The genetic profiling techniques used in MST are similar to those used in DNA profiling (a.k.a. "DNA fingerprinting") of criminal suspects. However, criminal investigators have a big advantage: DNA from blood, saliva, hair, etc., at the crime scene can be compared directly to a suspect's DNA. In MST, by contrast, investigators are working at one step removed—they don't have DNA from the source animal itself, only from bacteria living in its intestinal tract.

Some of the more common genetic profiling MST methods are ribotyping, pulsed-field gel electrophoresis (PFGE), and repetitive element sequence-based PCR (rep-PCR). In these approaches, specific fragments of DNA are obtained from a bacterial strain and analyzed using a technique called gel electrophoresis. Briefly, the fragments are placed in a well at one end of a slab of gel whose consistency is like hard Jell-O. An electrical current is applied to the gel and the fragments (which are negatively charged) begin migrating. Because smaller fragments travel faster than large ones, the end product is a pattern of

bands in the gel, like a bar code. This band pattern is the profile or fingerprint of that particular strain of bacteria. It is compared to profiles in a library of bacterial strains from known host species. [*Note:* See page 14 for more on ribotyping.]

Limitations and caveats

None of the MST methods described above is foolproof. The genetic approaches generally give the most reliable results, but are also the most expensive. Antibiotic resistance and genetic profiling methods are both "library-dependent," requiring a reference database of hundreds or even thousands of different bacteria strains collected from host species in the region under investigation. With both these approaches, it is fairly common to either find no match or else find too many matches (i.e., the resistance pattern or genetic profile of the bacteria strain found in the water sample matches more than one potential host species). Moreover, matches that are found are not completely reliable.

Before contracting for any of the MST methods, it's important to hold thorough discussions with the contracting laboratory. Communities and volunteer groups should be sure they fully understand the methodology that will be used, including the meaning and limitations of the resulting data in the context of their project goals.

The wave of the future?

Methods that don't require a library, or even culturing of water samples in the lab, are probably the wave of the future.

Developing such methods depends on finding a specific genetic marker (i.e., DNA sequence) in a bacterial strain that unequivocally identifies that strain as coming from a particular host species. Bypassing the need for culturing the bacteria, investigators could extract DNA from filtered water samples and then use a molecular probe to zero in on the marker. The handy polymerase chain reaction (PCR) technique, which can quickly generate up to a million copies from a single piece of DNA, could be used to amplify the marker to detectable levels.

Currently, researchers are investigating and evaluating several promising markers, especially human-specific markers, in various species of fecal bacteria. Some of these techniques may be fine-tuned and ready for wide application within a few years.

Resources:
U.S. EPA. 2005. *Microbial Source Tracking Guide Document*. US EPA Office of Research and Development, Cincinnati, OH. EPA/600-R-05-064. PDF available at www.calcoast.org/news/MSTGuide.pdf. For more information contact Santodomingo.Jorge@epamail.epa.gov.

Scott et al. 2002. Microbial Source Tracking: Current Methodology and Future Directions, *Applied and Environmental Microbiology* 68(12):5796-5803.

Malakoff, David. 2002. Can the Poop Detectives Solve a Pollution Mystery? *Cacapon* (Cacapon Institute newsletter), September 2002 issue. Available online from www.cacaponinstitute.org/.

Illustrations in this article ©Jean A. Hamilla

Source Tracking: Start with the Obvious

by Todd Callaghan

The availability of fee-for-results laboratories providing DNA fingerprinting analysis of contaminated water samples has led to increased interest in this technique among municipalities and environmental groups. But communities looking for ways to identify the sources of bacteria contaminating their surface waters should be cautious not to rely upon the increasingly popular high-tech methods at the expense of obvious and inexpensive approaches. The expectation is that comparing the DNA fingerprints of *E. coli* found in a waterbody with the DNA fingerprints of *E. coli* from a library of potential source species is a reasonably precise science. However, this expectation may not hold true because:

1. *E. coli* is an opportunistic colonizer that is not truly host-specific. Therefore the same strains may be found in multiple hosts.
2. Variability in the DNA within a given strain of *E. coli* is known to occur over short timeframes; thus DNA fingerprint libraries are expected to have diminished value over time.
3. The assemblage of strains of *E. coli* within an individual and within groups of individuals is known to fluctuate over time and distance (e.g., due to colonization and extinction events).

With these limitations in mind, and understanding the budgetary constraints of most volunteer groups, advocates for cleaner beaches and waterways should be encouraged to carry out a number of traditional or low-tech investigations before contracting for DNA-based source tracking.

1. Conduct repeated bacterial sampling over a couple of years, during wet and dry weather, and across multiple spatial scales. Sampling at regular intervals along a beach, a streambank, or the centerline of a stream can identify bacterial gradients that may lead to potential sources.
2. Conduct sanitary surveys and address the obvious sources. Sites that suggest likely contamination include stormwater outfalls that flow during dry weather, dog-walking areas, places where wildfowl congregate, farms and livestock areas without riparian buffers, marinas and docks, and areas where stormwater runs off paved surfaces toward the water. Potential bacteria hot spots can also be identified by talking to local officials (e.g., boards of health, shellfish officers) and residents, and by looking at GIS-based land-use maps, local sewer infrastructure maps, and NPDES Stormwater Phase II municipal storm drainage infrastructure maps.
3. A low-tech method to determine whether human sewage is present is to place absorbent pads in the water for a period of time, then view them under ultraviolet light to detect fluorescent whitening agents ("optical brighteners") that are associated with human wash water. Presence of the whitening agents is an indicator of potential human sewage contamination. [*Editor's note:* For more on low-tech optical brightener monitoring, see *The Volunteer Monitor* Summer 2003, page 16.]

A more advanced method of testing for optical brighteners uses high performance liquid chromatography (HPLC), a laboratory technique that is able to detect less than 0.5 micrograms per liter of each of the five commonly used commercial whitening agents. This method also avoids the false positives that can occur in the low-tech method when pads absorb natural organic matter that fluoresces under ultraviolet light.

Observations and data collection by volunteer groups and municipality staff often identify opportunities for actions that will help keep bacteria from waterways—for example, providing dog waste bags, discouraging wildfowl feeding, and installing fences and vegetative buffers to keep livestock from wallowing in streams. Volunteers can also use their observations to encourage municipalities to fix cracked sewer lines and remove illegal connections from stormwater pipes. Funds that would otherwise have been allocated to high-tech laboratory analysis may be better used to pay for remediation and prevention measures.

Todd Callaghan is a biologist with the Massachusetts Office of Coastal Zone Management. He can be reached at todd.callaghan@state.ma.us or 617-626-1233.

Low-Tech Source Tracking in Action

When routine monitoring detects bacteria problems on Maine's coastal beaches, Maine Healthy Beaches Program staff members spend a lot of time on source tracking, working in partnership with state agencies, nonprofits, and local officials to try to find out where the bacteria are coming from. Participation in the voluntary Maine Healthy Beaches Program has grown rapidly, from just a few beaches monitored in 2002 to 45 in 2005. Participating communities test local swimming beaches for enterococci, an indicator of fecal contamination.

In some communities, low-tech methods have been successful in pinpointing major sources of fecal contamination in the watershed. "A lot of source tracking is just walking the area and using common sense to pinpoint potential sources of bacteria," says Keri Lindberg, a University of Maine Cooperative Extension marine professional who helps coordinate the Maine Healthy Beaches Program. "You locate where water flows following rain or snowmelt, and mark the pathways on a watershed map. You observe how farms are managing their manure piles. You look for evidence of domestic or wild animals on or near the beach." Lindberg also recommends paying attention to whether public restroom facilities are available at a beach—if not, swimmers (especially children) may be more likely to use the water as a bathroom. Other sources to watch out for are pipes, stormwater discharges, and the "wrack line" of seaweed and debris deposited at the high tide line, where birds and animals often defecate.

In areas with in-ground septic systems, determining the location and status of the system is key. "Sometimes it's as simple as pushing a stick into a septic leach field, pulling it up, and using the old sniff test to check for odor," says Lindberg. Some visual indications of septic system failure include odd plant growth or changes in vegetation, soil dampness or atypical coloration, and seeps or breakthrough spots. The code enforcer or local plumbing inspector can also inspect the plumbing and connections for sewer and septic systems. Lindberg stresses that letters are sent to property owners in advance of visits, and that Maine Healthy Beaches staff or volunteers never go onto private property without permission or unless accompanied by local or state officials who have the authority to make inspections.

Fluorescent dye is poured into a septic tank that may be failing.

Low-tech methods can be used to identify and prioritize problems that need follow-up testing. For example, after preliminary inspection of septic systems in the Lincolnville Beach area, the local inspector partnered with the Maine Healthy Beaches Program and the Maine Department of Environmental Protection to conduct fluorescent dye testing of seven questionable systems. Six demonstrated failure—and all have been, or soon will be, replaced or upgraded.

One simple and effective approach to tracking (and remediating) fecal pollution sources is to talk to people. For example, Lindberg's conversation with the owner of a business next to a brook led to the discovery that the man was feeding the ducks. He was surprised to learn that this practice could be contributing to fecal bacteria pollution of nearby public swimming beaches. Once he stopped feeding them, the ducks moved away from the area.

Another low-tech technique is placing pads in the water to detect optical brighteners used in laundry detergent. This method can pick up graywater (i.e., household wastewater excluding water from toilets) discharges from washing machines and sinks. In partnership with the Maine Healthy Beaches Program, teachers and 8th-grade students on Mount Desert Island conducted an optical brightener study in a local brook that empties onto a swimming beach to determine if septic leakages or sewer line breaks could be responsible for high enterococci counts in the brook.

When low-tech methods are not enough to pinpoint sources, the Maine Healthy Beaches Program will often call upon the expertise of program partners. In Kennebunkport, the Maine Geologic Survey used acoustic doppler profiling to find out how river flows, bay eddies, and tidal stage were affecting the transport of bacteria to swimming areas along the beach. The program is also looking into the possibility of using new techniques such as a handheld fluorometer for detecting optical brighteners and genetic methods to determine whether sources are human or animal.

Whether using low-tech or high-tech methods, not all source tracking mysteries can be solved. But you can do a lot with patience, persistence, a keen eye, and common sense.

For more information on Maine's Healthy Beaches Program visit www.mainehealthybeaches.org or contact Esperanza Stancioff (esp@umext.maine.edu) or Keri Lindberg (klindberg@umext.maine.edu) at University of Maine Cooperative Extension/Sea Grant; 207-832-0343.

[*Note*: For more on low-tech source tracking methods, see *The Volunteer Monitor* Fall 1997, pages 18-20.]

Monitoring Water Pollution from CAFOs

by Rita Jack

Liquefied manure from CAFOs is sprayed onto agricultural fields, sometimes miles away from the CAFO.

Farms were always part of the beautiful rural landscape near Hudson, Michigan. Then 10 new mega-dairies began operations there in the late 1990s, with huge long barns to house thousands of animals, and huge lagoons to store their manure and wastewater. The EPA calls such large-scale livestock facilities Concentrated Animal Feeding Operations, or CAFOs.

One large CAFO houses at least 700 dairy cows or 1,000 beef cattle, but most house between 2,000 and 4,000 animals. A single dairy cow produces the same amount of waste as 13 to 20 humans, meaning that a CAFO produces as much waste as a town. Yet this waste is not treated in a wastewater treatment plant. A vast amount of liquid manure is spread onto fields around Hudson, and much of it runs off and degrades water in ditches and streams.

In response to this situation, a group of community volunteers who live in the Hudson area came together to form the Environmentally Concerned Citizens of South Central Michigan (ECCSCM). Neither the Michigan Department of Environmental Quality (DEQ) nor the Michigan Department of Agriculture seemed to understand the scope of the problems, so the citizens decided to do water monitoring to prove that the CAFOs were contaminating water. With funding from a Sierra Club Community Action Grant, ECCSCM members began a two-year water monitoring project in 2001. The volunteers decided to monitor two parameters, dissolved oxygen and *E. coli*. Both are relatively easy to measure, and both indicate an input of manure to water and potential harm to either the aquatic ecosystem or humans.

Water samples for *E. coli* testing were collected in sterile 100-ml bottles and analyzed at certified microbiology laboratories. To avoid trespassing, the volunteers worked from the public right-of-way alongside the road, often from the top of culverts.

The ECCSCM volunteer monitors sampled over 70 sites, collecting more than 400 water samples over the course of the project. Sites were chosen based on manure-spreading activity and information provided by area residents. Each site was downstream from, or adjacent to, a CAFO or a field where CAFO waste was land-applied.

An extendable pole simplifies collecting a sample from the water 15 to 20 feet below. The sterile bottle for bacteria testing fits snugly into a "cup" cut from a piece of PVC pipe and attached to the bottom of the pole.

The volunteers persisted in their monitoring despite being chased by manure-hauling trucks and being blocked from the roadway by the trucks. One volunteer was charged with harassment, but the judge ruled that her water monitoring activities in her community were her constitutional right.

Outcomes

The ECCSCM water monitors often found high levels of *E. coli*, as well as depressed levels of dissolved oxygen. Many times the bacteria counts were in the thousands or even hundreds of thousands of colonies per 100 ml. The state standard for partial body contact activities (like wading and fishing) is 1,000 *E. coli*/100 ml, and the standard for swimming is 300 *E. coli*/100 ml.

Nearly all manure-application fields in Michigan are tile-drained, which means that plastic tubing with small perforations (called "tiles" for historical reasons) is installed 2 to 4 feet below the soil surface to drain water from the fields. The volunteers found contaminated discharges coming from the drainage tiles even in dry weather, showing that the volume of liquefied CAFO animal waste being applied was so great that it would reach field tiles without rainfall. The volunteers also found water quality violations during every month of the year, including winter.

The volunteers' data were reported to the DEQ whenever there was an exceedance of Michigan water quality standards, which occurred every sampling session, and over time the

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SIMPLE Approaches in Alabama & Internationally

by Bill Deutsch

There are many options that citizen groups can use to analyze water for bacteria. Methods range from the simple, inexpensive, and (some would argue) of questionable accuracy to the complex, expensive, and (some would argue) impractical.

When Alabama Water Watch (AWW) first began looking for bacteriological monitoring methods in the early 1990s, the state environmental regulatory agency and the health department were both using fecal coliforms as an indicator of contamination. Water Watchers knew that EPA had published guidance documents in 1986 which admonished states to move toward *E. coli* monitoring of water as a better way to detect human health risks. So, the citizen groups wanted to begin their bacteriological monitoring efforts with a simple way to quantify *E. coli* levels.

Coliscan Easygel

The Coliscan Easygel method was relatively new at the time, and its inventor, Jonathan Roth of Micrology Labs, was gracious enough to come to Alabama and give us some background and training in the technique. This method suited our needs well: it detected the indicator *E. coli*, it was easy and inexpensive, and it was suitable for home use. We adopted it in 1994 and it quickly spread throughout our network of volunteer groups who were already monitoring water for chemical parameters. The volunteers made their own incubators using cardboard boxes or Styrofoam or plastic coolers and a low-watt light bulb.

The AWW office moved toward a quality assurance plan for *E. coli* monitoring and, with the help of some microbiologists at Auburn University, conducted a side-by-side study of the method state agencies were using (membrane filtration for fecal coliforms) and the Easygel method. The comparability study was encouraging and resulted in a quality assurance plan for citizen monitoring of bacteria that was approved by EPA Region 4 in 1999.

Using the data

Very soon, success stories about cleaning up bacterial contamination of waterbodies in Alabama began popping up—a stream contaminated by a faulty wastewater treatment plant discharge, a lake swimming area contaminated by Canada geese, a broken sewer main discharging hospital waste into a stream. Citizen monitors were the first to detect these problems, with test results showing *E. coli* levels greatly exceeding standards, and their findings were quickly confirmed and acted upon by governmental agencies.

About 7,000 bacteriological records have been submitted by AWW monitors, most with three replicates to give us a measure of variability among samples. We typically find very good agreement among the replicates. Data are usually submitted online to the AWW database, where anyone may access the quality-assured data and create simple bar graphs for sites of interest. The graphs follow a "traffic light" model, with green-colored bars for less than 200 colonies of *E. coli* per 100 ml of sample water, yellow for 200 to 600, and red for greater than 600. Stream discharge data can be overlaid on the bar graphs to correlate flow with bacterial concentrations and explore possible sources and patterns of contamination in the watershed.

International experiences

Even before the AWW bacteriological techniques were officially approved by EPA, our partner citizen groups in the Philippines began monitoring with similar protocols. Many villages in the Philippines drink untreated water from springs, but before this project bacteria testing was conducted very rarely, and then only with presence-absence methods. Using the Coliscan Easygel method, village residents and community health workers test the water "from spring source to mouth," meaning that samples are analyzed from springs, community faucets, water-carrying containers, in-home water storage tanks, and utensils in the home.

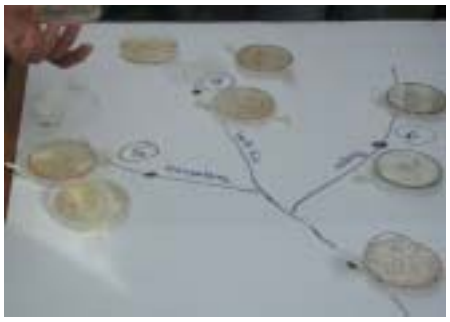
The monitoring generates a great deal of excitement. People are amazed to discover this powerful tool that lets them see bacterial contamination for themselves. Over the last 10 years, local train-

Bacteria plates from different sites near Xalapa, Veracruz, Mexico, are arranged on a drawing of the watershed to give a vivid perspective on the location of problems.

ers have conducted bacteriological monitoring workshops in about 40 Filipino villages, and this type of monitoring has also been enthusiastically received by citizen monitors in Ecuador, Thailand, Brazil, and Mexico.

We have found that an effective way to communicate bacteria survey results is to make a large drawing of the watershed on a tabletop or floor and then place the cultured sample plates in the appropriate spot within the watershed. Seeing the results visually laid out leads to vigorous discussions among monitors and observers, with a much greater understanding of watershed dynamics and human health risks.

A striking example of a success story among Filipino monitors occurred when a local official was planning to distribute water from three springs to about 300 households in a mountain village. Bacteria tests by local citizens revealed that one of the springs was highly contaminated with *E. coli* and it was, therefore, excluded as a public drinking water source.



All bacteriological techniques have pros and cons, but use of the Coliscan Easygel method has given us the flexibility, cost-effectiveness, and accuracy we need for basic watershed management. Even in developing countries, the expense is about 1/20th what laboratories charge for the same information, and results are obtained by local monitors, with local ownership and application of the data. When monitors have questions about interpreting the plates, they have transcended distance by emailing digital photos of cultured plates to the Water Watch office from around the world.

In summary, our simple bacteriological monitoring approach maximizes citizen participation and enables people to see and understand water quality issues in a new, often transformational, way.

Bill Deutsch is Program Manager for Alabama Water Watch (www.alabamawaterwatch.org) and coordinates a network of international volunteers called Global Water Watch (www.globalwaterwatch.org). He may be contacted at deutswg@auburn.edu; 334-844-9119 or toll-free 1-888-844-4785.

CAFOS, continued from page 19

CAFO in Huron County, Michigan.

volunteer monitors developed a working relationship with the agency staff. Because of the added attention garnered by ECCSCM's data, the DEQ stepped up its own sampling efforts. Based on these samples (because the agency can use only its own data for enforcement purposes), DEQ has cited every CAFO in the 10-square-mile area for illegal discharges—140 violations since 2001. In 2004, two streams affected by CAFO waste were added to the state's 303(d) list of impaired waters, and two more CAFO-impacted streams were listed in 2006. Data collected by the ECCSCM water monitors were cited in the report accompanying the 2004 listing. Yet the citations and listings have had little effect on CAFO practices, because water quality violations continue to occur, even from brand-new facilities.

According to an ECCSCM report posted at www.nocafos.org, "It's clear from our sampling, and from many other studies as well, that liquid manure pollutes. To protect rural watersheds and drinking water sources, to protect our Great Lakes, CAFOs (like every other industry) should be required to treat contaminated liquids." The monitors have called for a prohibition on the application of liquid manure to frozen ground and a phase-out of the application of liquid manure to tiled fields, and take every opportunity to talk to legislators and other decision-makers about problems with CAFOs. Since 2002, the Michigan Sierra Club Water Sentinels project has been working to "export" the work of the ECCSCM water monitoring project across the state, by training other volunteers to test water for *E. coli*.

Rita Jack directs the Water Sentinels Project at the Michigan Sierra Club (www.michigan.sierraclub.org). She may be contacted at 517-484-2372; rita.jack@sierraclub.org. For more information on the Environmentally Concerned Citizens of South Central Michigan, see www.nocafos.org.

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why pet waste is a threat to water quality. Next time, we'll kick off the study at school, where we can focus information and attention.

Several aspects of the study served us very well, however, including:

- Working with a school that places a high value on environmental learning and student empowerment.
- Emphasizing student recognition. As one parent observed after the children's report to the council, "The kids were just beaming. I think they felt very respected and appreciated."
- Striking a workable balance between research rigor and the students' need to have fun while learning.

And the ice cream. Can't forget that!

Judy Pickens is a founding member of the Fauntleroy Watershed Council in Seattle, WA, and a volunteer streamside educator. For more information, call 206-938-4203 or visit www.fauntleroy.net.

Monitoring Earns Respect for Texas Group

by Jason Pinchback

One late afternoon in the winter of 2000 I received a frenetic phone call from a person living in Rockport, a small town on the Texas Gulf Coast. He immediately began recounting his "saga" of letters and calls to local, regional, and state government agencies. His frustration was apparent as he described numerous telephone transfers, referrals to different agencies, and dead ends. Finally someone had recommended that he call Texas Watch, the statewide volunteer water quality monitoring network.

Concerns about marina

It turned out that this individual represented an impromptu coalition of citizens who were very concerned about a potential fecal pollution problem in Little Bay, the focal center of Rockport. The bay is popular for swimming, water skiing, boating, and fishing. As the caller explained, many houseboats docked at the Little Bay marina were occupied full-time and did not typically leave the dock slips or pump their latrine wastes, since pump-out services were not available at the marina. The citizens suspected that human waste from the houseboats was contaminating the nearby swimming beach and possibly other parts of the bay as well.

With 10 years of volunteer water quality monitoring coordination behind me, I'm accustomed to receiving emotional calls from worried citizens. Usually the first thing I have to consider is whether Texas Watch is able to take on a new project, given our limited resources. The decision is guided by questions like: Is there a potential health or safety concern? Is this a public waterway? How is the water body used by the public, industry, and municipalities? Are there local stakeholders who can assist with the project?

In the case of Little Bay, we decided the project was worth pursuing. The water is heavily used by the public and a municipality, there were local groups and agencies that could assist, and we knew that there were pathogen problems in adjacent bays. My search for existing data on the bay came up with nothing more recent than the 1980s. There was no current monitoring of Little Bay or its main tributary by state or local water authorities.

Baywide water quality study

Texas Watch began to work with the citizen group to develop a study design. Importantly, we did not focus exclusively on the perceived bacteria pollution issues at the marina. Instead, we designed a broader study whose goal was "to evaluate Little Bay and pertinent tributaries for aquatic life use and contact recreation conditions." We chose this route because initial hunches may not be true and often give way to hard data and analysis.

We established "fixed" sampling stations at four locations: the designated swimming area, the main tributary, the marina, and the center of the bay. Two other "rotating" monitoring locations were designated to periodically move to new places selected on the basis of data analysis. At each station, the volunteers took water chemistry and Secchi depth readings, measured flow, and collected samples for nutrient and bacteria analysis.

Little Bay Sentinels and others learn more about the Little Bay watershed at a Texas Watch meeting.

For the bacteria analysis, we opted to use Micrology Laboratories' Coliscan Easygel, a simple pour-plate method for detecting the indicator *E. coli*. Texas water quality standards for recreational waters actually recommend the indicator enterococci for tidally influenced waters, like Little Bay, and *E. coli* for freshwater. However, it was not feasible for the volunteers to test for enterococci because no simple enterococci testing method is currently available, and the group did not have the funds to pay for laboratory analysis. Even though we were not testing for the recommended saltwater indicator, and also were using a method that is not officially state- or EPA-approved, we felt confident that the data would be of sufficient quality for the purpose of identifying hot spots or areas of concern, especially since we would be comparing samples from the same subwatershed. In interpreting the data we did not attach much significance to any single data point but instead looked at trends and patterns based on multiple samples.

A team of volunteers collected water samples from the different locations and delivered them (on ice) to the home of one volunteer, who prepared the Easygel plates and incubated them in a simple homemade incubator. Having one person read and interpret all the plates eliminated person-to-person variation in interpreting the color reactions.

Pollution fears allayed

The first year of data collection turned out to be fruitful. The results from sites near the marina did not lend support to the group's concerns about fecal contamination from the houseboats. Counts were low at the designated swimming area, as well as throughout the rest of Little Bay. But the volunteers did find high bacteria counts, along with low dissolved oxy-

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___ Winter 2002 - Monitoring Beaches and Reefs
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gen and elevated nutrient concentrations, at the mouth of the main tributary.

The citizens, who by now had adopted the name "Little Bay Sentinels," were surprised but pleased to learn that their swimming, skiing, and marina areas were not contaminated with excessive pathogens. However, they continued collecting data to learn more about water quality in the bay.

Citizen group gets official role

Texas Watch encourages the citizen groups we work with to communicate their findings to local agencies and stakeholders. So about two years into the Little Bay Sentinels' monitoring effort we hosted a regional meeting where scientists, citizens, and agency staff gathered to discuss the results from the project. The meeting drew attention to the fact that the citizen group was the sole entity assessing local surface waters, and laid the groundwork for future cooperation among stakeholders.

A kayaker enjoys the tranquil waters of Little Bay.

Through their work, the Little Bay Sentinels have earned the trust of city officials and have come to be viewed as a valuable resource—as was clearly demonstrated when the city created a new water quality committee composed of the citizen group members and city staff. The coordinator of the Sentinels was appointed chair of the committee.

The story of the Little Bay Sentinels began with what turned out to be a misperception about a water quality problem. It has ended with a respected volunteer monitoring organization that enjoys city support and funding and has a designated role in protecting local water quality. Key steps leading from point A to point B were the adoption of a well-planned study design, careful data collection and analysis, communication of monitoring results to governmental agencies and other interested parties, and ongoing efforts to work cooperatively with various local stakeholders.

Jason Pinchback coordinates monitoring and watershed education projects for Texas Watch. He may be reached at 512-245-9148; JP30@txstate.edu. Texas Watch, a collaborative partnership between the Texas Commission on Environmental Quality and U.S. EPA Region 6, is headquartered at Texas State University in San Marcos.

Invasive Plant Control

The Weed Workers' Handbook is crammed with useful information about controlling and removing invasive plant species. In addition to detailed guidance on tools and techniques, the handbook provides insights and advice on organizing weed-removal projects and explaining such projects to the public. Published by the Watershed Project and the California Invasive Plant Council, the guide is geared toward San Francisco Bay Area invasive plants but will be broadly useful. Visit www.cal-ipc.org/ww_handbook/ to view the publication in PDF format or order a print copy (120 pages, laminated).

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test the water

Since the early 1990s, surfers have been monitoring bacteria at surfing beaches as part of the Surfrider Foundation's Blue Water Task Force Program. Currently about 20 of the Surfrider's 60 chapters are testing water quality along East Coast, West Coast, and Gulf Coast beaches, as well as in Hawaii and Puerto Rico. Most chapters use the IDEXX Enterolert Quanti-Tray method for bacteria testing.

Rick Wilson, who serves as Coastal Management Coordinator at the Surfrider Foundation's national headquarters in San Clemente, California, and also chairs the Laguna Beach chapter, says, "We regard the BEACH Act of 2000 as one outcome of our monitoring. Our water quality testing during the 1990s highlighted the bacteria problems at surfing and swimming beaches, and drew attention to the lack of official monitoring by government agencies. The Surfrider Foundation also actively lobbied for passage of the Act."

Some chapters, including Wilson's, implement their water quality monitoring through the "Teach and Test" program, in which chapters provide training and materials to local high schools. In Laguna Beach, the students found high counts in a storm drain that drained to the beach. They communicated their results with the city, which agreed to divert that storm drain to the sewer system except during rainstorms.

Several Surfrider chapters are collaborating with state or local agencies. In Oregon, Surfrider members served on a committee to shape the state beach monitoring program and helped decide which beaches should be tested. Surfrider chapters in Hawaii post the state's bacteria testing results on their websites because the state is not yet able to do this. Some Washington and California chapters are collecting samples for local counties or providing bacteria data for the counties to use.

Storm drain outfall discharging to beach.