Bacteria Monitoring Manual

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IOWATER Bacteria Manual

Introduction

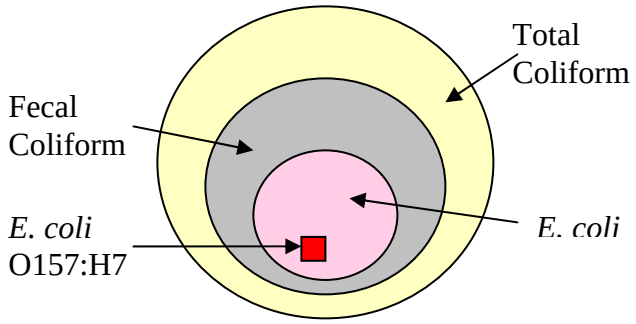
The presence of *Escherichia coli* (***E. coli***) bacteria in surface water is a major water quality concern in Iowa. Based on the Iowa Department of Natural Resources'(DNR) 2008 water quality assessment report, bacteria is Iowa's top cause of impairment for rivers and streams. For more information on the water quality assessment report visit: www.igsb.uiowa.edu/wqm/publications/TOCwaterQualityAssessment.htm

In this IOWATER workshop, you will learn what *E. coli* bacteria are; what sources of bacteria occur in Iowa; how bacteria enter our waterbodies; what health concerns are associated with bacteria; what concentrations imply increased risk for human health; how to monitor for bacteria; and how to interpret your results.

What are bacteria?

Bacteria are microscopic, single-celled organisms that are prevalent throughout the environment. Most bacteria are extremely small and difficult to see without the aid of a microscope. Bacteria live everywhere, in the air, soil, water, and in plants and animals. Bacteria can be both beneficial as well as harmful. Most bacteria provide beneficial uses, such as aiding in the digestion for many organisms, decomposing dead plants and animals, processing sewage sludge, aiding in the production of food products such as cheese and yogurt, and digesting hydrocarbons from oil spills. Some bacteria, however, are harmful and can cause human health problems. Those that are disease producing are referred to as **pathogens**.

The terms used to identify bacteria can be confusing and are defined below to help IOWATER volunteers distinguish between the types of bacteria discussed in the media, public meetings, or elsewhere.



Relationship among total coliform, fecal, *E. coli*, and *E. coli* O157:H7 bacteria.

Total Coliform bacteria are part of the Enterobacteriaceae **family** and are commonly found throughout the environment, including in plant and animal material and in soil.

Fecal coliform bacteria are a sub-group of total coliform bacteria that are present in the intestines and feces of warm-blooded organisms such as humans, livestock, and wildlife. The presence of fecal coliform bacteria in water suggests the presence of a relatively fresh source of fecal matter from a warm-blooded organism. Its presence in water, however, will not identify the host or source of the specific bacteria.

Escherichia coli (*E. coli*) is one subgroup of fecal coliform bacteria found in the intestines of warm-blooded organisms. While most are harmless, some can make you sick and cause diarrhea. The presence of *E. coli* bacteria in water serves as a marker or indicator of water contamination and the possible presence of disease-causing bacteria or other pathogens such as **viruses** or **protozoans**.

Note: While most *E. coli* bacteria strains are harmless and part of the normal bacteria found in the lower intestines of warm-blooded organisms, a form of *E. coli* bacteria called *E. coli* O157:H7 is one of the strains that can cause serious illness. *E. coli* O157:H7 is primarily a foodborne pathogen. Examples of foods that have been contaminated with *E. coli* O157:H7 include ground beef, unpasteurized milk and cheese, and fresh spinach. Ground meat can become contaminated with *E. coli* O157:H7 in the slaughter process; milk can be affected if the bacteria are on the cow's udders or milking equipment; and spinach can be affected if it has been exposed to water contaminated with the bacteria. When testing for *E. coli* bacteria as part of the IOWATER Program, the presence of *E. coli* bacteria does not necessarily mean that *E. coli* O157:H7 is present. *E. coli* O157:H7 can live in the intestines of healthy animals (primarily cattle, chickens, deer, sheep, goats, and pigs), but its natural occurrence in these animals is quite rare, so its presence in your monitoring is unlikely. However, whenever working with live bacteria, IOWATER highly recommends following the safety guidelines outlined in this manual and to thoroughly sanitize equipment and wash up after monitoring.

What are indicator bacteria?

Indicator bacteria are organisms used to measure or indicate potential fecal contamination, as they are found in human and animal waste. Commonly used indicator bacteria include fecal coliform, *E. coli*, and **enterococci**. These types of bacteria are referred to as indicator bacteria, as their presence in water indicates the presence of a relatively fresh source of fecal matter from a warm-blooded organism and the increased likelihood that pathogens or disease-causing organisms may be present.

The presence of indicator *E. coli* bacteria in water does not prove or disprove the presence of human pathogens, or disease-causing organisms, such as **Cryptosporidium** (protozoa), **Giardia** (protozoa), **Shigella** (bacteria), **Hepatitus** (virus), or **Salmonella** (bacteria). The presence of indicator *E. coli* bacteria merely suggests that as *E. coli* bacteria levels increase in water, there is an increased likelihood that disease-causing pathogens may be present. The U.S. Environmental Protection Agency (USEPA) has recommended that *E. coli* be used to assess the likelihood of risks to human health from exposure to water containing *E. coli* bacteria (USEPA, 1986).

Why not monitor pathogens?

Monitoring for pathogens in rivers, streams, and lakes is not frequently done as it tends to be expensive, requires large volumes of water, and the pathogens can often be difficult to grow in the laboratory and isolate for counting. *E. coli* bacteria monitoring, on the other hand, is less expensive, requires small volumes of water, and the bacteria tend to be present in large numbers and can easily be grown Additionally, *E. coli* bacteria are present in larger numbers than the pathogens and are easier to grow in the laboratory. U.S. EPA studies have also established the acceptable health risk associated with bacteria levels in recreational waters.

Common sources of *E. coli*

Sources of feces (and *E. coli* bacteria) in water can be quite varied. Common sources in Iowa include:

- Sewage overflows from municipal sewage treatment plants or sanitary sewer districts
- Routine discharges from municipal sewage treatment plants or sanitary sewer districts
- Animal waste such as livestock manure from cattle, hogs, or poultry
- Illegal **septic system**s from private homes or businesses
- Fecal accidents by beach goers ("poopy diaper" syndrome)
- Wildlife waste such as waterfowl, raccoons, etc.
- Pet wastes

How do bacteria enter waterbodies?
While a variety of sources of animal or human waste may be applied to or occur on the land surface, a pathway or route needs to exist for the bacteria to be transported from the source to the waterbody. Pathways include:

- Runoff from livestock feedlots containing manure
- Runoff from cropland with manure applications
- Animals directly discharging fecal waste into the waterbody
- Illegal septic systems from private homes or businesses which are linked to tile lines that discharge to nearby waterbodies
- Storm water runoff from urban areas carrying pet wastes
- Runoff of wildlife manure

How are bacteria levels measured?
Bacteria levels in water are measured as Colony Forming Units per 100 milliliters of water (**CFU/100 ml**). Since bacteria are too small to be seen by the naked eye, the bacteria must be allowed to reproduce and form "colonies" for counting. To do this, a water sample is collected, placed in a growth **media**, incubated at a warm enough temperature for the bacteria to grow, and the bacteria colonies are then counted after a specified length of time. The number of bacteria colonies is then converted to standardize the result to a number of bacteria colonies per 100 ml of water.

Human health risks and at-risk concentrations
Water quality standards for *E. coli* bacteria are based on acceptable risk levels to human health. The U.S. EPA conducted a series of studies to evaluate swimmer associated illnesses and bacteria levels in waters used by recreational bathers (USEPA, 1986). Based on these studies, the U.S. EPA established bacteria guidelines designed to help protect against disease from recreational exposure to water. The *E. coli* bacteria standard adopted by the State of Iowa for fresh water was established at 235 CFU/100 ml for a one-time sample maximum and 126 CFU/100 ml for a **geometric mean**. A geometric mean is similar to an average and is based on no fewer than five samples equally spaced over a 30-day period. The water quality standards are based on acceptable risk levels to human health. At these levels, 8 out of 1000 swimmers who come into contact with the water may become sick. As *E. coli* bacteria levels increase, so does the chance that someone will get sick.

What influences bacteria levels?
Bacteria levels in rivers, streams, and lakes can be affected by a variety of parameters, including weather, availability of sources, time of year, nutrient levels, and sediment.

- Weather – Often after rainfall events, snow melt, or any other time runoff occurs, bacteria levels increase as bacteria on the land are washed into nearby waterbodies.
- Availability of Sources – Identifying potential sources of bacteria within a watershed may help to interpret and understand the data.
- Time of Year – Different sources of bacteria may be evident during different times of the year. For example, during low-flow conditions (typically in the fall and winter), point sources such as inputs from illegal septic systems or routine discharges from municipal sewage treatment plants or sanitary sewer districts may be more apparent than during high-flow periods. Inputs from land-applied animal waste and other nonpoint sources, on the other hand, may not be evident until precipitation driven runoff carries bacteria to the waterbody.
- Nutrient Levels – Higher levels of nitrogen and phosphorus support more food sources, which in turn allow the bacteria to survive for longer periods of time.
- Sediment – Bacteria can survive a long time in bottom sediments, so disturbances of bottom sediments while sampling may re-introduce bacteria into the water column. Bacteria in bottom

sediments can also be re-introduced through wind and wave action, as well as from the turbulence created by boat motors. Bacteria can also attach to sediment, but not in all cases does more turbid water (or lower transparency) result in an increase in bacteria levels.

- Bottom sediments versus water column – Bacteria survive longer in bottom sediments than in the water column, so disturbances of bottom sediments while sampling may re-introduce bacteria into the water column.
- Freshwater versus seawater – Bacteria survive longer in freshwater than in seawater.
- Warmer temperatures – Bacteria generally survive longer in warmer rather than cooler water.

It was long believed that bacteria could only survive 24-72 hours outside a host, but research in recent years has shown that bacteria can survive much longer, and depending on environmental conditions, may be able to produce bacteria that are up to three generations removed from the original bacteria.

Bacteria source tracking

The presence of *E. coli* bacteria in water will not identify the source(s) of bacteria as human or animal. It merely indicates that a relatively fresh source of fecal matter from a warm-blooded organism is present. Newer procedures, called **bacteria source tracking** technologies, have the possibility of determining the source(s) of bacteria in water. When multiple source tracking methods are used in combination with each other, they provide supporting evidence in determining if the fecal source is human or animal.

DNA ribotyping compares DNA patterns of *E. coli* bacteria from contaminated waters to DNA fingerprints of *E. coli* bacteria from known sources of fecal matter in the nearby vicinity or watershed. **Multiple antibiotic resistance** analysis can be used to determine human inputs of fecal matter, as *E. coli* bacteria from human sources will have different patterns of antibiotic resistance than wildlife or domesticated animals.

Caffeine and cholesterol, both present in human feces, were at one time believed to be good indicators of human feces in water, however, both are more common in the environment than previously thought, so they are no longer believed to be as effective at distinguishing sources.

Optical brighteners are dyes present in most laundry detergents in the United States. They are added to laundry detergents to make whites brighter and fabrics cleaner. Their presence in a stream or other waterbodies indicates that laundry effluent is present. Elevated *E. coli* bacteria, in conjunction with the presence of optical brighteners, can indicate the presence of human sewage.

***E. coli* Testing Instructions**

The IOWATER Program uses the Coliscan® Easygel® method developed by Micrology Laboratories, Inc. The recommended frequency to test for bacteria will depend on your monitoring plan and why you are monitoring. If at all possible, monthly monitoring is encouraged. It is recommended that a Chemical/Physical assessment be completed when bacteria monitoring occurs.

<u>Sample Preparation</u>

Before going to the field, make sure to take the following with you:

- Sterile **pipette**s or other sterile collection container (1 per site)
- Bottle(s) of Coliscan® Easygel® media, thawed (3 per site)
- Permanent marker to label the media bottles
- Cooler with ice to keep sample(s) chilled until returning home
- IOWATER bacteria field data sheet
- IOWATER chemical/physical field data sheet (optional)

- IOWATER chemical/physical test equipment (optional)

1. All bacteria sampling equipment should be handled carefully to avoid contamination. *Avoid contact with the inside of the **Petri dish** and the tip of the eyedropper*.
2. Prepare incubator (cooler) by making sure the inside is clean and dry.
3. Check expiration dates on Coliscan® Easygel® media bottles.

Sample Collection

1. Using a sterile pipette (eyedropper), collect a sample of water from your transect from slightly below the water's surface while facing upstream in the main flow. Collect water samples in increments of 0.5 ml, with 0.5 ml being the minimum and 5 ml being the maximum volume sampled. For most samples, one ml of water is recommended. If you suspect high levels of bacteria, or if you have had high numbers of bacteria in the past, you may want to collect 0.5 ml of water.
2. Place the sample water directly into the bottle of Coliscan®Easygel® media. Invert the bottle a couple of times. This is Sample 1.
3. Using the same pipette, repeat Steps 1 and 2 of the Sample Collection procedure for Sample 2 and Sample 3. The same volume of water needs to be collected for all three samples.
4. It is recommended to chill your bottled samples on ice in the bottles until you return indoors.

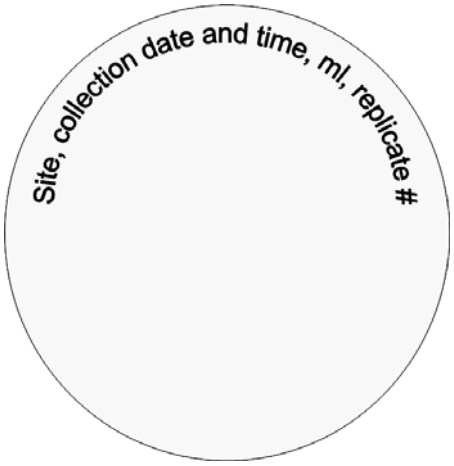
NOTE: If you are unable to prepare the samples for incubation upon returning home, it is possible to store the water for up to 24 hours. To do so, you **must** collect a water sample in a sterile container (collect ~ 50 milliliters of water) and then refrigerate the water sample until you are able to prepare and incubate the samples. IOWATER does not provide a sterile sample container, so you will need to locate one to use. **Do not** add the water to the media until you are ready to prepare and incubate the samples, as the bacteria can grow in the media, resulting in higher bacteria levels (false positives) than might actually be present in your water sample. When you are ready to prepare the sample, be sure to invert the water sample bottle a couple of times before transferring the same volume of water to each of the 3 media bottles.

Sample Incubation

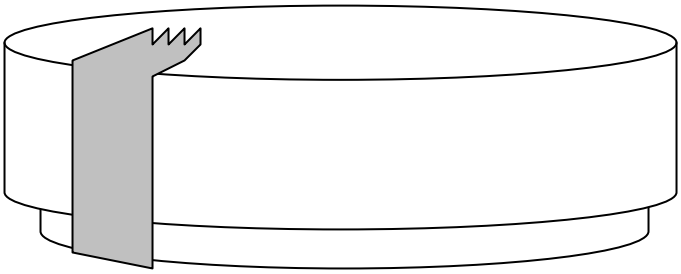
When returning from the field, be sure to have the following to prepare the samples for incubation:

- Pre-treated petri dishes from Micrology Labs (3 per site)
- Permanent marker to label the petri dishes
- Styrofoam cooler (incubator), meat thermometer, night light, and extension cord.
- Scotch tape to tape the petri dish lids.
- IOWATER bacteria field data sheet

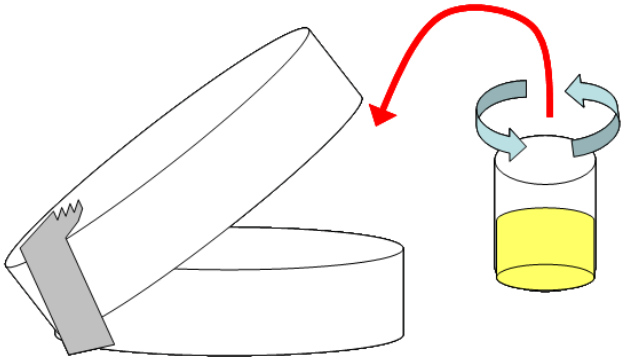
1. Prepare the incubator by turning on the night light and inserting the meat thermometer through the lid.
2. Label the bottom (smaller of the two pieces) of three Petri dishes along the edge using a permanent marker. This label should include the IOWATER site number, date and time of sample collection, volume of water collected, and sample replicate (you will be collecting three samples per site, so label them Sample 1, Sample 2, Sample 3).



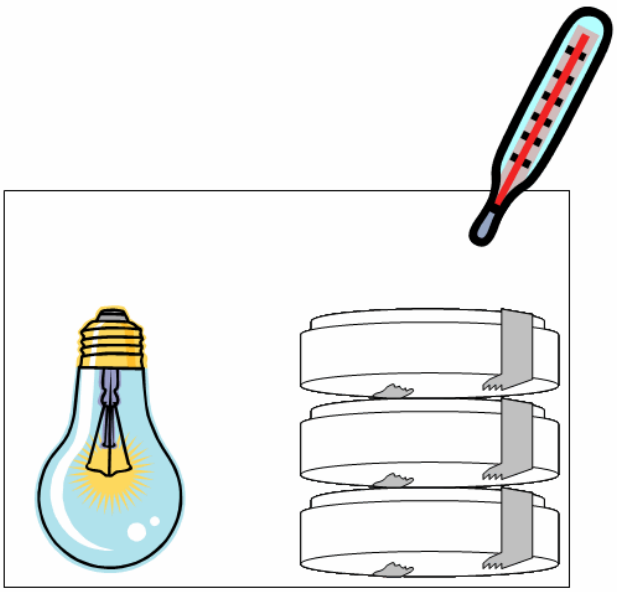
3. Tape the lid of the Petri dish to the bottom in any one location, creating a hinge.



4. Swish 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. Take care not to splash over the side or on the lid.



5. Tape the other side of the lid to the bottom of the Petri dish.
6. Place the Petri dishes on a level surface out of direct sunlight for up to 45- 60 minutes. The mixture will solidify on bottom of Petri dish. Avoid moving the dishes until they have solidified.
7. Check the incubator to make sure that the temperature is between 85-99°F. If the temperature is too hot, crack the Styrofoam lid slightly.
8. Once the mixture is solid, incubate the Petri dishes at **85-99°F** for **48 hours**. Petri dishes can be stacked in the incubator. Petri dishes should be placed upside down with the lid facing down to avoid condensation on the lid. Place the Petri dishes opposite the light source, allowing space between the Petri dishes and the sides of the incubator. Avoid placing the thermometer next to the light.



9. Monitor the incubator temperature for the first hour after placing the Petri dishes in it. Keeping the temperature between 85 and 99 °F is very important. If necessary, the temperature can be lowered by opening the Styrofoam lid a crack. If the temperature is not reaching the minimum 85 °F, place an additional night light in the incubator.

Sample Count

1. After the incubation period (48 hours), count all the dark blue-purple spots or "colonies" on the petri dish. Record the colony count on Bacteria Monitoring data sheet under **"*E. coli* Bacteria." Use the "Interpreting the Plates" guide to help determine what bacteria colonies to count as *E. coli*.**
2. When counting bacteria, use the laminated grid provided. Place the grid beneath your petri dish to use as a guide while counting. You only need to count to 100. If more than 100 colonies are present, record the colony count as 100.
3. Count all the pink spots or "colonies" on the petri dish with the methods described above. Record the colony count on the Bacteria Monitoring data sheet under **"General Coliform Bacteria."**
4. Repeat above procedure for other two samples Petri dishes.
5. Record on the Bacteria Monitoring data sheet the volume of water collected for each sample, the incubation time or period and the incubation temperature.

Sample Disposal

1. Carefully place about a teaspoon of straight 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.
4. Allow the Styrofoam cooler to air dry before storing. Avoid using the cooler for other uses. A dilute solution of bleach can be used to clean the cooler.

Note: Coliscan® Easygel® media ***does expire*** and ***should be kept frozen*** until used, but may be refrigerated up to two weeks before use. ***Check expiration dates frequently.*** Petri dishes do not expire as long as open packages are resealed after use and stored in a dry, cool location.

Interpreting Results
An important concept to understand is that feces are always present in our environment. Simply finding that indicator bacteria are present in your water does not warrant further investigation.

Designated Uses and Water Quality Standards in Iowa DNR
The federal Clean Water Act of 1972 gives authority to States and Tribes to establish water quality standards for waterbodies within their jurisdiction. These water quality standards are defined based on the existing and potential uses of a waterbody. (Examples of **designated uses** include recreation, aquatic life support, or drinking water.) Based on those uses, each state sets standards or criteria to ensure protection for those uses. For example, a stream or lake designated for recreation uses in Iowa would be protected by an *E. coli* bacteria standard.

Listed below are Iowa's current designated uses for surface waters:

- **Primary contact recreational use (Class "A1")**: Waters in which recreational or other uses may result in prolonged and direct contact with the water, involving considerable risk of ingesting water in quantities sufficient to pose a health hazard. Such activities would include, but not be limited to, swimming, diving, water skiing, and water contact recreational canoeing.
- **Secondary contact recreational use (Class "A2")**: Water in which recreational or other uses may result in contact with the water that is either incidental or accidental. During the recreational use, the probability of ingesting appreciable quantities of water is minimal. Class A2 uses include fishing, commercial and recreational boating, any limited contact incidental to shoreline activities and activities in which users do not swim or float in the water body while on a boating activity.
- **Children's recreational use (Class "A3")**: Waters in which recreational uses by children are common. Class A3 waters are water bodies having definite banks and bed with visible evidence of the flow or occurrence of water. This type of use would primarily occur in urban or residential areas or parks.
- **Aquatic life use**: uses of waters by fish, aquatic and semiaquatic species and wildlife. Surface waters in Iowa designated for aquatic life uses are termed Class "B" waters for which there are four different designated aquatic life uses. The Class B uses are protected by criteria for dissolved oxygen, pH, temperature, ammonia, toxic metals, and toxic organic and inorganic compounds.
- **Drinking water use**: surface waters that are used as a raw water source of a potable water supply. Surface waters in Iowa designated for drinking water use are termed Class "C" waters. The Class "C" uses are protected by criteria for radioactive substances, nitrate and nitrite, pesticides, toxic metals, and toxic organic and inorganic compounds.
- **High Quality**: Some surface waters with unique physical, biological or chemical features are also designated in one of two "High Quality" uses. Waters designated as High Quality (HQ) receive additional protection for their unique physical, biological and chemical features present. HQ waters have exceptional recreational and ecological importance. Waters designated as High Quality Resource (HQR) receive additional protection for their unique physical, biological features, but the chemical integrity will be maintained to the numerical criteria noted in the Water Quality Standards. HQR waters possess unusual, outstanding or unique characteristics that enhance the beneficial uses and warrant special protection. HQ and HQR waters are specifically protected in Iowa's antidegradation policy.

Prior to 2006, all surface waters in Iowa were classified for protection of general uses. These general uses included livestock and wildlife watering, non-contact recreation, crop irrigation, and industrial, agricultural, and other incidental water withdrawal uses. In addition, many surface waters had further protection by being designated for recreational, aquatic life, or drinking water uses. Surface waters with these designated uses needed to maintain flow throughout the year or contain sufficient pooled areas during intermittent flow periods to maintain a viable aquatic community.

In 2006, a new rule was passed in Iowa that designated all of the surface waters that were formerly considered "general use" to be Class A1 and Class B (WW-1 = warm water). This new rule, called **rebuttable presumption**, presumes that all waterbodies that maintain flow throughout the year or contain sufficient pooled areas during intermittent flow periods should support the "fishable, swimmable" goals of the Clean Water Act unless otherwise proven to not be capable of supporting these uses. Therefore, this rule will protect the recreational and aquatic life uses of all of the **perennial** rivers and streams that were not previously listed specifically in the Surface Water Classification.

Staff from the Iowa DNR are currently completing Use Attainability Analyses (UAAs) on some of the waterbodies that are now presumed to be Class A1 and Class B (WW-1) to determine whether or not those classifications are appropriate. The UAA process uses a set of guidelines that establish whether or not the waterbody should be Class A1 and Class B (WW-1) or another combination of the designated uses listed above.

Class A Water Quality Standard Table for *E. coli*

Use	**Geometric Mean**	**Sample Maximum**
Class A1		
March 15 - November 15	126	235
November 16 - March 14	Does not apply	Does not apply
Class A2 (Only)		
March 15 - November 15	630	2880
November 16 - March 14	Does not apply	Does not apply
Class A2 and B(CW) or HQ		
Year Round	630	2880
Class A3		
March 15 - November 15	126	235
November 16 - March 14	Does not apply	Does not apply
Class A1 – Primary Contact Recreational Use Class A2 - Secondary Contact Recreational Use Class A3 – Children's Recreational Use		

For more on Iowa Water Quality Standards visit: www.iowadnr.com/water/standards/index.html.

For Iowa waterbodies designated for Class A1 or A3 uses, the one-time *E. coli* maximum standard is 235 CFU/100 ml, and the geometric mean, a measure of five samples evenly spaced over a 30-day period is 126 CFU/100ml. To find out if your results exceed water quality standards, you must first know if your waterbody is designated as a Class A waterbody. For help in determining your stream's designated uses, contact IOWATER at iowater@iowater.net. If your *E. coli* bacteria exceed the water quality standards on a frequent basis, please contact IOWATER.

References

U.S. Environmental Protection Agency, 1986, Ambient Water Quality Criteria for Bacteria – 1986, U.S. Environmental Protection Agency, EPA-440/5-84-002, Cincinnati, OH.

Glossary

Bacteria –– Microscopic, single-celled organisms that are prevalent throughout the environment.

Bacteria Source Tracking – Technologies that have the possibility of determining the source (human or animal) of bacteria in water. Examples include DNA ribotyping, optical brighteners, and multiple antibiotic resistance.

CFU/100 ml – Colony Forming Units per 100 milliliters of water; a unit of measure used to express bacteria levels.

Cryptosporidium – A type of protozoa that can cause waterborne illness.

Designated Use – Water uses for streams, rivers, and lakes that are to be achieved or protected. Uses in Iowa include recreation, aquatic life support, or drinking water use. Based on those uses, Iowa sets standards or criteria to ensure protection for those uses.

DNA ribotyping – A bacteria source tracking that compares DNA patterns of *E. coli* bacteria from contaminated waters to DNA fingerprints of *E. coli* bacteria from known sources of fecal matter in the nearby vicinity or watershed.

***E. coli* –** One subgroup of fecal coliform bacteria found in the intestines of warm-blooded organisms. The presence of *E. coli* bacteria in water serves a marker or indicator of water contamination and the possible presence of disease-causing bacteria or other organisms such as viruses or protozoans. They are considered an indicator organism.

***E. coli* O157:H7** – A strain of *E. coli* bacteria that is a foodborne pathogen and responsible for the majority of human illnesses attributed to *E. coli* bacteria.

Enterococci – A subgroup of the fecal streptococcus group (occur in the digestive track of warm-blooded organisms) that are able to survive salt water, therefore are recommended for use as the bacteria standard for marine waters. Enterococci are considered an indicator organism.

Family – In the classification of living things, family falls between order and genus (kingdom, phylum, class, order, family, genus, species).

Fecal coliform – A sub-group of total coliform bacteria that are present in the intestines and feces of warm-blooded organisms such as humans, livestock, and wildlife and are considered an indicator organism.

Geometric mean – A type of mean or average in which the numbers in a data set are multiplied by each other and then the ^{n}th root (n = number of data points in the data set) is calculated to yield the geometric mean. The geometric mean calculated for Iowa's Water Quality Standards is based on no fewer than five samples equally spaced over a 30-day period.

Giardia – A type of protozoa that can cause waterborne illness.

Hepatitis – A type of virus that can cause waterborne illness.

Indicator Bacteria –- Bacteria whose presence in water indicates water contamination and the possible presence of disease-causing bacteria or other organisms such as viruses or protozoans. Examples of indicator bacteria include fecal coliform bacteria, *E. coli*, and enterococci.

Intermittent Stream – A body of water that contains flowing water for only part of the year.

Media – A liquid or gel that contains the material necessary for bacteria to grow.

Multiple antibiotic resistance – A type of bacteria source tracking used to determine human inputs of fecal matter, as human *E. coli* bacteria typically have the greatest resistance to antibiotics when compared to wildlife and livestock fecal bacteria.

Optical brighteners – Dyes present in most laundry detergents in the United States. Their presence in a stream or other waterbody indicates that laundry effluent is present.

Pathogens – A disease-causing organism such as bacteria and viruses.

Perennial Stream – A body of water flowing in a natural or human-made channel year-round, except during periods of severe drought.

Petri Dish – A shallow plastic, flat-bottomed dish with a loose cover which is used to grow bacteria. The bottom smaller portion of the dish used with the Coliscan® Easygel® is pre-treated and where the media should be poured.

Pipette – A small sterile plastic tube with graduated increments marked on the side which is used to collect water from your site and then be added to the bacteria media.

Protozoa – One-celled microorganisms that can be found in water. Giardia is an example of a protozoan that can cause waterborne illness.

Rebuttable Presumption – A new rule passed in Iowa in 2006 that presumes that all waterbodies that maintain flow throughout the year or contain sufficient pooled areas during intermittent flow periods should support the "fishable, swimmable" goals of the Clean Water Act unless otherwise proven to not be capable of supporting these uses.

Salmonella – A type of virus that can cause waterborne illness.

Septic System – A small scale wastewater system that treats wastewater from a home that is not connected to a centralized wastewater facility. It generally consists of a pipe from the house, a septic tank, and a drainfield.

Shigella – A type of virus that can cause waterborne illness.

Total coliform bacteria – Part of the Enterobacteriaceae family and commonly found throughout the environment, including in plant and animal material and in soil.

Virus – An infectious agent that acts as a parasite and is unable to replicate without a host cell.

Water Quality Standard – Defines the goals for waterbodies in Iowa by designating the beneficial uses that are to be supported in Iowa's streams, rivers, and lakes. Beneficial uses in Iowa include recreation, aquatic life support, or drinking water use. Based on those uses, Iowa sets standards or criteria to ensure protection for those uses.

Frequently Asked Questions for the Bacteria Workshop Manual

1. **Is there an expiration date associated with the Coliscan Easygel media?**
   Yes, the Coliscan Easygel media has an expiration date that should be indicated with the media you receive. If the media is thawed when you receive it, simply place it in the freezer until you use it.
2. **Is there an expiration date associated with the Coliscan Easygel pre-treated petri dish?**
   No, there is no expiration date associated with the Coliscan Easygel petri dishes.
3. **Why are triplicate samples collected?**
   Variability occurs with bacteria monitoring. Variability occurs in the bacteria levels present in a stream, as well as in the plating and culturing the bacteria, and counting the bacteria colonies. Replicates (triplicates) help identify and minimize variability in the sample.
4. **At what temperature should the petri dishes be incubated?**
   IOWATER recommends that the petri dishes be incubated in the Styrofoam incubator at a temperature between 85- 99°F for 48 hours.
5. **What is the minimum and maximum volume of water that should be collected?**
   It is recommended that anywhere from 0.5 to 5 ml of water be used. If you are just starting with bacteria monitoring, 1 ml is a good sample volume to initially use. The volume of water can be adjusted depending on previous experience and conditions at your site. If it has recently rained, bacteria levels are likely to increase, so it would be recommended to decrease the volume of water you use.
6. **Should the same volume of water be used for each of the triplicate samples?**
   Yes, the same volume of water should be used for each of the three samples collected from a site.
7. **How often should I monitor for bacteria?**
   The frequency at which you monitor for bacteria will depend on your monitoring plan. When you do monitor for bacteria, you are encouraged to complete a chemical/physical assessment at the same time
8. **After adding the media containing my water sample to the Petri dish, the plate does not solidify, what should I do?**
   Check the expiration date for the media. Confirm that you added between 0.5 and 5 ml of water. If too much water is added, the media may not readily gel. Confirm that you used a pre-treated petri dish that was provided. If you've confirmed everything, contact IOWATER staff.
9. **How do I dispose of expired media?**
   The expired media can be poured down your kitchen sink.
10. **What if no bacteria grow after 48 hours in the incubator?**
    Be sure to check the temperature in your incubator periodically while processing the samples. If the temperature is greater than the recommended 85- 99°F, the temperature can be too hot and kill any bacteria present. Be sure that the petri dishes were placed away from the light source in your incubator. While unlikely, it is possible that there are no bacteria present in your water sample.
11. **Once I add my water sample to the media bottle in the field, will it gel in the bottle?**
    No, the media will not gel until it has been poured onto the pre-treated petri dishes.
12. **As I am counting the bacteria, is it OK to open the petri dish to more clearly see the bacteria?**
    It is not recommended to open the petri dish to more clearly see the bacteria, as you are directly exposing yourself to live bacteria.
13. **How soon after a water sample is collected in the field should I add it to the petri dish?**
    It is recommended that you process your water sample that has been placed in the media as soon as possible after returning home. The longer the water sites in the media, the more bias you introduce to your sample, as the bacteria can start to grow. If you are not able to process the sample right away upon returning home, you can do the following. You **must** collect a water sample in a sterile container (collect ~ 50 milliliters of water) and then refrigerate it until you are able to prepare and incubate the samples. Do not add the water sample to your media until you are ready to process the sample.

14. **What is the proper storage of the petri dishes once the package has been opened?**
    It is recommended that you store unused petri dishes in a clean Ziploc bag in a cool dry location.

15. **I purchased petri dishes through a company for another use in my classroom. Can I use these petri dishes for my bacteria testing?**
    No, the petri dishes you receive for use with the Coliscan Easygel have been pre-treated and must be used with the Coliscan Easygel media. Use of other petri dishes will not cause the media to gel and bacteria to properly grow.

16. **The medium in my petri dish turned a uniform pink or blue/purple color or a color other than anticipated? Is this a problem and what causes it?**
    If this happens, contact IOWATER immediately. It could indicate gross pollution at your site.

17. **How long can I leave thawed media in my refrigerator before I should use it or put it back in the freezer?**
    The media can be thawed for 2-3 weeks with no adverse effects. Simply place it in the freezer until you are ready to use it.

18. **I want to sample my stream immediately, but the media is still frozen in the freezer? Is there a way to quickly thaw it so it can be used? The microwave?**
    Do not use a microwave. The best way to quickly thaw the media is to place the media bottles in lukewarm water for 5-10 minutes until they are no longer frozen.

19. **If bacteria levels are high in my stream, are the fish safe to eat?**
    The bacteria levels in your water have no affect on whether fish are safe to eat. The safety of consuming fish has more to do with the presence of contaminants such as metals, toxic organic compounds, and pesticides, as these compounds will bioaccumulate in fish tissue. Based on 25 years of fish contaminant monitoring in Iowa, data show that the state's fish are safe to consume.

20. **Can I use my Styrofoam cooler for other uses?**
    No, it is not recommended that your Styrofoam cooler be used for anything but your bacteria monitoring.

21. **I have mold growing in my Styrofoam cooler. What should I do?**
    Use a dilute solution of bleach to scrub the inside of your Styrofoam cooler. Be sure to leave the lid cracked for a few days to allow the inside to dry. It is recommended that you crack the Styrofoam cooler lid for a few days after each use.

22. **If I collect a water sample in a sterile container for use later in the classroom, how long can I hold the water until it should be added to the media and petri dish?**
    A water sample that has been collected in a sterile container needs to be refrigerated and can be held no longer than 24 hours before it should be added to the media and then poured onto a pre-treated petri dish.

23. **What is the maximum number of colonies that can be counted accurately on one petri dish?**
    IOWATER recommends that no more than 100 bacteria colonies be counted and recorded for general coliform bacteria as well as for *E. coli* bacteria.

24. *** Is the size of the bacteria colony of any importance in doing my counts?**
    Colonies that do not grow past a pin point size within 48 hours should not be counted.

25. ***There was a notice on the box of Coliscan Easygel media that said it must be frozen for storage, but when I opened the box, the medium was thawed and liquid. Do I have a problem?**
    No, you do not have a problem. The media can be thawed for 2-3 weeks with no adverse effects. It is, however, recommended that the media be stored in the freezer until used.

26. *** The pretreated plates which I received with my bottles of Easgyel® look strange. Some have water droplets covering the surface of the film on the inside bottom of the plate which gives the layer a hazy appearance. Some may also have water droplets collected in some plastic sleeves. Is this contamination or are these plates OK to use? (Conversely, you could receive plates which are so dry that the layer on the inside bottom has the appearance of frosted glass.)**
    No, this is not contamination rather it is caused by changes in temperature which cause condensate to form

from water in the pretreatment layers of the petri dishes. These plates can still be used. On rare occasions, the petri dishes may have a dry, frosted appearance which is caused by very dry conditions. These plate, too, can still be used.

For more Frequently Asked Questions, visit Micrology Laboratories' website at http://www.micrologylabs.com/Home/FAQ.

***** Modified from Micrology Laboratories' Frequently Asked Questions.

Tips for Bacteria Sampling

1) All of the bacteria sampling equipment need to be handled carefully as to avoid contamination. Avoid contact with the inside of the petri dish and the tip of the eyedropper.

2) The recommended volume of water to collect is 1 milliliter (ml). If collecting a sample after a rain event, collect less water (i.e., 0.5 ml). The same volume of water needs to be collected for all 3 replicates.

3) Collect water samples in increments of 0.5 ml, with 0.5 ml being the minimum and 5.0 ml being the maximum.

4) It is recommended to add your water sample to the medium bottle and then chill the sample until returning home; then transfer the medium to your petri dish. Allow the medium to solidify before placing the petri dish in the incubator (set the plates out of direct sunlight for up to 45 minutes).

5) Monitor the incubator temperature for the first hour after placing the petri dishes in it. Keeping the temperature between 85 – 99 °F is very important. If necessary, the temperature can be lowered by slightly opening the Styrofoam lid. If the temperature is not reaching the minimum 85 °F, place an additional night light in the incubator.

6) Petri dishes can be stacked in the incubator. Petri dishes should be placed upside down with the lid facing down. Place the petri dishes opposite the light source, allowing space between the petri dishes and the sides of the incubator. Avoid placing the thermometer next to the light.

7) When counting bacteria, use the grid.

8) After using the Styrofoam cooler, allow it to air dry before storing. Avoid using the cooler for other uses. A dilute solution of bleach can be used to clean the cooler if necessary.

Bacteria Worksheet

For the IOWATER database, enter from your field sheet the *E. coli* bacteria and general coliform bacteria counts for each of the 3 replicates, the volume of water added for each replicate, the bacteria incubation period (hours), and the bacteria incubation temperature (degree Fahrenheit).

Based on the information you enter, a total colifom bacteria number (Colony Forming Units/100 ml) will be generated for each replicate (Total coliform bacteria = *E. coli* + General Coliform bacteria), as will an average *E. coli*, general coliform, and total coliform bacteria number.

The attached worksheet is provided if you want to calculate the number of Colony Forming Units/100 ml for your samples.

COUNTS from petri dishes

Replicate Number	*E. coli* Bacteria (dark blue-purple)	General Coliform Bacteria (pink)
1		
2		
3		

Replicate (x) = 100 ÷ _____ = _____ * _____ = _____

Volume of water — Count of bacteria — # of bacteria per 100 ml

EXAMPLE: If a 0.5 ml sample displays 4 *E. coli* bacteria, then divide 100 by 0.5 = 200. Then multiply 200 by 4 = 800 Colony Forming Units/100 ml of *E. coli* bacteria.

Number of bacteria per 100 milliters (or CFU/100 ml)

Replicate Number	Number *of E. coli* Bacteria	Number of General Coliform Bacteria	* Number of Total Coliform Bacteria
1			
2			
3			
Average			

Number of Total Coliform Bacteria = Number of *E. coli* bacteria + Number of General Coliform Bacteria

Average = sum of 3 replicates ÷ 3