Citizens Monitoring Bacteria:

A training manual for monitoring *E. coli*

2nd Edition

A regional partnership between IN, IA, MI, MN, OH and WI

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Copies of this manual can be obtained on the web in pdf format at:

http://www.uwex.edu/ces/csreesvolmon/EColi/ProjectVolunteers.htm

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Preface

This manual is a result of a joint project to enhance citizen E. coli monitoring in streams of the upper Midwest. The partners involved in this project include the Iowa Department of Natural Resources IOWATER, Purdue University, the Indiana Department of Natural Resources, Hoosier Riverwatch, Michigan State University, the Ohio State University, the University of Minnesota Extension Service, Minnesota Water Resources Center, the Volunteer Stream Monitoring Partnership, the University of Wisconsin Extension, the Wisconsin Department of Natural Resources, and the Water Action Volunteers Program. Others who have lent support to this manual include local units of government, citizen leaders, and all the volunteers who have helped throughout this project.

Funding for this Citizens Monitoring Bacteria (CMB) project was granted from the U.S. Department of Agriculture's Cooperative State Research, Education, and Extension Service (CSREES) 406 Water Quality program. Additional funding was provided by the CSREES Great Lakes Regional Water Program.

Several excellent training manuals already exist that instruct citizens on monitoring various parameters of water quality in streams, and several are cited at the end of this manual. The content of this training manual will not provide a comprehensive approach to stream monitoring methods but will instead supplement other training manuals by focusing on the single parameter, *E. coli,* and provide detailed information on methods and analyses for *E. coli* stream monitoring.

Chapter 1: Introduction: Stream Monitoring

Why monitor streams?

Streams have been referred to as the arteries of the earth since they carry and transport the water that supports aquatic life. Humans also depend on this water for a multitude of activities including irrigation, drinking supply, energy production, recreation, industry, and aesthetics. Clean water is important to the health and livelihood of all people, and many groups and stakeholders are working together to protect water resources. However, 39% of the rivers and streams assessed in the United States in 2000 were polluted or had degraded habitat. According to the USEPA's 2000 National Water Quality Inventory, polluted water runoff from the land was the leading cause of water quality problems nationwide (USEPA, 2002a). Major pollutant sources were sediment, bacteria, heavy metals and nutrients. Stream monitoring programs can be invaluable in assessing current conditions and tracking changes in water quality over time to determine if remediation or protection actions have been successful.

Volunteer programs

State and regional agency staff as well as funds are often limited, yet stream monitoring needs can be vast. Volunteer monitoring programs can be an extremely valuable asset to states' water quality monitoring programs by expanding data collection efforts and resource assessment opportunities. Volunteer-collected data can provide important baseline information to assist with decision-making and resource assessment.

Volunteer monitoring programs are also a way to tap the expertise of volunteer monitors on local water quality conditions and history. Volunteer monitoring teams are often more "in-touch" with local settings and events and can be available to respond quickly when a pressing need for monitoring arises.

Volunteer monitoring programs are also a great opportunity for citizens of various backgrounds to become more involved in and to gain greater understanding of water quality issues. The training for and involvement in monitoring programs can empower citizens to become involved in informed debate, taking action, and making an impact in their

community. In fact, a study in Wisconsin found that experienced volunteer monitors are more active politically in their communities (Overdevest et al, 2004).

Goals of *E. coli* bacteria monitoring

Many parameters can be monitored to help assess a stream's condition or to follow trends in water quality. One that has received increasing attention as an important water quality indicator is *E. coli* bacteria. While other factors may be just as important to monitor, this training manual focuses on *E. coli* monitoring.

Citizens Monitoring Bacteria Program Goals:

- Build the capacity of volunteer monitoring programs to understand and use the most appropriate *E. coli* testing protocols (test kits, laboratory analysis, etc.) and watershed-based sampling strategies with their volunteers
- Enhance the public's understanding about the role of bacteria in water quality
- Increase awareness and acceptance of the use of volunteer-collected water quality data in various watershed programs, including watershed assessments and TMDL development and implementation
- Share results with other states across the country, primarily via the National Volunteer Monitoring Facilitation Project
- Demonstrate how to set up an appropriate watershed-based *E. coli* sampling strategy utilizing volunteer networks and begin collecting usable data

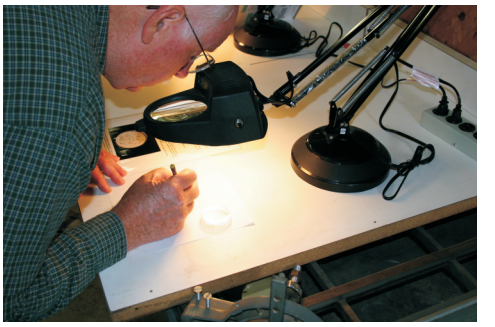
Setting goals and designing a sampling program

The objectives of this program are to provide citizens involved in *E. coli* monitoring programs with the scientific background, practical applicability, and tools needed to develop an understanding of the role of bacteria in stream water quality.

Before embarking on a bacteria monitoring program, it is suggested that your group first review and determine your own goals in terms of data collection and use. Where, when, and how often you sample will depend on these set goals. A reference you may wish to use is the Volunteer Water Quality Monitoring National Facilitation project website's Guide for Growing Programs. In the "Designing Your Monitoring Strategy," groups are introduced to goal-setting processes, and also referred to a number of valuable resources for working towards step-by-step goal making (www.usawaterquality.org/volunteer/).

The time involved with volunteer monitoring can be demanding, but rewarding. First assess how often your group is prepared to monitor. The amount of time allocated to volunteer monitoring depends on your group's goals. For example, one goal may be to conduct baseline monitoring. This plan would involve monitoring every few weeks over many years. You may also choose to monitor your selected stream to see if it is meeting water quality standards. This plan may call for more frequent monitoring but not necessarily for years and years. A short-term, intensive study, such as monitoring the effects of storm water runoff, is another option which may involve daily sampling. All these monitoring plans are not necessarily mutually exclusive.

If your group has the time and has set goals to monitor more frequently, such a plan will provide you with additional data. For example, many states have an active beach monitoring program because of the high level of full-contact recreational use of beaches. Standards have been developed by state and local agencies that indicate the level of risk to human health by swimming in beach waters. According to USEPA standards, when a one-time high count is reached (235 colony forming units (cfu)/100 milliliters (ml)) or a 30-day geometric mean (with a sample size of at least 5 samples per 30-day period or the total number of samples collected over the specified monitoring period) is exceeded (126 cfu/100 ml), the beach is closed until levels decrease (see Chapter 7 for a description of a geometric mean and how it is calculated). If your group has set a goal to determine a 30-day geometric mean, it is recommended that you monitor at least once a week.



Another group goal may include collecting data to further watershed management plans that will develop from coordination with other water quality monitoring programs. You may also want to work on fostering connections and partnerships with state agencies and other groups that promote sound land and watershed management.

In general, the time involved will include driving to and from the selected sites, taking water samples at these sites, and returning to your home or designated laboratory space to process and incubate the samples. You also must be available 24 to 48 hours later (depending on the test) to read the plates after incubation. Counting the *E. coli* colonies and recording them on a data sheet could take up to an hour.

Finally, remember that good sampling plans are flexible and can be updated and refined according to goals and objectives. You can visit the CSREES Best Education Practices (BEP) website for further information on this process (http://wateroutreach.uwex.edu/).

Other important water quality indicators

Bacteria monitoring, while an important and valuable water quality indicator, is only one part of total stream water quality. A comprehensive assessment program of stream water quality should consider monitoring for other water quality indicators.

Biologically and chemically, water quality is defined by a number of factors, and these parameters can generally indicate if a water body is degraded or polluted. How the water will be used may influence which or how many characteristics are used to determine water quality. In addition to bacteria, other common water quality measurements include clarity, conductivity, dissolved oxygen, hardness, nutrients (particularly nitrogen and phosphorus), pH, temperature, total suspended solids, and biological communities (see box, next page).

Various water quality standards exist based on many of these parameters, however the standards may vary depending on the use of the water. For example, drinking water and irrigation water have different standards for bacteria. Zero levels of *E. coli* are required in drinking water, but the presence of some *E. coli* are a tolerated risk in irrigation or swimming waters.

Other Important Water Quality Parameters

Temperature

Temperature varies depending on time of day, season, and vegetation along the stream. Temperature affects the oxygen content of the water since colder water can hold more dissolved oxygen than warmer water. Temperature also affects the rate of photosynthesis by aquatic plants, metabolic rates of aquatic organisms, and the sensitivity of organisms to toxic wastes and diseases.

Dissolved oxygen (DO)

Dissolved oxygen (DO) is necessary for the maintenance of a healthy aquatic ecosystem. Aquatic organisms differ in the amount of oxygen they require for survival. For example, fish such as trout and pike require higher concentrations of DO for survival, while carp and catfish are able to survive at much lower concentrations (less than 5 mg/L). Dissolved oxygen is supplied to a water body through the atmosphere where oxygen mixes with water through wind and wave action, and through photosynthesis by algae and other aquatic plants. Oxygen is more easily dissolved in cold water than in warm water; therefore, the amount of oxygen that water will hold increases as the temperature decreases. Low DO levels can have negative impacts on biota causing stress and sometimes death if levels fall below tolerance values for organisms.

pH

The pH is a measure of the acidity or the alkaline (basic) nature of the water. Since the scale is logarithmic, a drop in the pH by 1 unit is equivalent to a 10-fold increase in acidity. A pH of 7 is neutral. Thus a pH of 5 is 10 times more acidic than a pH of 6 and 100 times more acidic than a pH of 7. pH affects many chemical and biological processes in the water. Different organisms do well or poorly within different ranges of pH. The majority of aquatic animals prefer a pH range from 6.0-8.0. Outside this range reduces the diversity in the stream because it stresses the physiological systems of most organisms and can reduce reproduction. Low pH can also allow toxic elements and compounds to become mobile and "available" for uptake by aquatic plants and animals. This can produce conditions that are toxic to aquatic life, particularly to sensitive species such as salmon and trout. Changes in acidity can be caused by atmospheric deposition (acid rain), surrounding rock, and certain wastewater discharges.

Nutrients

Excess nutrients such as nitrogen and phosphorus can accelerate eutrophication in surface waters, a condition that often results in excessive plant growth, declining oxygen levels and changes in the aquatic community. Often, phosphorus is the nutrient in the shortest supply relative to the organisms' needs in fresh water systems, and even a modest increase in phosphorus can set off a chain of undesirable events. This includes accelerated plant growth, algal blooms, low dissolved oxygen, and the death of certain fish, invertebrates, and other aquatic animals. Sources of nutrients can be both natural and human. Natural sources include soil and rocks. Human sources include discharge from wastewater treatment plants, runoff from fertilized lawns and cropland, failing septic systems, animal manure inputs, storm water runoff and disturbed land areas.

Other Important Water Quality Parameters (continued)

Transparency/Water Clarity

Transparency or water clarity is a measure of how well light passes through the water column. Transparency is usually measured with a Secchi disk (for lakes) or transparency tube (for streams), although it can be measured in the field with a light meter. Secchi disk readings are probably the most commonly collected water quality data across the U.S. Transparency measurements are typically made *in situ* (on site) and can be affected by suspended sediment, by algae, and by the color of the water (i.e., humic acids that stain the water red or brownish).

Turbidity

Turbidity is a measure of how much light is scattered by particles in the water. Algal blooms or suspended sediment can increase turbidity because light is scattered by particles in the water, whether those particles are sediment or algae. Other sources contributing to turbidity include soil erosion, runoff from urban and agricultural areas, wastewater and storm water inputs, plant materials and sediment being stirred up by bottom feeders. Materials causing turbidity may also be responsible for clogging fish gills, reducing available habitat, interfering with egg and larvae development, smothering fish eggs and aquatic insect larvae, and suffocating newly-hatched insect larvae. Turbidity is most commonly reported in NTUs (Nephelometric Turbidity Units) and is most accurately measured with a nephelometer which may cost several hundred dollars.

Total Solids

Total solids consist of dissolved and suspended materials in water. Dissolved solids, or those particles that will pass through a filter with pores of around 2 microns (0.002 cm) in size, include calcium, chlorides, nitrate, phosphorus, iron, and sulfur. Total suspended solids (TSS) will not pass through a 2-micron filter and are a direct measurement of the particles suspended in the water - by weight. That means you must collect a sample and take it back to the lab where the water is filtered and dried in an oven, before being weighed. Suspended solids include silt and clay particles, algae, fine organic debris, and other particulate matter. Sediment weighs more than algae, so TSS is a more accurate measurement of how much sediment is in the water, whereas turbidity is affected equally by sediment or algae.

If you collect samples for turbidity or TSS, be sure to shake the container thoroughly before taking a measurement, so whatever has settled out is re-suspended. Neither TSS nor turbidity measurements are affected by colored water.

Biological Communities

Various biological communities can be used to assess stream ecosystem health. Aquatic macroinvertebrates, the animals without a backbone but larger than microscopic organisms, include the aquatic insects, mollusks, crustaceans, and aquatic worms. Macroinvertebrates often are used as indicators of water quality since their tolerance range to pollution varies among species, they are easy and inexpensive to collect, and many are sensitive to both physical and chemical changes in the water. Since they cannot easily escape pollution once it enters, they can be valuable in detecting pollution even after it is no longer detected by chemical methods. Fish may also be used as indicator species. Many fish cannot tolerate low dissolved oxygen concentrations or low pH. Others have narrow temperature tolerances. Some are also sensitive to high turbidity levels, which can clog their gills or interfere with their ability to see their prey.

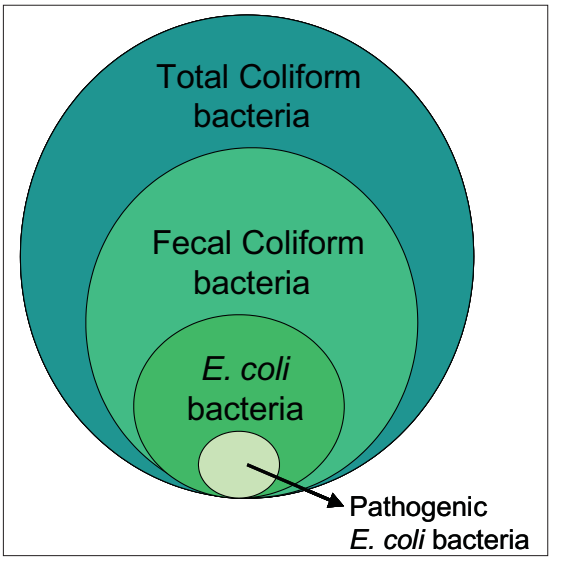
Chapter 2: Bacteria and Water Quality

What are bacteria?

Bacteria are microscopic, single-celled organisms that are the most numerous organisms on earth. They are so small that over five million could be placed on the head of a pin. Bacteria can live in numerous environments and perform many complex actions, some of which are beneficial and some harmful. Most bacteria, however, are not harmful and do not cause human health problems. Those that are disease producing are referred to as pathogenic. Viruses and some protozoans can also be pathogenic.

Coliform bacteria are part of the Enterobacteriaceae family and individual cells cannot be seen with the naked eye due to their small size (but colonies can be seen.) While some coliform bacteria can be naturally found in soil, the type of coliform bacteria that lives in the intestinal tract of warm-blooded animals and originates from animal and human waste is called fecal coliform bacteria.

Escherichia coli (*E. coli*) is one subgroup of fecal coliform bacteria. Even within this species, there are numerous different strains, some of which can be harmful. However, the release of these naturally-occurring organisms into the environment is generally not a cause for alarm. But, other disease causing bacteria, which can include some pathogenic strains of *E. coli*, or viruses may also be present in these wastes and pose a health threat.



Fecal coliform bacteria which include E. coli are part of a larger group of colifom bacteria.

What are indicator bacteria?

The use of an organism that can serve as a surrogate for another is called an indicator organism. Trying to detect disease-causing bacteria and other pathogens in water is expensive and may pose potential health hazards. Further, testing for pathogens requires large volumes of water, and the pathogens can often be difficult to grow in the laboratory and isolate. *E. coli* bacteria are good indicator organisms of fecal contamination because they generally live longer than pathogens, are found in greater numbers, and are less risky to collect or culture

in a laboratory than pathogens. However, their presence does not necessarily mean that pathogens are present, but rather indicates a potential health hazard.

The EPA has determined that *E. coli* are one of the best indicators for the presence of potentially pathogenic bacteria (EPA, 2002b). Because *E. coli* monitoring does not measure the actual pathogens, the assessment is not foolproof, however, it is a good approach for assessing the likelihood of risks to human health. Monitoring for these indicator organisms is an easy and economical method for citizens or professionals to assess health risks due to bacterial contamination of surface waters.

Common sources of *E. coli*

Bacteria in water can originate from the intestinal tracts of both humans and other warm-blooded animals, such as pets, livestock and wildlife. Human sources include failing septic tanks, leaking sewer lines, wastewater treatment plants, combined sewer overflow (CSOs), boat discharges, swimming "accidents" and urban storm water runoff. In urban watersheds, fecal indicator bacteria are significantly correlated with human density (Frenzel and Couvillion, 2002).

Animal sources of fecal coliform bacteria include manure spread on land, livestock in runoff or in streams, improperly disposed farm animal wastes, pet wastes (dogs, cats), wildlife (deer, elk, raccoons, etc.), and birds (geese, pigeons, ducks, gulls, etc.). If you are sampling in a watershed area without significant human impact and are finding *E. coli*, the source may be birds or wildlife. In a study comparing *E. coli* concentrations in waters from agricultural and "pristine" sites, contamination was found in both settings. The researchers deduced that the levels of *E. coli* at the pristine site likely came from wildlife, such as deer and elk, living the area (Niemi and Niemi, 1991).

Common routes of bacteria to streams

How does *E. coli* bacteria get into streams and rivers? Polluted water runoff from the land is the leading cause of water quality problems nationwide (USEPA, 2002a). Fecal material as well as other pollutants can be transported to waterways through runoff. How quickly they are transported partially depends on the type of land use. Non-developed lands including grasses and other vegetation tend to soak up rainfall, thereby increasing infiltration into the ground and reducing runoff to waterways. Developed lands such as streets, rooftops, sidewalks, parking lots, driveways, and other hard surfaces tend to create more impervious surfaces, and runoff increases. Lands that support domesticated animals, such as cattle, hogs, or horses, can also be a source of bacteria, particularly if animals enter the water for drinking or if heavy rains wash manure from the land into receiving waters.

Top: Cattle crossing on a stream in northeast Iowa. Bottom: The crossing keeps the cattle out of the stream. (Photos courtesy USDA NRCS)

Another source of bacteria pollution to stream waters comes from Combined Sewer Overflows (CSOs). Some sewer and storm water pipes are not separated. When a large storm event occurs, the wastewater treatment plants cannot handle the excess volume of water being pumped to them. As a result, untreated sewage along with storm water is dumped directly into rivers and streams.

The presence and levels of *E. coli* in a stream do not give an indication of the source of the contamination. However, it can be a good first step in investigating the watershed for potential sources.

Risks to human health

Most people are concerned about the risk that bacteria may pose to human health. When numbers are above health standards, people exposed to water that contain bacteria may exhibit fever, diarrhea and abdominal cramps, chest pain, or hepatitis. While *E. coli* by itself is not generally a cause for alarm, other pathogens of fecal origin that are health threats include *Salmonella, Shigella,* and *Psuedomonas aeruginosa.* Non-bacterial pathogens that may be present with fecal material include protozoans, such as *Cryptosporidium* and *Giardia*, and viruses.

There are some strains of *E. coli* that are pathogenic themselves. One that has received much attention is the *E. coli* strain named 0157:H7 that lives in the intestinal tract of cattle. This strain is primarily spread to people by eating contaminated, undercooked beef or drinking unpasteurized milk and is not generally found in surface waters.

Examples of at-risk concentration levels

Criteria for concentrations of indicator bacteria in recreational waters (USEPA 1986) have been developed by the USEPA. Initially, total coliform bacteria were used as the benchmark. However, because it was shown that *E. coli* were more closely correlated with swimming-related illnesses, the USEPA later recommended that *E. coli* be used as the indicator in freshwater recreational areas (USEPA 2002b).

Many states have since adopted this recommendation, however, some still use total fecal coliform bacteria when determining concentrations. The acceptable risk level for total body contact recreation, which involves activities such as swimming or water skiing, is 126 colonies of organisms (referred to as colony forming units or cfu) per 100 milliliters (ml) of water or less based on a geometric mean (calculated over 30 days with at least 5 samples) or a one-time concentration of 235 cfu/100 ml. The risk of getting sick increases as total numbers of colonies are exceeded.

The number of colony forming units of *E. coli* organisms per 100 ml of water and the method of determination may vary slightly by state based on State Public Health Codes and Water Quality Standards (See Chapter 7). The USEPA recommends a set of standards for *E. coli* in fresh water bodies as a single maximum allowable count. These rates correspond to an acceptable risk level of 8 people out of 1000 getting sick.

	Designated swimming	Moderate swimming area	Light swimming area	Infrequent swimming area
E. coli (colony forming units/100 ml of water)	235	298	410	576

(from USEPA 1986, 2002b)

Even with good watershed management measures, there will always be fecal material in the environment. If you repeatedly find unusually high levels of *E. coli* on a long-term, regular basis in your stream samples, you should alert and work with your local health agency.

Weather and seasonal influences

The number of bacteria colonies can be influenced by weather and seasonal effects. This variability makes the bacterial concentrations in natural water difficult to predict at any one time. Bacteria numbers often increase following a heavy storm, snow melt or other excessive runoff. *E. coli* bacteria are often more prevalent in turbid waters because they live in soil and can attach to sediment particles. Bacteria can also remain in streambed sediments for long periods of time. If the streambed has been stirred up by increased flow or rainfall, your sample could have elevated bacteria levels. This is why you should avoid disturbing the streambed as you wade out into the stream. You should also collect the water sample upstream from you. If you are collecting at several sites within the stream, collect the furthest downstream sample first and proceed upstream.

A number of other weather influences may affect bacteria levels in the stream. Higher *E. coli* counts may be found in warmer waters because they survive more easily in these waters. (*E. coli* are used to living in the warm environment of the intestines of warm-blooded animals). Ultraviolet rays of sunlight, however, can also kill bacteria, so a warm sunny day may produce numbers lower than expected.

Chapter 3: Preparation for Sampling

Selecting your equipment and supplies

There are several containers that can be used to collect your water sample. One recommended type is the pre-sterilized and disposable Whirl-pak® bags. These plastic self-seal bags are easy to use, carry, and transport. Because they are used only once, they are not re-sterilized. However, sterilized plastic bottles are also acceptable. They can be reused, and they're much sturdier than the bags. However, if bottles are re-used, then both the bottles and lids must be sterilized and sealed before collection. The sterilization procedure calls for the use of an autoclave for 15 minutes at 121°C (USEPA, 1997), which may require assistance from a professional laboratory.

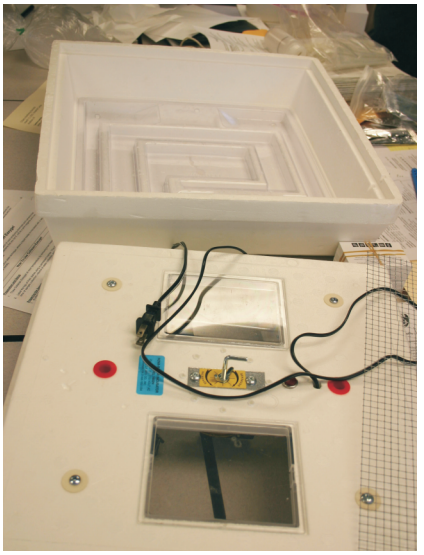
Equipment and supplies checklist

Before going out to a stream, refer to the check-list of the items needed, and make sure you bring them with you.

- ✓ Waders and/or rubber boots (depending on water depth)
- ✓ Bucket with rope or grab sample pole (if sampling from a bridge or water is too deep to enter)
- ✓ Sealed, sterilized, wide mouth bottles (plastic or glass) or Whirl-pak® bags
- ✓ Labels & clear tape to cover them
- ✓ Long rubber/latex gloves—elbow length if possible
- ✓ Clipboard and field data sheets
- ✓ Pencil and Sharpie® marking pen
- ✓ Cooler with frozen ice packs (or ice)
- ✓ Shipping containers
- ✓ First aid kit
- ✓ Personal flotation device (PFD)
- ✓ Monitoring reference sheet
- ✓ Chain of custody record
- ✓ Weather gear: sun-screen and hat for sun protection, rain gear, or cold weather gear
- ✓ Towel for drying off after sampling, if necessary
- ✓ Disinfectant hand wipes, antibacterial lotion or gel

Once you return from the field trip, you will need the following:

- ✓ Space for sample processing with good lighting
- ✓ Incubator or heating lamp and thermometer (if the sample requires incubation)
- ✓ Sterilized laboratory supplies
- ✓ Paper towels or Kimwipes
- ✓ Isopropyl alcohol
- ✓ Latex gloves
- ✓ Bleach and water-tight bag for sample disposal



A temperature-controlled egg incubator can be used for incubating the samples.

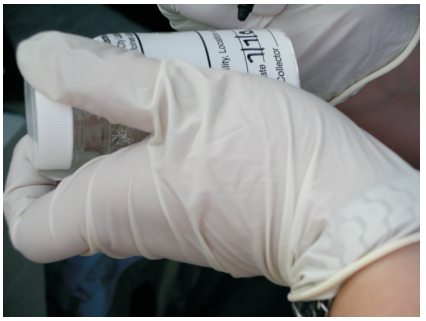
Use of an incubator

Several kits require that the sample be incubated. If this is the method you are using, you will need to either make or purchase an incubator to help the *E. coli* colonies grow once you have collected the water samples and plated them. You can buy an egg incubator for about $40 to $50. Use a small cup or tray to add water (deionized if possible) to keep the Petri plates/films from drying out. Incubation time will generally run 24 hours to 48 hours for *E. coli*, depending on the type of kit used.

Labeling and identification of bottles

It is advisable to use a specific system to assign a site number to your sampling locations. One option is to begin with the two-character abbreviation for your state. Next, use the assigned two digit county code that is pre-assigned for each county in a state. Follow this number with a sequential site number. For example, if Iowa's volunteers will be monitoring Prairie Creek in Boone County (county code 08) at 2 locations, the first site would be IA0801 and the second site would be IA0802. Organizations may have their own system of labeling.

When preparing the bottles:



- ✓ Stick tape over the lid to indicate that it has been sterilized
- ✓ Prior to collecting the sample, label each bottle with the location/sample number, time and date of sampling, initials of sample collector and type of sample
- ✓ Cover label with tape for water-proofing
- ✓ Wrap labeling tape around the circumference of the bottle. This will prevent the tape from coming off when the bottle gets wet. Do not, however, cover the lid with the tape
- ✓ Mark replicate samples with an *"R"* or appropriate marking
- ✓ Label 10% of your bottles as field blanks. Only distilled water will be added to these bottles

Safety is most important!

When sampling in a stream, always bring along a partner. It's also recommended that you inform people of where you are going and when you plan to return. It is advisable to carry a cellular phone with you in case of an emergency.

Other important tips include:

- ✓ Obtaining permission from the landowner, if needed
- ✓ Listening to weather reports prior to leaving and rescheduling the sampling if severe weather or temperatures are on the way. (Try www.weather.com for current weather conditions)
- ✓ Dressing appropriately for the weather conditions
- ✓ Bringing a first aid kit with you
- ✓ Parking your vehicle in a safe location so that you do not block traffic. Keep your keys in a safe and secure location
- ✓ Avoiding sampling in areas with very steep or unstable banks and making sure you can access the stream safely while wearing waders
- ✓ Wearing waders or rubber boots to help protect you from cold water and sharp rocks or surfaces in the streambed
- ✓ Making sure the water depth is not so deep nor the stream flow so swift that you risk losing your footing and being carried downstream
- ✓ Wearing a personal flotation device (PFD) while wading in the stream, if needed
- ✓ NOT entering the stream if you observe chemical, oil, or other hazardous substances in or discharging to the water

Once you return to your vehicle and/or home, wash your hands and be careful not to touch your eyes or mouth when processing your water samples.

You should consider reviewing the safety section of the USEPA's *Volunteer Stream Monitoring: A Methods Manual* (see Chapter 9) prior to field sampling.

Site selection

Your selected site should align with the goals of the study. When determining where you should sample, start with a USGS topographic map or similar map of your watershed and determine the extent of the stream and its tributaries (other streams entering the stream in question). If you have Internet access, several online sites listed at the end of this manual provide online maps that can give you latitude/longitude or other locational information. Sampling near a USGS gauging station will help with site identification and allow you to assess *E. coli* results with stream flow data (waterdata.usgs.gov/nwis/rt).

If your stream has many tributaries feeding into it, a site both upstream and downstream of the incoming water can help you determine if a specific tributary or sub-watershed is contributing more *E. coli* than another. If you are doing an impact assessment of a particular activity, you may also want to select sites

above and below the suspected area. However, try to select far enough downstream from stream convergences to allow even mixing of the waters.

As stated in your checklist, if the site is on private land, be sure to obtain written permission to sample prior to going on-site, or find a publicly accessible site instead.

When to sample

The number of times that you'll need to sample varies and depends on what you want to know. The more you sample, however, the better information you'll have when interpreting your data. At a minimum, it is recommended that you sample one time per month between May and September. You should also try to be consistent as to the time of day you sample and the interval of time between sampling. These factors help in the comparison of your data over time. If you have the opportunity to do so, also try to sample just after a relatively heavy storm. Remember that when and how often you sample will depend on the goals of your local program.

Wet versus dry weather sampling may help you identify general sources of the bacteria. For example, if you sample during dry weather, continuous sources will be more easily detected, such as leaking septic tanks or wildlife. If you sample after wet weather, sources that would increase in-stream bacteria levels due to runoff, such as storm water outfalls or field runoff, may be easier to identify. In a study of streams in agricultural and pristine areas, samples collected on wet days contained higher bacteria concentrations than on dry days (Frenzel and Couvillion, 2002).

Quality assurance/Quality control

You've likely heard the term QA/QC. It stands for Quality Assurance/Quality Control. Quality assurance is a method of maintaining quality in all practices and procedures used during your project. Quality control procedures assure that samples are being collected in a consistent and accurate manner at all sites and from all volunteer monitors.

Quality assurance measures include:

- ❑ Assigning responsibilities to volunteer members
- ❑ Training volunteers in collection techniques, handling of equipment, and analysis of samples
- ❑ Calibrating instruments
- ❑ Specifying procedures for field analyses
- ❑ Keeping accurate records of all procedures and conditions.
- ❑ Following chain of custody procedures or tracking samples from their collection in the field to final analyses or destination

Quality control measures include:

- ❑ Blank samples in the field: sampler fills a bottle at the bank of the stream with distilled water at 10% of your sampling sites or 10% of the times you sample. (This sample is plated as usual with the rest of your samples and helps identify contamination errors in the field)
- ❑ Field replicates: taking additional samples with another bottle(s) at 5-10% of your monitoring sites. (This method helps assess variability in the stream)
- ❑ Control plates: plating with distilled water to assure no lab contamination, or plating with a known quantity of sample
- ❑ Split samples: two different analyses from the same sample. In this case, it could involve sending the same sample to another lab for independent analysis
- ❑ Lab replicates: plating two or more separate plates from 1 bottle. (This technique helps assess the variability of the techniques of the person doing the plating and reading)
- ❑ Regular inspection of equipment, growth media, and other items being used

It is important that all volunteers use the same procedures so that samples within and between streams can be compared to each other. Consistency and keeping good field notes is key! Occasionally you may have staff from your local health agency taking side-by-side samples and readings with you to compare results.

The closer you adhere to QA/QC measures, the more confident you and others can be about your data results. Recognition of the importance and continued use of QA/QC protocols are good ways to assure agencies and the public that your data are worth considering.

The USEPA discusses the five key components of QA/QC:

- **Accuracy**: how similar your results are to a true or expected value.
- **Comparability**: the degree that data can be compared between sampling sites or across time.
- **Completeness**: how much data you planned to gather versus how much you actually were able to collect.
- **Precision**: how reproducible your results are, the level of consensus between repeated measurements.
- **Representativeness**: how much your data characterize the true environmental condition when the sample was collected (USEPA, 1996).

Why use replicates?

In the stream, bacteria concentrations can be highly variable since they often grow in clumps, so taking several samples can be very important. Variability can also occur during the transfer of water from one bottle or bucket to another bottle, during plating and culturing the bacteria, and in counting the colonies. Replicates (in duplicates or triplicates) help identify and minimize variability in the sample. Replicates can be two or more samples taken from the same collection bottle or bucket and transferred to other collection bottles or be two separate samples with separate containers taken at the same time at the same place. Split samples always come from the same collection bottle. When sending a replicate to a laboratory for verification, you should use a split sample. As a general rule, replicate samples should be taken at 10% of your monitoring sites or 10% of the time you sample.

Chapter 4: Field Sampling

Site assessment - Choosing a site within a stretch of stream

Safety should be a priority when selecting a sampling site. First make sure the stream has flowing water and that you can reach the site without difficulty. Look for uniform flow across the main streambed. Walk about 60-100 feet upstream and downstream to assess each site and conditions of the bank. Check for any obvious pollutant sources, such as storm water outfalls, lake/pond outflows, or sewage input. If the source is too close to your sampling site, your bacteria samples may not be representative of the stream overall. If the site is acceptable, take pictures, if possible, and be sure to thoroughly describe the site on your datasheet. Identify landmark features, such as crossroads and bridges or unique vegetation, that will help you or another person find your site again.

In-stream field collection

Once you're in the field, it is important to record all information. Forms may include a bacteria data sheet and site description form.

There are several methods for obtaining a sample from the stream depending on stream access, the depth of water, and safety. If you can safely enter the stream, you should obtain your sample where the main current is flowing. As you are wading into the water, try to disturb as little sediment as possible so that the sample is not contaminated by bacteria attached to or living in the soil. You should position yourself downstream of the sampling point (i.e. hold the bottle upstream of your body) so that if sediments are stirred up they won't affect your sample. If a stream site is curved, sample near the outside of the curve. Before entering the water, make sure your sample bottles are labeled correctly and completely.

If you cannot safely access the water, you should sample from a bridge following the procedures at the end of this section. If conditions are safe and you are a skilled boater, you may also sample from a canoe in the stream. If possible, do not take the sample at the stream bank's edge since the water may be stagnant or not well mixed with the rest of the water.

If sampling within the stream, follow these steps:

- ❑ Take 1-2 steps upstream, reach out your arm, and collect the sample upstream from where you are standing. It is recommended that you wear rubber gloves.
- ❑ Open the bottle and remember to not touch the inside of the bottle or the cap with your hands.
- ❑ Rinse the bottle and lid three times.
- ❑ Hold the bottle near its base and plunge it with the top facing downwards into the water to 3-5 inches below the surface or at approximately wrist level. Don't worry if you cannot get the bottle to this exact distance. Just try to avoid sampling water from the surface.
- ❑ Turn the bottle into the current (upstream) and wait for it to fill.
- ❑ Bring the bottle up, pour out some water so that there is 1 inch of air space and close and tighten the bottle with its lid or cap.
- ❑ Place the sample in a cooler with ice packs to be transported back to your house or wherever the tests will be done.
- ❑ Be sure to record all necessary information on field data sheets.

If Whirl-pak® bags are being used instead of bottles, follow these steps:

- ❑ Correctly label the Whirl-pak® bag with indelible marker.
- ❑ Remove the perforated seal from edge of Whirl-pak® bag.
- ❑ Use the two small white tabs to open the bag.
- ❑ Place the bag in the water below the surface and allow the water to flow into the bag.
- ❑ Grab the ends of the twist ties and "whirl" the bag shut.
- ❑ Make sure the bag is securely closed by testing the seal.
- ❑ Place the Whirl-pak® bag in a cooler with frozen ice packs.

If you are collecting your sample with a bucket or other container from a bridge, the following steps are recommended:

- ❑ Attach the bucket/container to a secure rope and lower it into a fast flowing section of the stream.
- ❑ Rinse the bucket/container three times with the stream water.

- ❑ Rinse the sample bottle three times.
- ❑ Do not let the rope, bucket/container or bottle touch the ground.

To minimize exposure to potential pathogens in the water, use disinfectant wipes or gel to wash up after sampling, as a preventive measure.

If you are taking a pipette sample directly from the water, you should:

- ❑ Unwrap the sterile pipette and do not touch its tip
- ❑ Squeeze the bulb of the pipette, lower it into the water to wrist level, and then release the bulb while the pipette is under water
- ❑ Remove the pipette from the water and adjust water volume in the pipette to the exact marking (1 ml)
- ❑ Squirt the water from the pipette into the collection bottle

Packaging your water samples for shipping

All samples taken should be analyzed within 24 hours. So, if you need to ship your water samples to an analytical lab, try to collect them in the early part of the week and no later than a Wednesday to allow time for the lab to process them prior to the weekend. Make arrangements with your mail carrier prior to sampling to make sure the samples will be collected promptly and delivered within 24 hours. On the day of sampling, you will need to sample early in the day so the samples can be shipped out the afternoon of the same day.

When shipping, make sure the bottles are secure, cold, and not going to leak. You should consider:

- ✓ Using a plastic garbage bag to line the shipping container to prevent leaks of water.
- ✓ Sealing each sample in its own plastic bag to prevent any cross-contamination and to contain the sample in case of leaks or breakage.
- ✓ Packing the samples with ice or ice packs.
- ✓ Using a Styrofoam container, cooler, cardboard box, or specialized water sample shipping container.

Be sure to fill out the sampling form completely, the chain of custody form, and any other paperwork, and place them on the top of the container before sealing the box. You may want to first seal the paperwork in a large zippered storage bag. Finally, attach the provided pre-addressed, pre-paid mailing label and ship overnight.

Chapter 5: Use of Kits

Value of volunteer analyses

The expense of sending E. coli samples to a commercial laboratory for analysis can be costly over time. Completing the analyses at your "home lab" is one way to determine E. coli levels in your stream without excessive costs. Through your work, you also help extend limited agency resources for water quality assessments.

General methods and procedures with kits

For the most reliable results, USEPA recommends that you should prepare your sample for analysis within 6 hours of taking it (USEPA, 1997). In many cases it is not possible to meet this recommendation, but samples should not be held longer than 24 hours. In all cases, you should store your samples on ice before lab analysis, and the quicker you get your sample processed the less chance there is for variability. Make sure you indicate on the data sheet the length of time between collecting and processing.

Regardless of the kit used, it is essential that you maintain sterile conditions while filtering and plating, since this is the time with the greatest potential for external contamination of the samples. Thus, it is recommended that you do your plating all at once in the lab and not at the field site. Sanitize your working surface by spraying or wiping it with a 70% isopropyl alcohol solution or with bleach.

You should also:

- ✓ Wash your hands thoroughly with soap
- ✓ Have the following with you: paper towels or wipes; isopropyl alcohol, distilled water, waste container, permanent marker and gloves
- ✓ Label both your bottles and plates/films with the date, time, sampling site number, and replicate number (if applicable). For the petri dishes, make sure the written information does not interfere with your ability to read the plate.
- ✓ Always shake your sample bottle before drawing a sample with a pipette

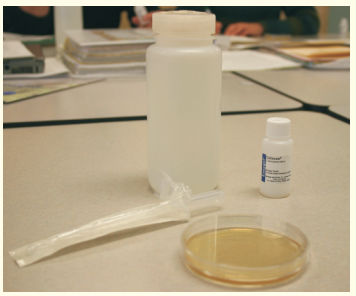
There are many kits on the market that are being used for determining *E. coli* numbers in water. During the research phase of this project, five kits and variations within the kits were tested by volunteers. Their results were compared with laboratory results. Four of the five methods were found to be acceptable. However, when ease of use, volunteer preference, and economics were added to the equation, one kit, 3M™ Petrifilm™, stood out over the others.

Methods and procedures using Coliscan® Easy Gel® (incubated)

The following information comes from the Indiana Hoosier Riverwatch Program and the Iowa IOWATER program.

Coliscan media incorporates a patented combination of color-producing chemicals and nutrients that make *E. coli* colonies appear blue, coliform bacteria that are not *E. coli* as a pink magenta and non coliforms as white or teal-green colonies. Coliscan® Easygel® employs a pour plate technique, where a liquid media is inoculated with a sample and poured into a Petri dish to solidify.

Preparation and Setup



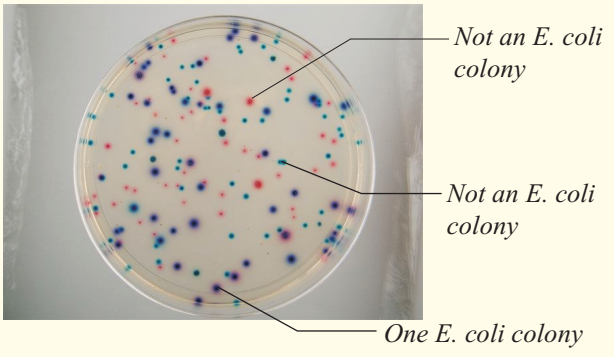
1. Thaw Coliscan® Easygel® at room temperature by removing from freezer before sampling.
2. Label the bottom of Petri dishes using a permanent marker. This label should include site ID, date and time of sample collection, volume of water collected, and sample number.

Preparing the Sample

1. Always SHAKE sample collection bottle before drawing a sample with a pipette!
2. Using a sterile pipette, transfer 0.5 – 5 mL of stream sample directly into the Easygel bottle.
3. Swirl the Coliscan® Easygel® bottles to mix the contents and pour each bottle into the already labeled Petri dishes. Gently swirl the mixture in the Petri dish making a figure eight on the tabletop with the dish until the mixture is evenly distributed, being careful not to splash over the side or on the lid.
4. Place the Petri dishes on a level location out of direct sunlight for 45 minutes to 1 hour. The mixture will solidify on the bottom of Petri dish.

Incubation and Interpretation

Invert the Petri dish(es) and incubate at 35 degrees Celsius for 24 hours. After incubation is complete, count the colonies. Do not count "pin-point" sized colonies. *E. coli* colonies appear blue, dark blue, or purple. Other coliforms appear pink/magenta, and non-coliforms appear white or teal green.



Sample Disposal

1. Carefully place about a teaspoon of household bleach onto the surface of the Coliscan® Easygel® of each plate.
2. Allow to sit at least five minutes.
3. Place in watertight bag and discard in normal trash.

Methods and procedures using 3M™ Petrifilm™

The following information comes from the Indiana Hoosier Riverwatch Program and the Iowa IOWATER program.

Storage and Disposal

Store unopened Petrifilm plate pouches at temperatures <8°C (46°F) – REFRIGERATE!

Official 3M Instructions

Return unused plates to pouch. To prevent exposure to moisture, do not refrigerate opened pouches. Store resealed pouches in a cool, dry place for no longer than one month. Exposure of Petrifilm plates to temperatures greater than 25°C (77°F), and/or humidity greater or equal to 50% relative humidity can affect the performance of the plates.

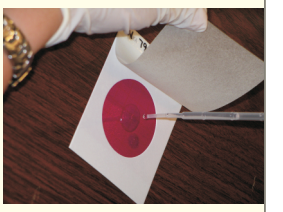
Citizens Monitoring Bacteria Research Project Instructions

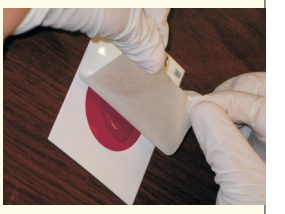
Store plates from opened packages in sets of no more than 8 in a small "snack-size" ziplock or similar type storage bag. Place a weight on top of the package to keep it from curling. Plates may be stored for up to a year.

Allow pouches to come to room temperature before opening – at least 10-15 minutes.

Do not use plates that show orange or brown discoloration.

Expiration date and lot number are noted on each package. (Example expiration date: 2007-10, would expire in the 10th month (October) of the year 2007. The lot number is also printed on individual plates.





Plating

Inoculate and spread one Petrifilm plate before inoculating the next plate.

1. Place a Petrifilm plate on a level surface.
2. Lift the top film and dispense 1 ml of sample or diluted sample on the center bottom film.
3. Slowly roll the top film down onto the sample to prevent trapping air bubbles.
4. With the smooth side down, place the plastic spreader near the top of the plate.
5. If necessary, distribute sample evenly using gentle downward pressure on the center of the plastic spreader.
6. Remove the spreader and leave plate undisturbed for at least one minute to permit the gel to solidify. Incubate plates in a horizontal position, with the clear side up in stacks of up to 20 plates. Incubator should be humidified with distilled water. Incubate 24 hours at 35°C.

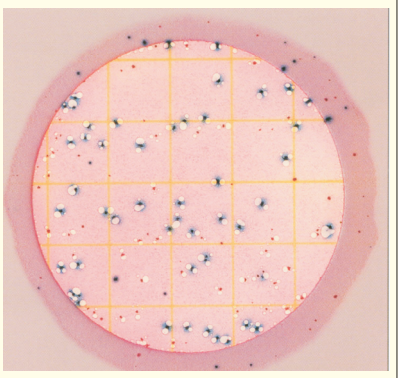
Count blue colonies with gas bubble(s) after 24 hours at 35°C

3M™ Petrifilm™ (continued)

Interpretation

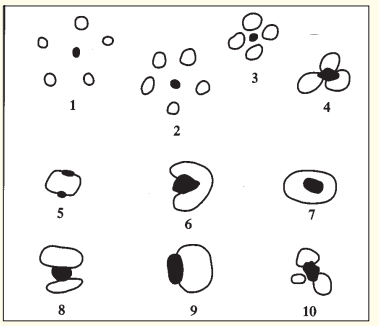
1. Petrifilm *E. coli* plates can be counted on a standard colony counter or other magnified light source. Only count colonies within circle. Do not count artifact bubbles. Approximately 95% of *E. coli* produce gas.

2. In general, *E. coli* colonies are blue to blue-purple and closely associated (approximately one colony diameter) with entrapped gas. General coliform colonies are bright red and closely associated (approximately one colony diameter) with entrapped gas (figure below). **Only count colonies that have one or more associated gas bubbles!**



This plate has 49 E. coli colonies as depicted by blue colonies with gas. (From 3M™ Petrifilm™ interpretation guide)

3. The circular growth area is approximately 20 cm^2. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine total count per plate.



All 10 examples depict various bubble patterns associated with gas producing colonies. Each numbered picture would be counted as one colony. (From 3M™ Petrifilm™ interpretation guide)

4. Petrifilm *E. coli* plates with colonies that are too numerous to count (TNTC) have one or more of the following characteristics: many small colonies, many gas bubbles, and deepening of the gel color. High concentrations of *E. coli* will cause the growth area to turn blue while high concentrations of coliforms (non-*E. coli*) will cause the growth area to turn dark red. When any of these occur, you will not be able to count the sample – and should write TNTC on the data sheet. Next time, you may want to use less sample if the stream is under similar conditions.

Disposal

Place the Petrifilm plate in a sealed Ziplock or similar type bag with the Easygel plates that have already been treated with bleach. The excess bleach will spill out and disinfect the Petrifilm plates, too. Discard with regular trash.

Further Information

http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/products/petrifilm-plates/

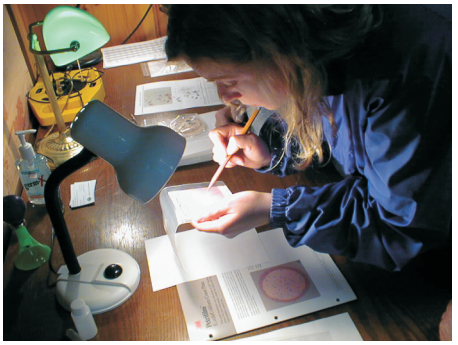
Other Kits

Other kits on the market are being used for *E. coli* analysis. Appendix D (beginning on page 45) provides information on three additional kits. Further information on these and other kits can be obtained from the manufacturer or on various web sites.

Chapter 6: Sampling Results

Reading the Results

After removal from the incubation unit, colonies of bacteria with a particular color are counted. The normal incubation time is 24 hours, but if the colonies are not developed enough, wait a total of 48 hours. The E. coli colonies will stand out from general coliforms because they will turn a distinct color. The exact color depends on the test method used. Place the plate on a grid and place a white sheet of paper as a background. Count colonies that are visible to the naked eye. Be sure to have adequate lighting. Sometime it helps to use a pen to mark on the outside of the plate the colonies you have already counted. If there are more than 200 colonies per plate, report this as "too numerous to count" (TNTC) since the colonies are not considered distinct enough for an accurate reading.



The standard reporting unit is colony forming units per 100 ml of water sample (cfu/100ml). To determine the number of colony forming units (cfu) per 100 ml of water sample, the following steps should be taken:

STEP I. Count the number of colonies of the color specified in the test kits you are using and record that number:	Let's assume you counted 6 colonies
STEP II. Take the amount of sample water used and divide it into 100 since you want to report your sample per 100 ml of water:	Assume you used a 5 ml sample Thus, 100 / 5 = 20
STEP III. Now, multiply the number of colonies you counted in step #1 by the number you obtained in step #2:	6 x 20 = 120
STEP IV. You have now determined the number of colony forming units per 100 ml of sample:	120 cfu / 100 ml

Averaging Samples

If you want to obtain an average of replicate samples, and the amount of sample used varies in each replicate, you must first count the total number of colonies in each sample, add them together, and then divide by the total milliliters of sample. Then, multiply both numerator and denominator by 100 to obtain total number of colonies per 100 ml. In the example below if you simply took an average of the three replicate sample totals (1200 + 1100 + 900)/3, your answer would be 1066.6 colonies/100ml which would be incorrect.

Sample Number	Number of ml Used	Colonies Counted	Total # / 100 ml	Average # / 100ml
1	1	12	1200 / 100 ml	**12 + 33 + 45 / 1 + 3 + 5 = 90 colonies / 9ml or 10 colonies / ml** **Thus, the average equals 1000 colonies / 100 ml**
2	3	33	1100 / 100 ml	
3	5	45	900 / 100ml	

Disposal safety

After counting the colonies of bacteria on the plates, add ¼ teaspoon of household bleach using either a dropper or other dispensing unit to each plate. Be careful not to get the bleach on your hands or clothes. Place the plates in an airtight ziplock or sealable plastic bag and seal it shut. Finally, dispose of the bag in the trash. Do not be overly apprehensive with this step, since in general, *E. coli* do not pose a huge health risk.

Chapter 7: Interpreting Results

State standards

Using guidance provided by the USEPA, states have developed standards for fecal coliform bacteria and/or E. coli. Compliance is often based on the arithmetic mean of three or more samples taken during the same sampling event at representative locations within a defined sampling area or on the geometric mean based on at least five samples taken over a 30-day period or a total number of samples collected over a specified monitoring period.

State	*E. coli or* Fecal coliform	Water Use	One-time Standard	30-day Geometric Mean
Indiana	*E. coli*	Primary bathing contact. This standard only applies April to October (the recreation season). From November to March, there is no standard.	235 colony forming units (cfu)/100ml	125cfu/100ml
Iowa	*E. coli*	Full contact recreation	235 cfu/100ml	126cfu/100ml
Michigan	*E. coli*	Full body contact recreation	300 cfu/100ml (3 or more samples)	130cfu/100ml
Minnesota	*E. coli**	Full body contact recreation	1260 cfu/100ml	126cfu/100ml
Ohio	*E. coli*	Primary bathing contact	298 cfu/100ml (not exceeded in more than 10% of samples)	126cfu/100ml
Wisconsin	Fecal coliform	Recreational Waters	400 cfu/100ml (not exceeded in more than 10% of samples)	200cfu/100 ml
	*E. coli***	Beach Closures	235 cfu/100ml	126 cfu/100ml
YOUR STATE				

**Proposed in September 2007*

***EPA Guidelines* (see page 10 for other *E. coli* standards in fresh water bodies)

Determining the geometric mean

E. coli concentrations are reported as colony forming units (cfu) per 100 ml of water sample. When measuring *E. coli* concentrations over time, using the geometric mean is a useful reporting tool. The geometric mean takes into account that a few extreme counts may be found among many more closely grouped values. Calculating a geometric mean provides a number that is more representative of the median (or that number where half the samples are higher and half are lower) and helps reduce the effect of a few extreme values. Also, use of a geometric mean over time (often 30 days) minimizes fluctuations in the levels of bacteria in the water or one-time high counts. The 30-day geometric mean helps determine if a stream has a continually high level of *E. coli*.

The geometric mean (GM) can be calculated as follows:

$$GM = (s1 \times s2 \times s3 \times sn)^{1/N}$$

Where "s" is the number of *E. coli* colonies per 100 mls for samples 1, 2, 3, though the n^{th} sample, and N is the number of samples collected.

For example, let's say you have 5 samples and your counts of cfu/100ml at one site over a 30-day period were:

5, 10, 120, 20, 2600

The geometric mean would be determined by taking the 5th root of the product of the 5 readings:

$$(5 \times 10 \times 120 \times 20 \times 2600)^{1/5} = 50$$

If you had just taken an average of the five samples for the 30-day period, your answer would be:

$$(5 + 10 + 120 + 20 + 2600) = 2755$$

and

$$2755/5 = 551$$

The simple average does not reflect the typical value of the set of numbers as well as the geometric mean does, nor does it take into account the one result that is much higher than the others.

Note: The geometric mean can only be used with positive numbers greater than zero.

Getting "high" bacteria counts

If you find a "high" bacteria count (over your state's standard for a one-time sampling), it may be a one-time event or occurrence. This information is useful, but before taking further action, you should return to the site to take more samples. When you return, pay careful attention to anything out of the ordinary at the site. Look for the presence of animals and be alert for any unusual odors. Walk the banks again to look for obvious sources of pollution (see Chapter 2), and note past and current weather conditions. Continue to sample and contact your local health agency if numbers remain high. Be sure to wear long rubber gloves while sampling and wash your hands carefully afterwards.

If you do find a high *E. coli* count what steps should you take? Generally, you should:

- ❑ Continue to monitor the site. This will help identify if there is a chronic bacteria problem or a high count resulting from a one-time event.
- ❑ If you continue to find a high count, work through your volunteer monitoring program to alert your local agency.

You may wish to alert your local watershed group or local agency about your monitoring efforts and the results so far. These groups will likely have an interest in your results regardless of whether or not you have detected a problem. They may be able to work with you on determining the possible sources of *E. coli* pollution if a problem does exist.

Tracking, storing and retrieval of data

Keep track of your *E. coli* data on a spreadsheet (electronic, if possible) or data form (see Appendix B for a sample data sheet). An electronic spreadsheet may be advantageous in that it allows for easy calculations to show ranges, pollutant loads, or to make graphs. After entering the results on your data sheet, mail or fax this to your program leader as promptly as possible.

Alternatively, you can enter the data on the *E. coli* electronic database website developed as a part of this project. It can be accessed at www.iwr.msu.edu/cmb. The site is password protected; however, the password can be obtained by emailing any of the contacts listed near the beginning of this manual.

Source tracking

One method for determining sources of *E. coli* is called bacterial source tracking. Bacterial Source Tracking (BST) is a collective group of new methodologies being developed to determine sources of fecal pollution in environmental samples. Sources of fecal pollution may come from domestic pets, cows, deer, geese, hogs, other wild animals, and humans.

If used successfully, BST methodologies have the potential to turn nonpoint (diffuse) sources into point sources. Current BST research is being driven by the recent implementation of the Total Maximum Daily Load (TMDL) concept by EPA. BST methods represent the best tools available for determining sources of fecal pollution in water and should be an integral part of any project that involves TMDL development for fecal coliform. BST methods can also be used in the design and implementation of Best Management Practices to reduce fecal loading in water.

Currently, both molecular (genotypic) and biochemical (phenotype) BST methods are under development. DNA fingerprinting has received the greatest publicity, but numerous methods show potential. Most researchers believe that some combination of BST methods will be needed to provide the most accurate and reliable source identification answers. It is doubtful that any one BST method will emerge as the "best" method for all situations.

While this is not a procedure that the volunteers will be conducting, it is a procedure to be aware of, and a possible step that state agencies might take. At this point, it is still an emerging and costly technology, even for agencies, so it is not used routinely.

Pollution prevention actions you can take

Our valued streams and rivers are subject to pollution stress from land uses in the watershed. These pollutants come from many sources, including those around our own homes. You can practice certain activities that can help reduce water pollution risks from bacteria. Some examples may include:

- Planting any bare soil with native grasses, shrubs, or other plants. The roots of these plants will help contain the soil from running off into the nearest stream.
- Cleaning up after your pet. Pet wastes can be a source of *E. coli* and excess nutrient contamination in our waterways. Pet wastes can make their way from the lawn to a river, so dispose of wastes in the toilet or trash.
- Draining roof downspouts onto vegetated areas, not on the street or pavement, so that water can soak into the ground.
- Limiting paved surfaces; landscape with rocks, plants, or gravel.
- Supporting active interaction, communication, and education between technical advisors and land users.
- Encouraging community appreciation of watershed health through community events, e.g. outdoor sports, river cleaning, and other events.

Chapter 8: Conclusions

The purpose of this training manual is to discuss sampling and monitoring techniques for E. coli and to highlight the test kits that are reliable, economical and usable by volunteers. However, it is important to keep in mind that bacteria monitoring is only one component of water quality monitoring, and that E. coli data alone do not indicate the ecological health of your stream. They do, however, provide valuable information that can be used in concert with other monitoring data to help assess overall ecosystem health.

Volunteer time is valuable, and the remarkable power of your efforts is your positive impact on the environment and the enthusiasm and commitment of your teams. By using standardized sampling and analysis procedures along with acceptable test kits, the *E. coli* data you collect as a volunteer can be very useful and utilized in various watershed programs. The bacteria monitoring data you collect and disseminate will help determine baseline conditions, provide continued data on your stream, and assist in assessing future water quality trends. It can help build partnerships with agencies and other groups from the local to federal level.

By remaining vigilant in your monitoring efforts, water quality problems can often be targeted and addressed before they become major.

Notes

Chapter 9: Resources for Further Information

Internet sites

Center for Disease Control's information on the pathogenic *E. coli* 0157:H7
www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.htm

The **Center for Watershed Protection** provides local governments, activists, and watershed organizations around the country with the technical tools for protecting our streams, lakes and rivers.
www.cwp.org/

Volunteer Water Quality Monitoring National Facilitation Project is designed to expand and strengthen the capacity of existing Extension volunteer monitoring programs and support development of new groups. www.usawaterquality.org/volunteer/

Building Capacity of *E. coli* Monitoring By Volunteers: A Multi-State Effort is the web site that complements this training manual. www.uwex.edu/ces/csreesvolmon/EColi/index.html

EPA: **Microbiology** homepage: www.epa.gov/nerlcwww/

EPA: **National Newsletter of Volunteer Water Quality Monitoring**
www.epa.gov/owow/monitoring/volunteer/issues.htm

EPA: **STORET** (short for STOrage and RETrieval) is a repository for water quality, biological, and physical data. www.epa.gov/storet/

EPA: The **Volunteer Monitor's Guide To Quality Assurance Project Plans**
www.epa.gov/owow/monitoring/volunteer/qappcovr.htm

Michigan State University's **Digital Watershed**: Type in any address and obtain an aerial photograph as well as data on the watershed. www.iwr.msu.edu/dw

Purdue University's **stream delineation** site: Pick your stream from an interactive map. Click on a portion of the stream and the tool delineates the watershed of the stream from that point to upstream.
pasture.ecn.purdue.edu/~watergen/owls/htmls/select_your_state.htm

U.S. Geological Survey's **Water Science Glossary** of Terms.
ga.water.usgs.gov/edu/dictionary.html

Water Resources of the United States (U.S. Geological Survey) Access to water-resources data.
water.usgs.gov/

Volunteer stream monitoring manuals

Volunteer Stream Monitoring: A Methods Manual, US Environmental Protection Agency
www.epa.gov/volunteer/stream/stream.pdf

Volunteer Stream Monitoring Training Manual, Hoosier Riverwatch, Indiana Department of Natural Resources - http://www.in.gov/dnr/riverwatch/trainingmanual/

Volunteer Surface Water Monitoring Guide, Minnesota Pollution Control Agency
http://www.pca.state.mn.us/water/monitoring-guide.html

Vermont Citizen's Guide to Bacteria Monitoring in Vermont Waters, Department of Environmental Conservation - http://www.anr.state.vt.us/dec//waterq/lakes/docs/lp_citbactmonguide.pdf

Washington State's Department of Ecology, A Citizen's Guide to Understanding and Monitoring Lakes and Streams - http://www.ecy.wa.gov/programs/wq/plants/management/joysmanual/

Watershed Watch (University of Rhode Island) - http://www.uri.edu/ce/wq/ww/Manuals.htm

Wisconsin Water Action Volunteers Citizen Stream Monitoring
http://watermonitoring.uwex.edu/wav/monitoring/methods.html

Other Guides to Volunteer Monitoring can be found on the National Volunteer Monitoring website at:
http://www.uwex.edu/ces/csreesvolmon/links.html

Watershed and stream management guides

A **Beginner's Guide to Water Management - Bacteria,** University of Florida
edis.ifas.ufl.edu/FA103

Developing a Watershed Plan for Water Quality: An Introductory Guide (Michigan)
www.deq.state.mi.us/documents/deq-swq-nps-Watershe.pdf

Getting to Know Your Local Watershed - A Guide for Watershed Partnerships
www.ctic.purdue.edu/KYW/Brochures/GetToKnow.html

Indiana Watershed Planning Guide from the Indiana Department of Environmental Management, August 2003. http://www.in.gov/idem/catalog/documents/water/iwpg.pdf

Michigan Department of Environmental Quality's **Stormwater Management Guidebook**
http://www.deq.state.mi.us/documents/deq-water-sw-links-SW_Management_Guidebook.pdf

Minnesota Shoreland Management Resource Guide - www.shorelandmanagement.org/quick/

Ohio **Stream Management Guide** fact sheets - www.dnr.state.oh.us/water/pubs/fs_st/streamfs.htm

Rapid Watershed Planning Handbook: A Comprehensive Guide for Managing Urbanizing Watersheds. 1999. Center for Watershed Protection. Ellicott City, MD

U.S. Geological Survey: **National Field Manual** for the collection of water-quality data
water.usgs.gov/owq/FieldManual/

Wisconsin Department of Natural Resources **Runoff Management**
http://www.dnr.state.wi.us/runoff/about.htm

Chapter 10: References

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Niemi, R.M. and J.S. Niemi (1991) Bacterial pollution of waters in pristine and agricultural lands. Journal of Environmental Quality. 20:620-627.

Overdevest, C., C. Huyck Orr, and K. Stepenuck (2004) Volunteer stream monitoring and local participation in natural resource issues. Human Ecology Review. Vol. 11(2): 177-185.

USDA Natural Resources Conservation Service photo gallery http://photogallery.nrcs.usda.gov

USEPA (1986) Ambient Water Quality Criteria for Bacteria EPA 440/5-84-002. Office of Water. Regulations and Standards. Criteria and Standards Division. January 1986.

USEPA (1996) The Volunteer Monitor's Guide To Quality Assurance Project Plans. (http://www.epa.gov/owow/monitoring/volunteer/qappcovr.htm) EPA 841-B-96-003. Office of Wetlands, Oceans and Watersheds. September 1996.

USEPA (1997) Volunteer Stream Monitoring: A Methods Manual. (www.epa.gov/OWOW/monitoring/volunteer/stream) EPA 841-B-97-003. Office of Water. November 1997.

USEPA (2002a). Water Quality Conditions in the United States: 2000 National Water Quality Inventory. EPA-841-R-02-001. August 2002.

USEPA (2002b). Implementation Guidance for Ambient Water Quality Criteria for Bacteria (Draft). May 2002 (www.epa.gov/waterscience/standards/bacteria/bacteria.pdf).

United States Geological Survey (2004). National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chaps. A1-A9 http://pubs.usgs.gov/twri

Appendix A: Glossary of Terms

Agar - A gelatinous medium on which to grow *E. coli* colonies.

CFU - Colony Forming Units (bacteria colonies).

Colony – Visible growth of microorganisms.

Culture - Growing microorganisms (i.e., *E. coli*) in a nutrient medium that encourages their growth.

Delineate - To define or portray, often by drawing.

E. coli - A species of fecal bacteria that lives in the intestinal tract of warm-blooded animals and is essential in digestion.

EPA - The U.S. Environmental Protection Agency, a government agency who's mission is "…to protect human health and the environment."

Gastroenteritis - Irritation of the digestive tract, often resulting in abdominal pain, vomiting and/ or diarrhea.

GIS - Geographic Information Systems. A software program that combines different layers of information (streams, land use, cities, counties, elevation, etc.) for analyses.

GPS - Global Positioning System. Hand-held or larger devices that triangulate your position on earth from satellites in orbit. One can take reading(s) at a sampling site, and later download this data into a software program.

Imperviousness - Impenetrable surfaces such as driveways, roads, etc.

Pathogen – A disease-causing life form such as a virus, bacterium, or other microorganism.

Replicate – Samples collected in the field in duplicate, triplicate, or more. Or samples plated in the lab in duplicate, triplicate, or more. Replicates help identify any variability in the stream or lab procedures.

TMDL - Total Maximum Daily Load. A TMDL is a regulation that specifies the sum of the pollutant contributions from point source discharges, *non-point* (diffuse) sources, and natural background levels that a water body can process and still meet water quality standards.

TNTC - Too Numerous To Count. If there are too many *E. coli* colonies on a plate, they are considered as too many or numerous to count.

Tributary - Smaller streams that feed into a larger portion of the main stream or river.

Watershed - The area of land that drains to a common water body.

Appendix B: Forms

Survey tools and other forms have been developed to help in the implementation of a volunteer monitoring program. These include: pre-post knowledge surveys given to volunteers at the start and end of the training sessions, to assessments done following the training, to those following a season of monitoring to assess user preferences in regards to using the test methods. These tools are available at www.uwex.edu/ces/csreesvolmon/EColi/SurveyTools.htm as pdf files.

Various forms have been developed for recording data, gathering information about your volunteer samplers, and keeping track of sites to be sampled and the data collected from these sites. A summary sheet that provides a step-by-step approach for sampling has also been developed. An example Data Sheet to record site conditions and bacteria data, and a Sampling Plan Summary are included beginning on the following page.

Citizens Monitoring Bacteria Data Sheet

Date ____/____/____ Volunteer ID _____

Collection Time _____:_____ (am/pm) Site ID _____

Monitor's Name _____

Stream/River Name _____

Stream Flow

❒ High ❒ Normal ❒ Low

Air Temp _____ (°C)

Water Temp_____ (°C)

Current Weather

❒ Clear/Sunny ❒ Overcast ❒ Showers ❒ Rain (Steady) ❒ Storm

Worst Weather in Past 48 hrs.

❒ Clear/Sunny ❒ Overcast ❒ Showers ❒ Rain (Steady) ❒ Storm

Transparency ____ (cm) or ____ (NTU) *(optional)*

Stream assessment comments and observations:

For each method, record the volume of water (in mL) used when plating the Easygel samples. Note the incubation temperature and the time samples were placed in the incubator. After incubating for 24 hours, count how many colonies you see on the plate. Repeat after 48 hours. To calculate the number of *E. coli* colony forming units (CFUs) per 100 mL, divide 100 by the number of mL of sample you used and multiply that result by the number of colonies you counted. You now have the estimated number of CFUs in 100 mL of sample. To properly average your replicates, see page 26.

Test Method	Sample Volume (mL)	Number *E. coli* colonies counted @ 24 hours	Number *E. coli* (calculated) CFU /100mL @ 24 hours	Number *E. coli* colonies counted @ 48 hours	Number *E. coli* (calculated) CFU /100mL @ 48 hours
EASYGEL – Sample 1		**A**		**A**	
EASYGEL – Replicate 2		**A**		**A**	
EASYGEL – Replicate 3		**A**		**A**	
3M Petrifilm – Sample 1	**1 mL**	**B**		**B**	
3M Petrifilm – Replicate 2	**1 mL**	**B**		**B**	
3M Petrifilm – Replicate 3	**1 mL**	**B**		**B**	

Incubation Temperature

_____°C

Time Samples Placed in Incubator

_____

A = count dark blue and purple colonies; **B** = count blue (or blue-purple) colonies with gas bubbles

Comments, observations and concerns about the sample prep or the analysis (include the time samples were counted if different from 24 or 48 hours):

Citizens Monitoring Bacteria Sampling Plan

Note: *This sampling plan includes steps for both Easygel and Petrifilm tests. Volunteers may decide to just use one of the tests. The sampling plan also includes steps to take if you are sending split samples to a laboratory for comparison of results. Depending on your location, you may need to sample on Monday, Tuesday, or Wednesday to get samples shipped overnight to the lab in time for them to complete the tests.*

Before You Go Out to Sample

1. Take 3 bottles of Easygel per each site out of freezer to thaw – if rapid thawing is required, they may be rinsed in warm water.
2. Take 3M™ Petrifilm™ out of the refrigerator – 3 for each site.
3. Turn on incubator – be sure the lid is tight and that it's the correct temperature (35°C) Fill appropriate channels in plastic tray with distilled water and set in bottom of incubator. Place wire tray on top.

Take to the Sampling Site

- ❑ soap, antibacterial lotion or wipes
- ❑ plastic gloves
- ❑ waders
- ❑ cooler with ice
- ❑ Sharpie® or permanent marker (to label bottles)
- ❑ shipping containers/ice packs and forms
- ❑ sterile collection containers (one per site)
- ❑ sterile lab sample bottles (one per site)
- ❑ 2-3 data sheets (one per site) on clipboard
- ❑ 1 or 2 thermometers
- ❑ transparency tube
- ❑ sampling device with rope (if sampling from bridge)

At the Site

1. Hang thermometer where it is not in direct wind or sunlight (for air temperature reading) – it may take about 5 minutes to stabilize
2. Complete top of data sheet, stream flow stage, and stream assessment comments
3. Take water temperature (hold approximately 2 minutes in main stream flow) – record on data sheet
4. Rinse labeled sterile collection bottle (500mL bottle) three times with sample water using proper sample collection technique – lower in upside down position to a depth of 3-5 inches below the water's surface (or approximately up to your wrist), fill at an angle facing upstream – be sure your hand and or fingers are not in front of the mouth of the bottle
    - ❑ If sampling from a bridge – rinse sampling device with stream water 3 times, then collect a sample and rinse the collection bottle three times – then fill collection bottle (be sure the bucket and rope do not come into contact with the ground during this process)
5. After rinsing the bottle 3 times, collect sample and top with lid after removing from stream – place collection bottle in cooler with ice for transporting
    - ❑ If shipping samples to lab before returning home/office, SHAKE COLLECTION BOTTLE TO MIX THE SAMPLE, then fill the lab sample bottle to its shoulder from the collection bottle (DO NOT rinse the laboratory sample bottle; it may be filled with a preservative) – also put this bottle in cooler on ice.
6. Record air temperature reading on data sheet

7. Take transparency reading and record on data sheet
8. Wash hands when finished

Tips for Preparing/Plating the Samples

1. Prepare table by cleaning with bleach or isopropyl alcohol
2. Wash hands thoroughly with soap
3. Items to have at home/office "lab" station

- ❑ paper towels or Kimwipes
- ❑ isopropyl alcohol/bleach
- ❑ distilled water
- ❑ rinse/waste container
- ❑ Sharpie® or permanent marker
- ❑ gloves
- ❑ pipettes
- ❑ Petrifilm spreader

4. Set up stations for each site you sample:
   - ✓ You should have one collection bottle and one lab sample bottle **per site**
   - ✓ You should have 3 Petrifilm plates and/or 3 Easygel bottles and 3 Easygel petri dishes, and 1 pipette **per site**
   - ✓ Label Easygel bottles with site #s; label bottom of petri dishes and Petrifilm plates with site #, replicate number, date, and volume (mL) of sample to be used.
5. ALWAYS SHAKE SAMPLE BOTTLE BEFORE DRAWING A SAMPLE WITH A PIPETTE!
6. Add an appropriate volume of sample water (using a sterile pipette and drawing from the collection bottle) to the three duplicate Petrifilm plates and/or Easygel bottles. You will always use 1mL for the Petrifilm. You can chose between 0.5 mL up to 5 mL for the Easygel bottles. (Note: you can use the same pipette to transfer the sample water to each of the appropriate tests if you use sterile technique.). Each site you sample requires using a new sterile pipette.
7. Complete the Petrifilm test by using the spreader as described on page 23.
8. Complete the Easygel tests by inverting each bottle, pouring each into a separate petri dish and swirling each as described on page 22.

Incubation (Remember to write down what time incubation begins!)

- ✓ Place plated samples in incubator: Easygel petri dish (upside down) and 3M™ Petrifilm™ (right side up) – three per site. **Remember:** Easygel needs to sit for at least 45 minutes to gel before placed in incubator upside down
- ✓ After 24 hours, count *E.coli* colonies on the Petrifilm plates and Easygel petri dishes
- ✓ After 48 hours, count *E.coli* colonies on Petrifilm plates and Easygel petri dishes (*optional*)
- ✓ After use, rinse incubator with dilute bleach or distilled water and let it dry
- ✓ Dispose of petri dishes and plates in a ziplock bag with a teaspoon of bleach added

Which items need to be sterile?

- ✓ Collection bottles and any bottle sent to the lab for confirmation
- ✓ Pipettes

Don't forget to **take photos** (or have someone take photos of you) at your site and while performing the methods – these can be used for a variety of purposes!

Appendix C: Sample Training Agenda

Below is a recommended agenda for an *E. coli* volunteer monitoring workshop. We recommend that you cover these essential topics, but you may wish to add additional information of your own.

1. Introduction
2. What the Citizen Monitoring Bacteria Project is
3. Implementation of the Pre-Test Survey and Demographics Survey; Liability and Photo Release Forms
4. Bacteria 101 – What is bacteria, why should we monitor for it, what do we know about bacteria, and how do we monitor for bacteria?
5. Site selection – how to pick a site to monitor (where, how, why). Sampling frequency
6. Safety
7. How to collect a field sample – hands on; QA/QC, field replicates
8. Lab protocol – how to collect a lab sample, how to ship the sample FedEx, chain of custody, shipping instructions
9. Field parameter instructions
10. How to use the kits – hands on
11. How to use the incubator and other bacteria equipment
12. Practice reading the plates
13. Data sheets
14. Disposal of kits
15. What does the data mean – interpretation of results
16. Post-Test Survey; End of Training Volunteer Assessment; End of Training Staff Assessment
17. Contact information for questions; wrap up; hand out kits and supplies

Appendix D: Other Methods

IDEXX Colisure

Because of the equipment costs associated with the IDEXX Colisure, it was not selected for use by volunteers. However, its accuracy when compared with laboratory analyses was as good as the two methods selected.

Preparation and Setup

1. Turn on IDEXX Quanti-Tray® Sealer.
2. Label Quanti-Trays using a permanent marker. This label should include site ID, date and time of sample collection, and sample number.

Preparing the Sample

1. Water samples are collected in 100 ml plastic IDEXX bottles by filling the bottles up to the 100 ml graduation.
2. Add Colisure reagent and two drops of anti-foam solution into sample.
3. Mix thoroughly until reagent is dissolved.
4. Pour sample into Quanti-Tray.
5. Place Quanti-Tray on rubber insert, and seal with Quanti-Tray Sealer.
6. Remove from back of sealer as soon as sealing is completed.

Incubation and Interpretation

Incubate at 35 degrees Celsius for 24-48 hours. After incubation is complete, read results. Wells containing total coliforms will turn from yellow to magenta. Wells containing *E. coli* will turn from yellow to magenta and fluoresce under UV radiation. If wells appear pink or orange, return tray to incubator and reexamine in 4 hours.

After all positive wells are counted, refer to a table of Most Probable Numbers (MPN) to determine total coliform MPN and *E. coli* MPN.

Sample Disposal

Because Quanti-Trays need to be sterilized by autoclaving, used trays are stored in large Ziplock bags and returned for disposal during each subsequent sample transfer.

IDEXX Colilert

Because of the equipment costs associated with the IDEXX Colilert, it was not selected for use by volunteers. However, its accuracy when compared with laboratory analyses was as good as the two methods selected.

Preparation and Setup

1. Turn on IDEXX Quanti-Tray® Sealer.
2. Label Quanti-Trays using a permanent marker. This label should include site ID, date and time of sample collection, and sample number.

Preparing the Sample

1. Water samples are collected in 100 ml plastic IDEXX bottles by filling the bottles up to the 100 ml graduation.
2. Add Colilert reagent and two drops of anti-foam solution into sample.
3. Mix thoroughly until reagent is dissolved.
4. Pour sample into Quanti-Tray.
5. Place Quanti-Tray on rubber insert, and seal with Quanti-Tray Sealer.
6. Remove from back of sealer as soon as sealing is completed.

Incubation and Interpretation

Incubate at 35 degrees Celsius for 24. After incubation is complete, read results. Wells containing total coliforms will turn from clear to yellow. Wells containing *E. coli* will turn from clear to yellow and fluoresce under UV radiation.

After all positive wells are counted, refer to a table of Most Probable Numbers (MPN) to determine total coliform MPN and *E. coli* MPN.

Sample Disposal

Because Quanti-Trays need to be sterilized by autoclaving, used trays are stored in large Ziplock bags and returned for disposal during each subsequent sample transfer.

Coliscan Membrane Filtration

Coliscan media incorporate a patented combination of color-producing chemicals and nutrients that make *E. coli* colonies appear blue, coliform bacteria that are not *E. coli* as a pink magenta and non coliforms as white or teal-green colonies.

There are two methods of Coliscan® : Coliscan-MF (membrane filter) and Coliscan® Easygel®. Coliscan-MF uses a sterile soaked pad in Coliscan medium as platform growth. Coliscan® Easygel® forms a gelled surface on which bacteria grows.

The Coliscan-MF method can be used when the water being tested has very few coliforms and/or *E. coli*. About a half cup (115 ml) of sample water is drawn through a membrane filter apparatus that traps bacteria on the surface of the filter. The filter is placed within a small petri dish on a sterile pad saturated with Coliscan-MF. The incubated colonies grow on the surface of the filter and are then counted.

Equipment

- ✓ 1.8 - 2 ml Coliscan-MF from a 20 ml bottle
- ✓ Membrane filter apparatus with holding pad
- ✓ 1 sterile dropper
- ✓ membrane filter with grid
- ✓ 2 inch petri dish with sterile pad
- ✓ forceps or tweezers (alcohol for sterilizing)

How To Use Coliscan-MF

Preparation and Setup

1. Thaw Coliscan-MF at room temperature by removing from freezer the night before sampling. (Note: Unused MF medium may be refrozen.)
2. Carefully open petri dish and use a sterile dropper to add less than 2 ml (1.8 ml) Coliscan-MF to soak the pad in the petri dish. Replace lid. (Note: the same pipette may be used to transfer the MF medium to each petri dish – one per site – if all are done at the same time following sterile technique.)
3. Twist the funnel to remove it from the collection container. Place a sterile holding pad on the top blue circle of the container. (Note: This pad does not have to be sterile, but should be clean. Store in Gelman plastic container or Ziplock bag. Use tweezers to transfer to the blue filter top. Only one pad will be used for each day's sampling. The same pad can be used for different sites because only sterile water is passed through the membrane filter. Discard holding pad after one day's use.)
4. Wipe forceps with alcohol to sterilize. Open a sterile filter envelope and remove the membrane filter with clean forceps. Be sure to separate the filter from the 2 blue protective backings when taking the filter from the filter envelope. Handle the filter carefully with tweezers or forceps so the filter does not tear. Place the filter grid-side up on top of the holding pad on the collection container. Be sure there are no air spaces between filter and pad.
5. Firmly push the funnel back down onto the filtering device bottom to hold the membrane filter in place and to create a seal. Double check that the funnel is securely against the blue filtering plate, over the red "O" ring, and touching the bottom vessel before filtering the water. Press down firmly.
6. Attach the hose to the collection container by pushing the end of the hose onto the side port of the container. Be sure the syringe plunger is pushed in.

Preparing the Sample

1. ALWAYS SHAKE SAMPLE COLLECTION BOTTLE BEFORE DRAWING A SAMPLE WITH A PIPETTE!
   Option 2a.) Using a sterile pipette, transfer 0.25 – 5 mL of stream sample to the filter funnel, then add distilled water (about 10-15 mL) to the filter funnel and gently swirl to mix.

   Option 2b.) Using a sterile pipette, transfer 0.25 – 5 mL of water sample to a pre-labeled bottle of diluent (sterile water) and shake vigorously to mix well. Mixing the sample with 10 – 99 mL of diluent helps distribute the colonies over the membrane filter more evenly. (Note: You will calculate the number of colonies/100 ml using the original sample size, disregarding the added volume of sterile water.)

Filtering the Water

1. Create a vacuum by pulling out the plunger of the syringe or by squeezing the handle of the pump.

   The water will be pulled through the filter, depositing any microorganisms present onto the filter. If all of the sample water is not drawn through the filter after the plunger has been pulled out, remove the plunger hose from the collection container, push the plunger back in, reattach the plunger hose and pull the plunger out again
2. When the water sample has been completely passed through the filter, disconnect the syringe and remove the funnel. With clean tweezers, remove the filter (grab near the edge) and place it grid-size up directly on top of the pad in the dish which was soaked with 2 ml of Coliscan-MF earlier. Place the lid on the dish, and place the dish in the incubator.
3. The filtered water in the collection container should be emptied and the filter apparatus prepared for repeat use by sterilization.

<You now need to sterilize the filter funnel for use during your next sampling event.>

Option 1. Rinse the funnel with isopropyl alcohol and let air dry

Option 2. Immerse in boiling water for at least 5 minutes and let dry

Place caps on funnels and store filtering device in plastic bag or sealed container until next use.

Incubation and Interpretation

Incubate the prepared dish (do not turn upside down) at 35^{o}C for 48 hours. After incubation is complete, count the colonies. *E. coli* colonies appear blue, dark blue, or purple. Other coliforms appear pink/magenta and non-coliforms appear white or teal green.

Confirmation Media Double Checks for Presence of *E. coli*

When using the Coliscan MF method, if the color of a colony is in question, you can add a drop of Kovac's reagent on or at the edge of the colony in question. A bright red zone will develop within 5 seconds if the colony is *E. coli.* An unused toothpick, plastic loop or small wire may be used to transfer the drop. The red color must be observed within the first minute after transferring the drop.

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