# A Beginner's Guide to Water Management — Bacteria

**Information Circular 106**


**Florida LAKEWATCH**

Department of Fisheries and Aquatic Sciences
Institute of Food and Agricultural Sciences
University of Florida
Gainesville, Florida



**February 2003**
**1st Edition**


UF | UNIVERSITY of FLORIDA
IFAS Extension

Florida LAKEWATCH

**This publication was produced by:**

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**As always, we welcome your questions and comments.**

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## In addition to reading this circular, we encourage you to read the five publications that precede it:

♦ **A Beginner's Guide to Water Management – The ABCs (Circular 101)**
This 44-page publication provides a basic introduction to the terminology and concepts used in today's water management arena, in a user-friendly glossary format.

♦ **A Beginner's Guide to Water Management – Nutrients (Circular 102)**
A basic introduction to the presence of phosphorus and nitrogen—two nutrients commonly associated with algal growth and other forms of biological productivity in lakes. Limiting nutrients are also discussed, along with conceptual and mathematical tools that can be used to achieve a variety of water management goals. The booklet is 36 pages in length.

♦ **A Beginner's Guide to Water Management – Water Clarity (Circular 103)**
Anyone interested in the subject of water clarity can benefit from reading this 36-page circular. Topics include techniques for measuring water clarity, the factors that affect it, as well as a discussion of the techniques needed and/or used for managing it.

♦ **A Beginner's Guide to Water Management – Lake Morphometry (Circular 104)**
Knowledge of the size and shape of a lake basin (i.e., lake morphometry) can tell us a great deal about how a lake system functions. It can also help us appreciate lakes for what they are and manage them with more realistic expectations. This 36-page booklet is recommended for anyone interested in learning more about the terminology and techniques currently being used to study lake morphometry in Florida.

♦ **A Beginner's Guide to Water Management – Symbols, Abbreviations & Conversion Factors (Circular 105)**
This 44-page booklet provides the symbols, abbreviations and conversion factors necessary to communicate with water management professionals and scientists in the U.S. and internationally. Included are explanations for expressing, interpreting and/or translating chemical compounds and various units of measure.

**Copies of any of these publications can be obtained by contacting the Florida LAKEWATCH office at 1-800-LAKEWATCH (1-800-525-3928). They can also be downloaded for free from the Florida LAKEWATCH web site at:**

**http://lakewatch.ifas.ufl.edu/LWcirc.html**

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Joe Richard

# Introduction

After years of working with Florida LAKEWATCH volunteers and discussing lake management issues with them, we've come to the conclusion that bacterial contamination is one of the major concerns, if not the biggest, among citizens who live on or use our state waters. Such concerns are certainly understandable; for centuries, waterborne diseases have ravaged human populations worldwide and, in some countries, continue even today. Fortunately, within the United States and Florida, advances have been made in the treatment of human waste that have greatly reduced incidences of disease from contaminated water.

**So why are people still worried?**

It may have something to do with the swimming beach closures that occur every summer due to high bacteria counts, as well as periodic reports of bacterial contamination in drinking water supplies. As sporadic as these incidences may be, it is evidence that even with modern technology and the improvements made in wastewater treatment, problems do occur. (As usual, it's the rare problems that we remember, rather than the many successes.)

These occasional problems seem to underscore a general apprehension among some Floridians that changes in land use and unprecedented population growth could be contributing to an increase in the contamination of our local waters. When one considers that the state's population has increased by more than 115% in the past thirty years (i.e., since the 1970s), with even more growth expected during the 21$^{st}$ century, it's no surprise that people are beginning to wonder about the effects that such growth may be having on our lakes, rivers and coastline.

The widespread development of permanent homes and businesses, many of them built on or near lakes, has been accompanied by a dramatic increase in the number of septic tanks and/or municipal sewage treatment plants. With the record pace at which many of these systems were installed, concerns are re-emerging amongst citizens and some scientists.

**Are these concerns warranted?**

As Professor Dan Canfield[1] likes to say, "Yes, no, and maybe." No individual or agency can guarantee with absolute certainty that recreating in a given waterbody is completely without risk. While this may be an unsettling thought to many individuals, it is important to remember that in most monitored waters, there is a very, very low risk of becoming ill. When you drive your car a few miles to the grocery store and back home, you are exposing yourself to a much greater health risk than one would normally experience while recreating in Florida's waters.

**What should one do if bacterial contamination is suspected?**

The first thing to do is to stay well informed and this circular provides a good place to start. (See the outline provided at the end of the introduction for an overview of the topics covered.)

Secondly, if you or a group of homeowners suspect recurring contamination and you have the financial capability to pay for testing by a private laboratory, we recommend that you do so, as many public health agencies are limited in what they can do. If these agencies do test for bacteria, it usually involves only one or two indicator tests. Then, due to lack of funding and personnel, their only option is often restricted to simply ordering the site closed until re-testing indicates the water is again safe for use.

This is unfortunate, especially if there is a

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**1** *Dr. Canfield is director of Florida LAKEWATCH, a citizen-based volunteer water monitoring program at the University of Florida's Department of Fisheries and Aquatic Sciences.*


Joe Richard

chronic problem that needs to be identified and eliminated.

Whether you hire a laboratory or decide to collect water samples and do the testing yourself, we strongly suggest that you refer to the Part 7 of this booklet for an easy, relatively inexpensive approach to guide you through the process. Even if you have the financial resources to pay for more complicated testing methods (also described in this circular), we think our Four Step approach is a good way to begin and may save further expense in the long run.

Unless there is a catastrophic failure of a major sewage collection line or septic system, finding a leak is not always easy or cheap. In some instances, contamination can be the result of outdated or improperly installed septic tanks and at other times, miles of leaky sewer lines may be the culprit. Or it could be from an entirely different point of origin; sometimes, bacterial contamination is the result of naturally occurring animal waste from birds and other wildlife living nearby. “False alarms” are also common.

That’s why good detective work is required from both professionals and the general public. Everyone must recognize that, even if the source of the problem may seem obvious at first, it’s important to remain objective and not jump to conclusions before doing one’s homework.

**The bottom line?**

Correcting the problem should be the most important management objective once the public has been warned about possible contamination! The solution to this problem should not be limited to simply foregoing the use of the waterbody, but being aware and committed to tracking down the source.

Lastly, remember that prudence should always be the watchword when it comes to human health. If you have been swimming in a lake, river or coastal waters and become ill, go see your doctor and be sure to tell her/him that you have been in contact with recreational waters. You’ll probably find out that your illness is not related to a waterborne disease, but if it is, most illnesses can be treated quickly and effectively.

Should you have any questions or concerns regarding bacterial contamination in your lake, please call Florida LAKEWATCH:

**1-800-LAKEWATCH (1-800-525-3928).**

# Included in this circular:



Joe Richard

Joe Richard

# Part I
# A Brief Lesson on Bacteria

When we think about bacteria, many of us often think of pathogenic (disease-causing) organisms that are notorious for causing illnesses in humans such as cholera, tuberculosis, typhoid fever, etc. While admittedly, these diseases can be quite serious, it is important to recognize that the bacteria responsible for such illnesses represent a relatively small fraction of the thousands of "species" that are known to exist.

It also helps to keep things in perspective by acknowledging that bacteria have been around for a very long time. In fact, fossil remains tell us that one group of bacteria, known as *Cyanobacteria*, was among the first life forms to have been established on earth more than three billion years ago. Some scientists even theorize that these organisms helped to create the earth's unique life-giving atmosphere by producing so much oxygen, via photosynthesis, that eventually the atmosphere became habitable for other creatures.

Bacteria can be found in virtually every environment you can think of including air, soil, and water. Some strains have even been found in volcanic vents and deep inside arctic ice flows—environments once thought to be barren of any life. However, bacteria are also found much closer to home. Did you know that a single teaspoon of topsoil is thought to contain more than a billion bacteria and one square centimeter of human skin holds an average of 100,000 bacteria cells!

### Bacteria in Lakes

Bacteria are a natural component of life in all aquatic systems including freshwater lakes, rivers, streams and oceans, where they serve as "decomposers," helping to break down dead plant and animal tissue and continually releasing nutrients back into the water. For example, *Cyanobacteria* play a critical role in the photosynthetic production that occurs within many aquatic ecosystems,[2] while other bacteria are crucial to important chemical processes in water such as nitrogen fixation and denitrification.[3]

### Why the Concern?

Like most things in life, it only takes a few troublemakers to spoil the fun. In this case, health officials are mostly concerned about a small number of bacteria strains that are enteric (i.e., of or related to the intestines of warm-blooded animals, including humans), as well as opportunistic viruses and protozoa that can cause illness in people, particularly those with weakened immune systems.

Bacterial contamination generally refers to instances in which human or animal wastes are found in concentrations greater than the receiving waters can handle (i.e., when the volume of water is not enough to dilute waste products to an acceptable level). In such instances, humans

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**2** *Because cyanobacteria are aquatic and capable of making their own food via photosynthesis, they are sometimes called* ***blue-green algae****.*

**3** *Some bacteria are known to convert gaseous nitrogen into nitrates or nitrites. The resulting products are released into the water, making it possible for some plants to capture these nutrients. This process is known as* ***nitrogen fixation****. When bacteria metabolize nitrates and turn them into nitrogen gas or nitrous oxide, it is known as* ***denitrification****.*

drinking from, swimming in, or eating shellfish from a contaminated waterbody, run a greater risk of being exposed to harmful bacteria or pathogenic viruses that may also be present.

Because it is impossible to eliminate all harmful bacteria from aquatic environments, U.S. government health agencies have set standards for acceptable levels allowed in public waters.

**See Part 6 on page 29 for more on the criteria used to assess coliform contamination of Florida waters.**

These standards tend to be conservative and experience has shown that they are effective in preventing human health problems nearly all of the time. However, even if risk levels may be deemed acceptable, meeting the standard does not completely eliminate the possibility of becoming sick. Along those same lines, just because a bacterium enters a waterbody, it doesn't necessarily mean the risk of contracting a disease is increased. It simply means that there is the potential for a problem.

### Viruses and Protozoa in Water

Bacteria are not the only microbial concern related to water usage. Pathogenic viruses and protozoa such as amoebas may also be present and can be even more difficult to detect. For example, an infectious dose for a virus is far lower than that of bacteria—by at least one order of magnitude (i.e., 1/10$^{th}$ of the concentration). This means that detecting a virus in a waterbody is akin to finding a microscopic-sized needle in a haystack. Adding to the challenge is the fact that some enteric viruses can remain infective for several months in both sediments and water and tend to be somewhat resistant to disinfectants.

Testing for protozoa can also be tricky as they are present in relatively low concentrations, even in polluted waters, and the number of organisms can change quickly over time. Current methods for detection are not well standardized, so there has been a lack of consistency when it comes to setting water safety standards for these organisms. Because of the difficulties and the expense associated with this type of monitoring, most efforts have been limited to work being conducted by researchers, as opposed to public health agencies. However, if epidemiological evidence indicates that sampling is needed, some public health organizations are equipped to do extensive and detailed sampling.

If you have questions regarding viruses or protozoa, contact your public health department.

Joe Richard

# Amoebas in Lakes

Every summer, questions surface about an aquatic amoeba (***Naegleria fowleri***) with a bad reputation. This organism is part of the larger protozoa group mentioned on page 2. Over the past 30 years, there have been 34 deaths recorded in the United States due to exposure to this nasty little organism. Fifteen of the deaths occurred in Florida.


Joe Richard

Fortunately, the chances of coming in contact with ***Naegleria***, or contracting the resulting illness (Primary Amoebic Meningoencephalitis—PAM, for short) are quite slim. In Florida, health officials estimate that there is only one case for every 2.5 million hours that people spend in freshwater. Drowning and boating accidents pose a much greater threat to our state's water enthusiasts. With that said, there are a few precautions swimmers can take to decrease their chances of exposure even more.

The first thing you should know is that, with the exception of Antarctica, this amoeba is believed to exist in virtually every lake and river around the world. It is also found in spas, hot tubs, thermally enriched waters and poorly chlorinated swimming pools. So, if you're thinking of simply avoiding these aquatic environments, you might get a little lonely.

### So, How Does One Avoid the Amoeba?

The best way to prevent exposure to ***Naegleria*** is to avoid stirring up bottom sediments, as this is where the amoeba lives and feeds on bottom sediments composed of fallen leaves and dead plants. Once sediments are mixed into the water column, the amoeba could be forced up the nose of a swimmer who jumps or falls into the water. This increases the chance for it to enter into an ear or nasal passage where it can follow the olfactory nerve and gain entry into the brain, where it has been known to cause problems.

It's important to note that swimmers who have contracted PAM usually got it after rooting around the lake bottom, in heavy silt where the amoeba lives. Therefore, keeping one's face away from the bottom of a lake, river, canal, etc. and keeping swimmers from jumping off a dock into shallow water—or any other scenario that would result in the disruption of bottom sediments—will significantly reduce the risk of exposure to ***Naegleria***. Young children are at the highest risk of exposure as they tend to engage in such activities.

Everyone can be further protected by wearing ear plugs and a nose clip (or a dive mask that covers the nose) when swimming. Remember, exposure to bottom sediments is the single MOST important factor that increases chances for infection.

During most of the year, concentrations of ***Naegleria*** are rarely high enough to cause public health problems. However, as water temperatures rise during the summer (82-86 degrees Farenheit), it provides a more accommodating environment for the amoeba to feed and multiply. So, if possible, avoid swimming in warm shallow waters during this time.

### Diagnosis

Early diagnosis is the best bet for survival. In the two known cases where patients survived infection from ***Naegleria***, the family doctor recognized the symptoms immediately and was quick to react with appropriate antibiotics. Persons who complain of severe headaches, rigidity of the neck, impaired sense of smell and taste, nausea, vomiting and/or a high fever, and who have been swimming in a lake should be taken to a doctor. If the treatment is going to be effective, it needs to be administered quickly.

***Note:*** *You cannot get PAM by eating fish from a lake.*

Kevin McCarthy

Joe Richard

City of Orlando Streets, Drainage and Stormwater Utility

Joe Richard

# Part 2
# Sources of Bacterial Contamination

There are numerous potential sources of bacterial contamination in Florida lakes—and other lakes, for that matter. In a booklet such as this, it's impractical to list every one of them. However, they can be grouped into three general categories: human waste, domestic animal waste, and naturally occurring contamination from wildlife. Of course, contamination can also result from a combination of sources.

### Human Waste

The disposal of untreated human waste into the nearest waterbody was once a common practice throughout the world, including the United States. Even as recently as the mid-20th century, it was common practice for U.S. cities and towns to discharge untreated human waste into rivers, streams, lakes, or oceans. For years, dilution was considered to be the "solution to pollution." This practice is no longer condoned and now, there are legal requirements for the treatment of human wastes.

In less developed countries, some communities continue to discharge both human and animal wastes into local waters. This is not always a problem if the amount of waste is small relative to the volume of the receiving water. In some instances, wastes are used as fertilizers for terrestrial crops and/or even as fish food for aquaculture crops.

In developed countries where more financial resources are available, large municipal wastewater treatment plants are used to treat large volumes of human waste. These types of treatment plants are extremely effective at removing disease-causing bacteria from wastewater discharges (often greater than 99.9% of the time). However, there is still a risk that a pathogen or virus could be released in the water discharged from the treatment plant.

In rural and suburban areas of Florida, septic tanks are the most common treatment system used for human waste. However, despite their prevalent use, septic tanks are often maligned when issues of nutrient enrichment and bacterial contamination are discussed among lake users. This is unfortunate because, while there is certainly evidence that septic tanks can add nutrients and bacteria to lakes, the contribution is usually not as great as many people think.

**See Part 3 The Wastewater Treatment Debate on page 9.**

### Domestic Animal Waste

The improper disposal of human waste is not the only possible source of bacterial contamination. Domesticated animals are warm-blooded and their wastes can, at times, harbor pathogens known to adversely affect humans. So, unmanaged stormwater runoff from sites with high concentrations of domesticated animals such as animal feedlots, cattle and pig grow-out operations, etc. can also be potential sources for bacterial contamination. Given the high visibility of these facilities, and the odors they tend to emit, it's natural for area residents to point to these areas first when bacterial contamination issues arise. However, such operations are not always to blame and bacterial testing must be conducted before any conclusions are made.

Domesticated animals living on open rangeland or pastureland, such as cattle or horses, can also contribute to high bacteria counts in lakes and waterbodies. Often the contamination is related to the animals entering the water for drinking or cooling purposes, and then defecating directly into the water. This can be corrected relatively easily by fencing the animals away from the water.

However, the animals will then need to be provided drinking and cooling water, which can be expensive. In these situations, the problem, and resulting tensions among neighbors, can sometimes be resolved more quickly if the effected community is willing to assist the landowner(s) in obtaining financing that will help correct the problem.

Perhaps the most pervasive problem associated with domesticated animals is the runoff that follows heavy rains. This is often referred to as "non-point source" runoff. However, it must be remembered that this source of contamination is not solely limited to agricultural lands. In urban areas, contaminated stormwater runoff is believed to originate from animal waste generated by pets, particularly in parks where people bring their pets to exercise.

This type of non-point source runoff is difficult to control. In some areas, attempts are being made to reduce the problem by preventing the direct flow of stormwater into a waterbody. Swales (a shallow depression in the landscape), man-made wetlands, and stormwater retention ponds are a few of the methods that have been widely used in recent years. However, because use of such techniques is not always possible, the safest approach is to avoid recreational activities in your neighborhood lake for two to three days following exceptionally heavy rainfall. While it may not eliminate the possibility of contracting an illness, it can reduce the probability.

**If bacterial contamination is suspected, bacterial testing is a good first step to try and locate the source(s).**

**See Part 7 on pages 31-35 for a step-by-step approach to determining if bacterial contamination has occurred in a waterbody.**

### Naturally Occurring Contamination from Wildlife

When issues of bacterial contamination occur at a lake, the focus almost immediately turns to sources such as septic tanks, runoff from livestock holding pens, or leaky sewer lines. While these should always be considered, it should also be remembered that there are naturally occurring sources of bacteria. For example, large concentrations of wildlife such as deer or birds represent a significant potential source for bacterial contamination of a waterbody.

A case in point is the bacterial contamination problem that occurred at Lake Fairview in Orlando, Florida. It was originally thought that the contamination was due to leaky septic tanks. This resulted in discussions concerning the need for a municipal water treatment plant. However, after an extensive bacterial study, it was determined that the source of contamination could be traced to bird droppings from large numbers of seagulls that were using the lake. Apparently, the seagulls were feeding at a nearby landfill during the day and then congregating at Lake Fairview to roost each evening.

An observant biologist happened to notice that when the seagulls were absent, there was no contamination. In this instance, it's evident that eliminating septic tanks would not have solved the bacterial contamination problem as it was essentially a natural phenomenon that was difficult to control. It is also a good lesson for those who may balk at the expense of additional bacterial sampling for a lake or waterbody. In the case of Lake Fairview, the cost of the extra sampling paled in comparison to the cost of a new wastewater treatment plant and expenses associated with long-term maintenance of the plant and its sewage collection pipes.

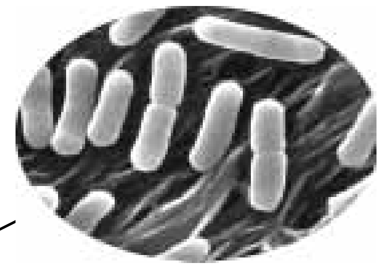
# A Taxonomic Headache

**One of the problems related to the study of bacteria is the difficulty in isolating and describing the thousands of organisms that exist. It's hard enough to locate and identify insects, birds and mammals; imagine working with microscopic organisms, many of which have a half-life* of one hour!**

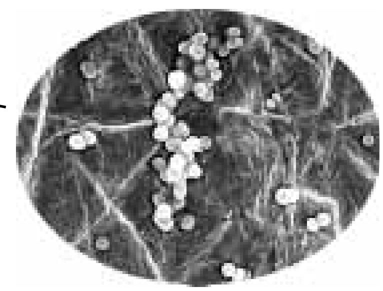
### So how does one differentiate between different types of bacteria?

Classification by shape and/or structure is one way. Fortunately for us, bacteria are essentially limited to three basic shapes:

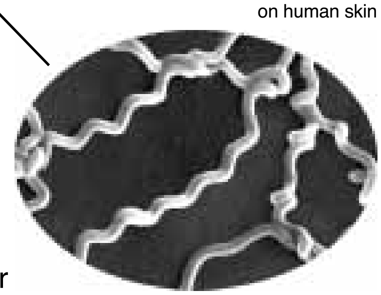
- ♦ Rod or stick-shaped bacteria are referred to as **bacilli** (pronounced ***buh-sill-eye***);
- ♦ Sphere shaped bacteria are classified as **cocci** (pronounced ***cox-eye***); and
- ♦ Spiral shaped bacteria are classified as **borrelia** (pronounced ***boar-el-eeya***).



*Esherichia coli (0157:H7)*



*Staphylococcus aureus* on human skin



*Leptospira interrogans* (a spiral shaped prokaryote)

Some live as individual cells while others tend to group into pairs, chains, squares or other configurations.

The composition of a bacteria's cellular wall is also an important defining characteristic: **Gram positive** bacteria have multi-layered cell walls, while **gram negative** bacteria tend to have much thinner cell walls.

### Adding to the challenge

Recent advances in microbial research are presenting another dilemma. Thanks to new information gained from DNA and RNA sequencing, many bacteria are being renamed and/or re-classified. As a result, many old lengthy complicated names are being changed to new lengthy complicated names. This can be quite confusing, as many of these outdated references are still in use.

If you should find that the science has indeed gotten ahead of this publication, please don't hesitate to let us know! And remember, your questions and comments are always welcome. Call Florida LAKEWATCH at 1-800-LAKEWATCH (1-800-525-3928).

* **Half-life** – *the time required for half of the atoms of a substance/organism to disintegrate.*

Robert May

Amy Richard

# Part 3
# The Wastewater Treatment Debate: Septic Tanks vs. Wastewater Treatment Plants

There can be no doubt that advances in wastewater treatment over the last 50 years have contributed greatly to the reduction in waterborne illnesses. With this great success, one might wonder why we're not treating all human waste with advanced treatment processes and why opposition to upgrading existing wastewater plants always seems to emerge during public discussion. The following is a brief overview of the on-going debate, including some of the advantages and disadvantages associated with each system.

### Septic Tanks

The septic tank is the most common wastewater treatment system in many rural and suburban areas of Florida. Yet despite its prevalent use, it is often maligned among lake communities when issues of nutrient enrichment (eutrophication) and bacterial contamination are discussed.

Certainly, there is evidence that septic tanks can add nutrients and bacteria to lakes, but the contribution of these materials to local waters is usually not as great as many people think. Properly functioning septic tanks generally contribute only small amounts of nutrients, if at all. In fact, some long-term lake studies have suggested that septic tanks may have only a limited impact on nutrient levels in most lakes. The same seems to be true regarding the issue of bacterial contamination, but each situation must be examined carefully.

Failed septic systems are primarily associated with effluent leakage to the soil's surface from the drainfield and are usually detectable by

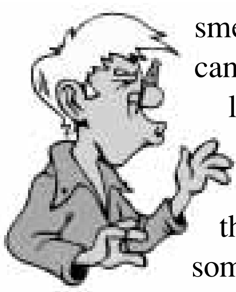
A **septic system** is a self-contained, underground wastewater treatment system that uses natural processes to treat and dispose of wastewater. These systems are also referred to as **onsite** or **decentralized** wastewater systems.

Septic systems are simple in design, consisting of two main components—a watertight tank and a drainfield.

The tank is usually made of concrete or fiberglass, with an inlet and outlet pipe. Wastewater from the home or building flows to the inlet pipe and septic tank through a sewer pipe. Once inside the tank, the wastewater eventually separates, forming three layers. Solids that are lighter than water, such as greases and oils, float to the top while solids heavier than water settle to the bottom, leaving a middle layer of partially clarified wastewater.

Naturally occurring bacteria that live in the wastewater continually work to break down the solids. Any sludge that cannot be broken down is retained in the tank until the tank is pumped.

The middle layer of clarified liquid flows from the tank via the outlet pipe to the drainfield, which usually consists of a series of pipes placed in trenches lined with gravel or course sand. The drainfield treats the wastewater by allowing it to slowly trickle from the pipes into the gravel and down through the soil, which serves as a biological filter.



smell. As a general rule, if you can smell waste, there is most likely a problem that needs to be addressed quickly. If there is leakage, it tends to be near the ends of the drainage field or sometimes it's due to the lack of soil over the drainage area. In these situations, wastewater is subject to being washed to a nearby lake via surface water runoff. Fortunately, this type of problem can be easily fixed by importing more soil and covering the area.

In other instances, leakage is due to the drainage field becoming clogged over time. This can be remedied by providing a new field or expanding the existing field. Homeowners often balk at this solution because it is expensive, but health concerns should override any monetary concerns.

There may also be times when solid waste will need to be pumped from the septic tank itself. This is usually dependent on the amount of wastewater generated, based on the number of people using the system and the amount of water used. Kitchen garbage disposals, for example, are infamous for increasing the amount of solids in a septic tank, making it more difficult for bacteria to do their job of breaking down the waste.

In the 21st century, homeowners now have a choice between below-ground septic tank systems or above-ground systems. While the below-ground type may seem risky for lakefront communities, there are systems that are specially designed for waterfront property. The tanks are setback considerably from the lake shoreline to minimize the possibility of untreated waste or nutrients entering the lake as seepage or runoff. If maintained properly, they can provide reliable cost-effective wastewater treatment for years.

Above-ground septic tanks are becoming popular in low-lying areas where soils remain wet for prolonged periods of time. When working properly, they are considered to be quite effective at treating wastewater. Some people believe these new elevated above-ground septic systems are better than the in-ground versions. This is a dangerous assumption as improperly constructed elevated drainage field mounds have been known to leak through the sides. For that matter, well-constructed elevated drainage fields have had leakage problems. Therefore, even the newer systems should be carefully examined for potential problems.

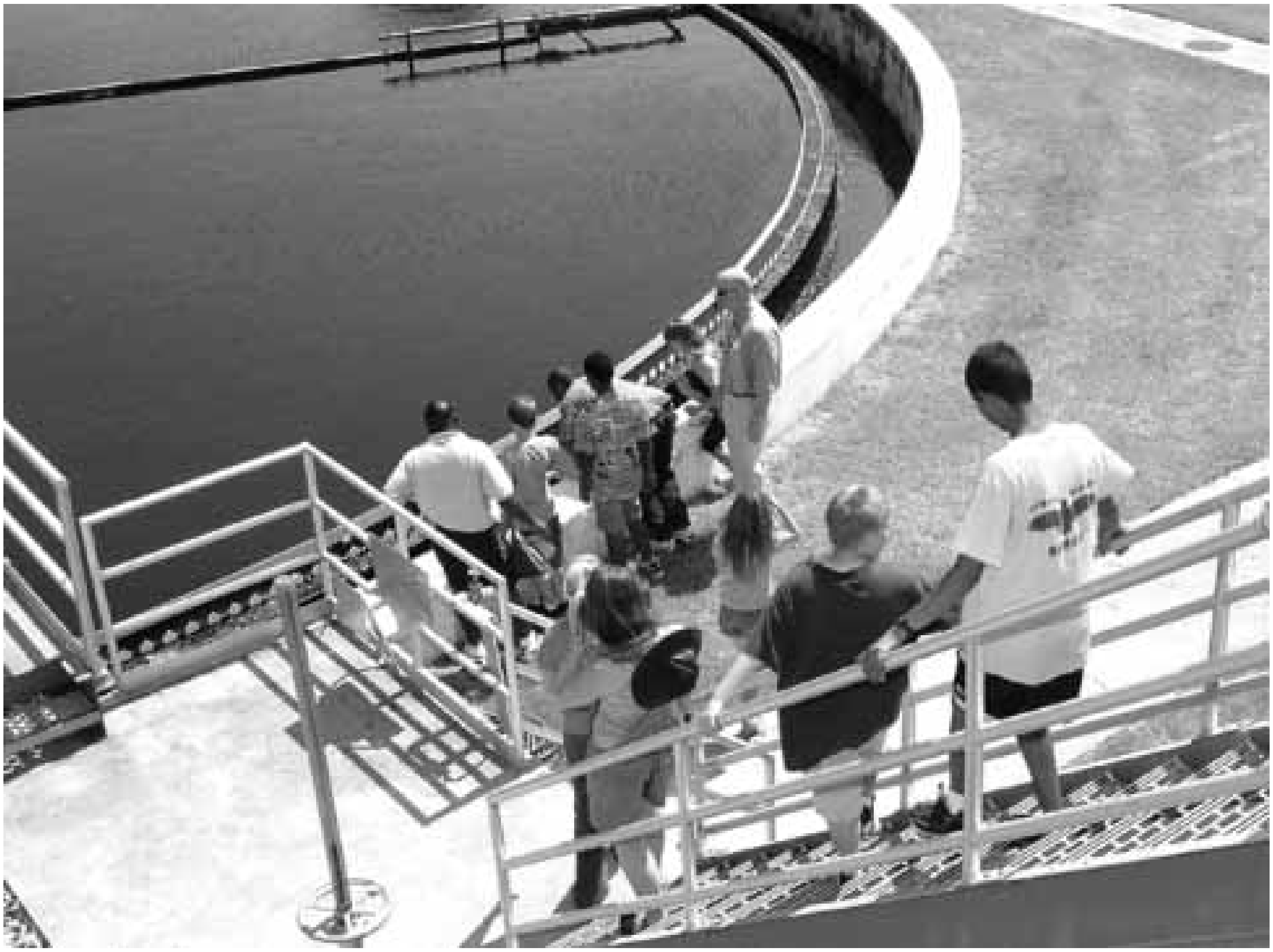
Admittedly, there is no perfect septic disposal system and there probably never will be. However, the failure of one septic system should not be used to condemn this method of waste treatment. Expensive municipal wastewater treatment plants have problems of their own.

**See pages 11-12 for more information on municipal wastewater treatment plants.**

Rick Bernowski

In 1997, the US EPA reported that "adequately managed decentralized wastewater systems (i.e., septic tanks) are a cost-effective and long-term option for meeting public health and water quality goals, particularly in less densely populated areas." The agency also recognized that poor septic tank management is a major part of the problems and/or criticisms associated with these systems. As a result, in 1998 the EPA was challenged to produce a set of voluntary national management standards for citizens to follow. These guidelines are available in the EPA publication entitled **Onsite Wastewater Treatment Systems Manual** (EPA 625R 00008). Free copies can be ordered by calling 800-490-9198. For more information, check out the EPA website:

**http://www.epa.gov/owmitnet/mtb/decent/summary.htm**



Joe Richard

**Wastewater Treatment Plants**

Perhaps the strongest argument in favor of wastewater treatment plants is their effectiveness. Today's advanced treatment processes are about 99.9% effective in removing pathogens from wastewater. However, their effectiveness during normal operation should not always be the primary focus of concern. There are other issues that should also be considered.

One major concern for Floridians should be the potential for complete disruption of services during natural disasters, such as hurricanes. During these periods, the public is asked to avoid contaminated water and be patient until services are restored. Depending on the problem, this can sometimes take weeks. (Parents should be especially vigilant to keep children from playing or swimming in potentially contaminated water following such storm events.)

Another point of contention relates to the financial burden that wastewater treatment plants can have on a community; they are expensive to build and to operate. And while government grants are often available for the construction of the plants, the cost of properly maintaining these systems for the long haul is borne by the community. This means that any community contemplating the construction of a wastewater treatment system must be prepared to expend significant amounts of money on maintenance for both the treatment plant and its collection system. If long-term expenses are overlooked, problems often become apparent years after the construction of a wastewater treatment plant when bacterial contamination is discovered in a nearby lake or in the groundwater.

After many meetings and expensive upgrades are made to the main treatment facility, some communities are surprised to find that the source of contamination still may not have been eliminated. Instead, the problem could very well be within the collection system—miles and miles of sewer pipes that, when deteriorated, can begin to release small amounts of untreated waste. When this happens, additional capital will be needed to pay for the upgrade or repairs.

There is another aspect concerning the construction of new wastewater treatment plants that many people don't think about. While citizens may be anxious to modernize their community's sewage treatment facilities, they may not realize that by building a new system or expanding an existing one, they might also be opening the door to the dramatic development of an area, including an increase in the local population. If septic systems are suspected sources of bacterial contamination, one might first assess the cost of improving those systems before jumping on the wastewater treatment plant bandwagon.

As with septic systems, municipal wastewater systems also have a limited life expectancy and must be maintained and repaired constantly to retain their effectiveness and integrity. It has even been speculated that aging wastewater collection systems (sewer pipes, etc.) represent the greatest threat of fecal contamination to lakes—even more than septic tanks.

Sometimes extensive monitoring is necessary to determine if waste is leaking into a local waterbody or the groundwater supply. Fortunately, there are several methods that have been developed in recent years that make this task a little easier.

Lastly, it's important to remember that no human invention is foolproof. All wastewater treatment plants can experience failures in treatment processes. When severe failures do occur, wastewater plant operators must release untreated wastes to the nearest waterbody because they cannot treat the waste or they risk damage to a section of the treatment plant. This doesn't occur often, but it will most likely occur at some time during a plant's history.

**See Part 7 on pages 31-35 for more on identifying suspected sources of contamination.**

Joe Richard

**Wastewater treatment plants** are centrally located facilities, usually built and operated by city or county municipalities and are primarily designed to do one thing: Remove harmful pollutants from domestic and industrial liquid waste so that it is safe to return to the environment. This is accomplished by pumping wastewater from private homes or businesses through many miles of sewer pipes to waste treatment plants. It is then pumped through a series of treatment processes to remove unwanted materials and chemicals.

The removal of harmful materials, including micro-organisms, is accomplished with strictly regulated control processes and specialized equipment such as control pumps, valves, etc.

Once the wastewater is treated, it is returned to streams, rivers, and oceans, or re-used as "gray water" to irrigate landscaping. Industrial facilities sending waste to municipal treatment plants must meet certain minimum standards to ensure that wastes have been adequately pretreated and will not damage municipal treatment facilities. Waste from private homes is currently not regulated.


Amy Richard

## **Septic Systems for Dogs** (What Will They Think of Next?)

With the increasing popularity of dog parks around the nation, concern about surface runoff from doggie waste has also cropped up. Some park planners are thinking ahead by incorporating waste treatment systems into their plans. Underground septic tanks located at the parks are used to prevent seepage into low-lying areas or possibly into nearby streams or lakes. Pet owners are asked to collect pet waste and deposit it into clean-up stations located throughout the park; the waste is ultimately disposed of via the septic system. According to estimates, septic tanks only need to be pumped out once a year or on an as-needed basis. The system costs about $700, and is reimbursed from annual fees paid by park users.

Joe Richard

# Part 4
# Indicators Used to Detect Bacterial Contamination in Recreational Waters

Detecting pathogenic bacteria and viruses in water can be a challenging endeavor. Even with today's advances in microbiology, it is extremely difficult and expensive to isolate specific organisms. Some species are rarely found in large enough numbers for detection, while others are nearly impossible to cultivate in a laboratory as they require just the right combination of environmental conditions to grow.

So, instead of trying to identify elusive pathogens in a water sample, nearly all monitoring programs test for the presence of non-pathogenic bacteria that are far more numerous and easier to detect. This approach is based on the theory that if certain non-harmful **indicator organisms** are present in a water sample, then harmful bacteria or viruses may also be present. The concept was introduced in 1892 and continues to be the basis for most water quality standards today.

For many years, public health agencies have largely relied upon the presence of two coliform bacteria groups, **total coliforms** and **fecal coliforms**, as indicators of bacterial contamination in water. As you can see in Figure 4-1 on page 16, coliforms are classified as two sub-groups of the **Enterobacteriaceae** family (pronounced Enter-o-bac-teer-ee-a-see-ay). Their classification within this family means that, aside from their genetic similarities, coliform bacteria share several common traits that make them useful indicator organisms, including:

♦ Many of these organisms are known to exist in the intestines and feces of warm-blooded animals, including humans, and therefore serve as fairly reliable indicators that fecal waste may be present;

♦ They tend to live longer and are found in greater numbers than pathogens, making them easier to detect in a laboratory sample;

♦ They are generally non-pathogenic and therefore less risky to deal with when collecting samples and analyzing in a laboratory; and

♦ Laboratory methods used for detecting and counting these organisms are relatively simple and inexpensive.

Continue reading the rest of this section to learn more about the Enterobacteriaceae family, total coliforms, and fecal coliforms. Learn why they may or may not be appropriate indicators for specific bacterial contamination concerns. Also, learn about other bacteria groups, including the Enterococcus family, that are being considered for use as indicator organisms.

The indicator organisms discussed in this circular are primarily used for the detection of bacteria in "non-potable" waters — waters intended for swimming or bathing. While several of the same organisms may be used for monitoring potable (drinking) water or even wastewater, there may be some variation, more than we have room for in this publication. For more information about monitoring criteria and techniques used for detecting bacteria in drinking water or wastewater, please refer to the Selected References section in the back of this publication.

# Enterobacteriaceae

As scientific names go, the word Enterobacteriaceae is a definite tongue-twister. However, it's really not as hard to decipher as one might think. One helpful hint is the prefix *entero* which tells us that these bacteria are enteric—of or relating to the intestines. Also, its lengthy name is certainly appropriate as this family represents an expansive group of organisms that includes nearly a dozen separate genus groups and more than 40 "species."

**See Figure 4-1 for a general idea of the hierarchy of the Enterobacteriaceae family.**

Fortunately for us, there are only a few groups within this family that we need to know for bacterial monitoring purposes:

♦ The larger **total coliform** group includes many different species and strains of coliform bacteria, originating from a variety of sources (i.e., fecal and non-fecal) including both plants and animals.

♦ The **fecal coliform** group includes bacteria that usually originate from fecal matter (i.e., animal or human waste).

♦ ***Escherichia coli (E. coli)***[5] is just one of the many types of bacteria within the fecal coliform group. This "species" has recently surfaced as a particularly useful indicator organism.

For years, most public health agencies largely depended on the first two groups, total and fecal coliforms, as indicators for detecting potential bacterial contamination. Why?

Ease of testing is one reason. Because these groups include a broad spectrum of closely related organisms, scientists were able to develop a fairly simple testing method for estimating the number of coliform bacteria colonies in a volume of water. The tests are commonly referred to as **total coliform counts** and **fecal coliform counts.**

Traditionally, health officials using these counts have assumed that if high numbers of coliforms are detected in a water sample, then recent fecal contamination is present and represents a health threat. (Remember the theory: if indicator organisms are present in a water sample, disease-causing bacteria or viruses may also be present.)

Years ago, when many U.S. cities and towns were discharging untreated waste into public waters, this assumption was most likely correct.

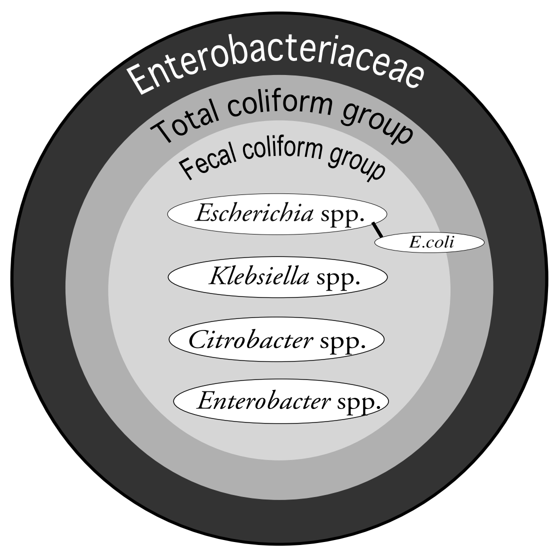



**Figure 4-1**

**Bacteria within the Enterobacteriaceae family belong to at least four genera[4]: *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter*. Within these groups, there are many different "species" and/or strains—far too many to list here.**

**4** *The term **genera** is plural for **genus**. A genus is comprised of one or more species and is one of the primary ranking categories used for classifying living organisms within the animal kingdom. An organism's genus name helps to rank it within the larger family group, but is still one level above its "species" name.*

**5** *The strains of* E. coli *discussed in this circular are not the same as those associated with cases of severe food poisoning.*

However, now that the vast majority of municipalities have wastewater treatment plants—effectively eliminating most major health threats—total and fecal coliform counts are no longer considered to be as useful. (i.e., They are not considered "sensitive" enough for detecting small amounts of bacteria.) In fact, the U.S. Environmental Protection Agency (U.S. EPA) and many state health agencies are now recommending that total and fecal coliform tests be phased out and replaced with *E. coli* tests.

For the time being however, it's important to remember that fecal and total coliform counts remain the legal standard for Florida waters. These standards continue to be used for several reasons:

(1) They are the least expensive tests to perform;

(2) They are commonly used as water safety criteria in many state legal standards;

(3) They can provide valuable clues on what is really going on in the lake or waterbody.

As you will learn from reading the rest of this section, there are a few drawbacks related to the use of total and fecal coliform counts. However, they still provide a good screening mechanism to begin with if bacterial contamination is suspected in a waterbody. If one does find high total and fecal coliform counts, steps can then be made to investigate further, perhaps using other indicator organisms and tests.

If you are deciding whether to do the testing yourself or hire a private laboratory, equipment requirements will undoubtedly be a major factor. Many testing methods require the use of an incubator, an autoclave (for sterilizing) and membrane filtration devices—all of which involve a large initial investment. However, once the equipment is obtained, routine testing is fairly inexpensive, especially if water samples are collected by volunteers.

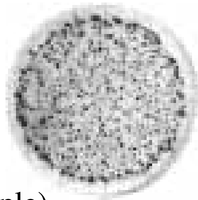
The good news is there are several companies working to develop reliable bacteria detection methods that involve less equipment and are easier to use. So stay tuned.

Amy Richard

Jennifer Donze, with Florida LAKEWATCH, places bacteria samples into an incubator where they will "bake" for 24 hours. After incubation, samples are observed and bacteria colonies counted. LAKEWATCH has been monitoring bacteria for survey purposes, on a limited number of lakes, since the year 2000. Total coliform counts, fecal coliform counts and *E. coli* counts have been the bacteria testing methods of choice for the survey.

For years, many state governments, including Florida, have come to rely on total and fecal coliform counts for the detection of bacterial contamination. Many have enacted legislation establishing numerical bacteria standards or guidelines for determining if a waterbody is "safe" for recreational activities. See page 29 for the current Florida standards, but also remember to watch for changes that may occur in the near future.

## Total Coliforms



Jennifer Donze

The term **total coliforms** refers to a numerical count of the total number of coliform bacteria that exist in a measured amount of water (i.e., from a sample). This count generally includes both fecal and non-fecal coliforms and an expansive assemblage of closely related organisms within the Enterobacteriaceae family.

**See Figure 4-1.**

For years, total coliform counts have been considered tried-and-true indicators of bacterial contamination, mainly because they include fecal coliforms, which tend to be more prevalent and longer-lasting than the elusive pathogens that sometimes co-exist in fecal waste.

Also, their ability to carry out lactose fermentation at fairly low temperatures (95-97 degrees F) makes it relatively easy and inexpensive to process samples in a laboratory: Samples are incubated to "trigger" a fermentation process, which causes coliform bacteria to grow. Once growth has occurred, colonies can be counted. For those with a limited budget, a sampling method that can be achieved without expensive high-tech incubators is certainly preferred.

Joe Richard

As an indicator, total coliform counts are most effective at red-flagging contamination in drinking water. World Health Organization guide-lines for drinking water allows a maximum of 0-2 organisms per 100 mL as acceptable for piped water supplies and a maximum of 10 per 100 mL for unpiped water supplies. It also states that "frequent occurrences of high coliform counts signify the need for an alternative water source or sanitary protection of the current source." [6]

When using total coliform counts as an indicator of contamination in recreational waters, remember that even though coliforms are found in fecal waste, there are other bacteria within the same group that naturally occur in aquatic plants and soils. Because of this, high total coliform counts cannot always be considered an indicator of fecal contamination. It's also the reason why total coliform counts are no longer considered as useful for determining the safety of recreational swimming or the consumption of shellfish.

**Does this mean that total coliform counts should no longer be used as indicators?**

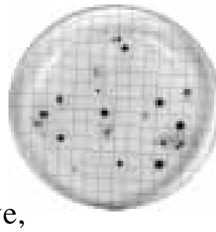
Not necessarily. While high total coliform counts may not always be an indication of fecal contamination in a waterbody, they may still be an indication of a potential health risk. For example, high total coliforms may sometimes indicate the presence of plant material and an associated bacteria known as *Pseudomonas aeruginosa*. This bacteria is considered to be non-fecal in origin and, therefore, unlikely to pose any severe health threats. However, it is known to be a major cause of ear infections in humans and is also associated with skin rashes.

So, even if a lake shows no sign of fecal contamination, a high total coliform count could indicate a potential risk for swimmers, water skiers, or others that come in contact with the water for a prolonged period of time.

**See page 21 for more on *Pseudomonas aeruginosa.***

**6** *Hach Company. 2000. The Use of Indicator Organisms to Assess Public Water Safety. Technical Information Series – Booklet No. 13.*

## Fecal Coliforms



Jennifer Donze

As illustrated in Figure 4-1, fecal coliforms are a subset of total coliforms. They are also the group of bacteria that, from a human health perspective, people are most concerned about because they indicate the presence of fecal matter in a waterbody.

When comparing the effectiveness of total coliform counts versus fecal coliform counts, it could be said that fecal coliform counts are considered to be a more reliable indicator of possible contamination within a waterbody. Use of this test has long been based on two assumptions:

(1) Fecal coliforms originate only from warm-blooded animals; and

(2) Fecal coliforms do not survive for an extended period of time in water and are, therefore, fairly reliable indicators of recent contamination.

When dealing with large-scale human contamination from untreated wastes or an inoperative wastewater plant, these assumptions are typically true. However, they've also become dogma among many public health workers, even when studies have shown otherwise. For instance, several studies now show that fecal coliform counts sometimes include bacteria that are not necessarily fecal in origin. An example is the free-living strain of the *Klebsiella* bacteria that is often present in soils.

The presence of such organisms in a fecal coliform count can result in false positive readings. In other words, the test results will suggest fecal contamination, when there is none. Studies have also definitively shown that fecal coliforms can survive and even multiply in the natural environment, therefore their presence does not necessarily indicate contamination from an outside source.

Perhaps the strongest criticism related to fecal coliform counts is the fact that these counts do not seem to correlate with the incidences of gastrointestinal illness experienced or reported by individuals using recreational waters.

**Does this mean that fecal coliforms should no longer be used as indicators?**

Again, not necessarily. Many public health agencies continue to use fecal coliform counts as indicator bacteria. Legal standards are one reason; a large number of states, including Florida, still rely on long-standing fecal coliform criteria to set the legal limits for water quality and safety. In many instances new standards or criteria have yet to be developed for several of the newer indicator organisms, including *E. coli*.

So, even if new indicator organisms are added to the testing regimen, fecal coliform tests still have to be used to meet the existing legal standards. Also, some groups have decided to continue fecal coliform counts in their testing regimen so that current data can be directly compared with historical data, which usually consists of fecal coliform measurements.

Joe Richard

## **Escherichia coli** *(E. coli)*

*Escherichia coli* (*E. coli*) bacteria represent a subgroup within the fecal coliform group. (See Figure 4-1.) Amazingly, even within this smaller *E. coli* bacteria group, there are hundreds, perhaps thousands, of different strains. Although most strains are harmless and live in the intestines of healthy humans and animals, there are a few known to cause problems. For example, many of us have heard rather alarming reports about *E. coli* O157:H7, a strain associated with an estimated 73,000 cases of food-borne illness each year.[7] This deadly organism is different from the *E. coli* used as an indicator for water quality.

Use of the "harmless" *E. coli* strain as an indicator organism has advantages over fecal coliform counts:

(1) It occurs only in the feces of warm-blooded mammals and is therefore a good indicator of the presence of fecal waste in water;[8]

(2) EPA studies have shown that, in fresh water, there seems to be a correlation between the presence of *E. coli* and swimming-related illnesses.

***Note:*** *Admittedly, this can be somewhat confusing; even though the indicator strain of E. coli is considered to be harmless, it can sometimes be accompanied by the toxic strain (O157:H7) and other organisms that can cause illness.*

**Does this mean that *E. coli* bacteria should always be used as indicator organisms?**

Not necessarily. The methods used for detecting *E. coli* do have a few drawbacks:

♦ Some methods involve two incubation steps, making it more time-consuming and expensive than total and fecal coliform counts.

♦ When counting colonies of *E. coli* bacteria in a laboratory sample, there are times when other naturally occurring bacteria, belonging to the group *Klebsiella*, may be present and inadvertently counted along with the *E. coli*.[9] This can result in false positives. There is also the chance that *E. coli* counts may be elevated due to the presence of bird feces.

When any of these scenarios occur, additional steps are required to definitively demonstrate that the vast majority of detected coliforms are, in fact, *E. coli*. Even with these drawbacks, the EPA is now recommending that public health agencies regularly use the *E. coli* test when monitoring for bacteria contamination.

Joe Richard

While researching testing methods, you may run across the phrase "EPA Approved." It is important to note that just because a testing method is EPA approved, it does not necessarily mean that it is adopted by your local public health organization. If your purpose is to determine if a sample meets state legal water safety standards, you need to check with your local health organization before deciding which method to use.

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**7** *From the Center for Disease Control: The combination of letters and numbers in the name of the* E. coli *bacterium refers to the specific markers found on its surface and distinguishes it from other types of* E. coli. *Other known sources of infection from* E-coli O157:H7 *include the consumption of sprouts, lettuce, salami, unpasteurized milk and juice, and swimming in or drinking sewage-contaminated water.*

**8** *Similar to the total and fecal coliform indicator approach,* E. coli *testing is based on the assumption that if* E. coli *bacteria are found in a waterbody, there is the chance that pathogenic bacteria or viruses may also be present.*

**9** Klebsiella pneumoniae *is a naturally occurring free-living soil bacterium that can also be found in the human gut shortly after birth. There is no evidence to suggest that this species has caused healthy individuals to experience illness due to exposure.*

# Pseudomonas aeruginosa

Joe Richard

In addition to the indicator organisms mentioned in this section, a bacteria known as ***Pseudomonas aeruginosa*** (abbreviated *P. aeruginosa* often shows up incidentally in total coliform counts, even though it is not a coliform.

This bacterium can be found almost everywhere in nature and in some man-made environments, including the garden hose in your back yard. In lakes, *P. aeruginosa* is often found as a naturally occurring bacteria within aquatic plant communities and in the surrounding soils.

According to an informational bulletin published by the Hach Company,[10] a standard laboratory method has tentatively been accepted for *P. aeruginosa* testing. While it is not considered to be a particularly useful indicator of fecal contamination (i.e., it is rarely found in the feces of healthy humans and seldom isolated from animal feces), it can be useful for monitoring bathing beaches. This is because *P. aeruginosa* has been known to be a major cause of skin rashes and ear infections in swimmers and bathers.

**10** *Hach Company. 2000. The Use of Indicator Organisms to Assess Public Water Safety. Technical Information Series – Booklet No. 13. Page 26.*

# Enterococcus

As we enter the 21st century, an entirely different group of bacteria, belonging to the ***Enterococcus*** family are now being considered as indicator organisms (plural: **Enterococci**, pronounced enter-o-cox-eye).

Previously known as **group D streptococcus**, *Enterococcus* bacteria represent an entirely different strain of bacteria from the Enterobacteriaceae family. Because of their hardy nature, these bacteria occur naturally in almost every environment including soil, plants, water, and also within the gastrointestinal tract of many animals and birds. They have even been found in various food products such as cheese, raw and pasteurized milk, frozen seafood, frozen fruits, fruit juices, and vegetables.

Prior to 1984, Enterococci bacteria were grouped within the fecal *Streptococcus* genus and were often referred to as "fecal streps" by bacteriologists. However, with recent advances in genetics, microbiologists have found that certain bacteria within the fecal *Streptococcus* group were genetically unique enough to classify them as a separate genus.[11] As a result, approximately 17 different bacteria have been identified within this new *Enterococcus* group.[12]

### Enterococci as bacterial indicators

Recently, many professionals have come to consider the *Enterococcus* bacteria group as one of the preferred indicators of fecal contamination from warm-blooded animals. This is true for at least two reasons:

(1) In marine environments, Enterococci can survive longer than fecal coliforms, thus providing a more accurate indication of the presence of fecal waste; and

(2) Studies show a positive correlation between incidences of human gastrointestinal illness and concentrations of Enterococci found in public waters. However, there is one exception.

In tropical regions, some of these organisms are commonly found in unpolluted waters, making them less reliable indicators of fecal contamination. This may apply to Florida waters, so caution should be used before this method is selected over the total and fecal coliform tests presently used.

Within the *Enterococcus* group, chemists generally agree there are two main species that hold the most potential as bacterial indicators: *Enterococcus faecalis* and *Enterococcus faecium* (formerly known as S*treptococcus faecalis* and *Streptococcus faecium*, respectively). These species occur in large numbers in both human and animal feces and are thought to be appropriate indicators for determining the presence of fecal contamination in a waterbody.

It should be noted however, that two other bacteria within the same genus, *Enterococcus avium* and *Enterococcus gallinarium* (formerly known as *Streptococcus avium* and *Streptococcus gallinarium*, respectively), have been known to pose health problems, even though they are primarily associated with bird feces.

Until recently, the greatest hurdle in using Enterococci as bacterial indicators was the lack of reliable testing methods. This has changed with the development and (EPA) approval of a new mE culturing medium. While the process is similar to fecal coliform tests,[13] there are drawbacks: the medium used to test for Enterococci is more expensive than medium used for fecal coliform tests, and it contains a toxic ingredient.

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**11** *Richard R. Facklam and Sahm, D.F. 1995. Manual of Clinical Microbiology. Page 308.*

**12** *Richard R. Facklam and Sahm, D.F. 1995. Manual of Clinical Microbiology. Pages 308-309.*

**13** *Enterococci require incubation at 41° C (106° F).*

Joe Richard

## Other Indicators

Within the scientific community, there have been numerous discussions about using bacteriophages (viruses that attack bacteria) or other bacteria as possible indicators of contamination. These organisms include coliphages, such as *Bacteroides fragilis* viruses, and F-Specific coliphages, such as *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Aeromonas*, *Campylobacter jejuni*, and *Legionella*.

Many of these have merit as potential indicator organisms, but they also have major problems when it comes to detection methods. For example, some of the organisms are naturally occurring in aquatic environments, which would make it difficult to determine if there is an outside source of contamination. Additionally, the expense of detection and the use of new DNA sequencing techniques often places these tests outside the budgetary constraints imposed on most monitoring agencies. For now, it seems prudent to continue testing for total and fecal coliforms, to eliminate the most probable and immediate health risk, and let the researchers worry about detecting other organisms.

**In addition to bacterial indicators, there are a few chemical agents currently being considered as indicators of human fecal pollution:***

♦ Detergents and optical brighteners are associated with laundry discharge and their presence in surface water may indicate an upstream source of wastewater from leaky septic tanks or sewer pipes.

♦ Coprostanol—a by-product of the bacterial breakdown of cholesterol in the human body.

♦ Caffeine.

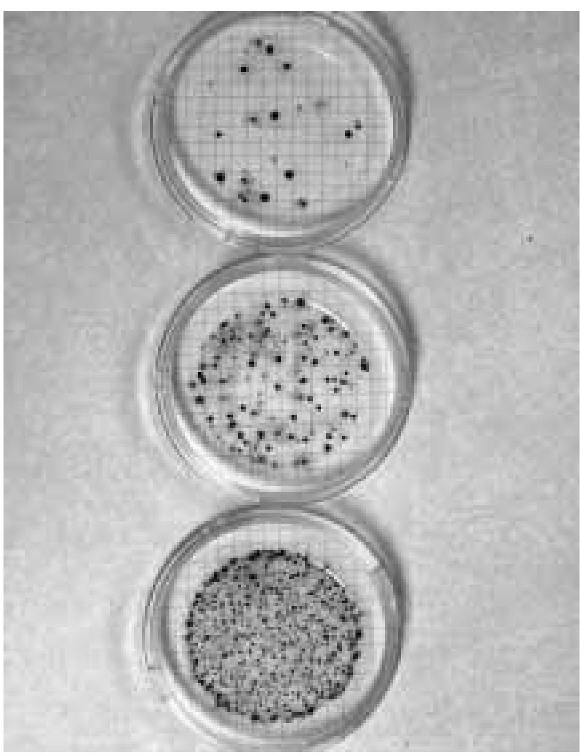
** Non-point Source News. Number 63. U.S. EPA, 2000*


Joe Richard

Disposable "Whirl-Pak" bags are popular for use in bacteria water sampling because they don't require sterilization.


Amy Richard

The membrane filtration method involves filtering samples through filters and incubating them for a specified time and temperature.



Jennifer Donze

Once filters have been incubated, coliform bacteria colonies are then identified and counted based on their coloration.

Amy Richard

Rebecca Varner counts bacteria for Florida LAKEWATCH's statewide bacteria survey project.

# Part 5
# Laboratory Methods for Counting Indicator Organisms

In addition to learning about indicator bacteria, described on pages 15-23, it's also helpful to know a little about the laboratory methods used for detection. The information may come in handy when trying to decide which method to use for bacterial monitoring. There are several variations, including:

- ♦ Membrane Filtration (MF)
- ♦ Most Probable Number (MPN)
- ♦ Plate Counts
- ♦ Presence/Absence (P/A)

Each of these methods generally involves the use of an incubator[14] as well as specific media (i.e., agars or broth) designed for supporting the growth of the targeted bacteria, while inhibiting the growth of others. The following pages provide brief descriptions of each method, with an emphasis on the two techniques most widely used in lake monitoring the **Membrane Filtration** method and **Most Probable Number** method.

### Membrane Filtration (MF)

The Membrane Filtration (MF) method involves filtering a measured amount of sample water through a membrane filter that is designed to retain the targeted bacteria. This is usually accomplished with an electric or hand vacuum pump that pulls sample water through the filter, leaving behind bacteria cells that are too large to pass through the pores.

After the filtration process, the filter itself is placed on an appropriate agar medium or a pad saturated with a special broth medium, and then incubated. If the targeted organism is present, colonies will grow. Filters are then examined and bacteria are identified by size, color, shape and sheen. Because bacteria colonies grow from a single bacteria cell, the number of colonies present is considered to be representative of the number of bacteria present in the water sample.

Results are usually reported as the number of **colony forming units** per 100 mL (**CFUs**/100 mL). This method is one of the easiest, least expensive methods for counting total coliforms.

### Most Probable Number (MPN)

The Most Probable Number (MPN) method usually involves 10-15 test tubes that are prepared with different amounts of bacteria growth medium and sample water. The medium is designed to support only the growth of a targeted bacteria species.[15] Once the test tubes are "inoculated" with sample water, they are incubated for up to four days and then examined.

The test tubes are observed for a positive or negative reaction from the target organism. A positive result would show bacteria growth and the presence of gas within the tube. (e.g., When

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**14** *Incubators range from large expensive "stand-up" models to something as simple as a box with a 40-watt bulb. See Suppliers List in the back of this circular for more information.*

**15** *Four types of media that are used for the MPN method include: minerals-modified glutamate medium, lauryl tryptose broth, MacConkey broth, and lactose broth.*

testing for fecal coliforms, a positive tube is one that shows evidence of growth and gas.) Tubes with positive growth are counted and the results are used to estimate the "most probable number" of bacteria in a water sample. This estimate is achieved by using statistical probabilities—by comparing the number of positive tubes with a table of statistically determined numbers.[16] As with anything statistical, the accuracy of this method is improved by increasing the number of inoculated test tubes for each group of samples.

Compared with the MF method, the MPN test has several disadvantages, especially for individuals wanting to do their own sampling and bacteria testing:

(1) it's considerably more expensive and labor intensive;

(2) because it involves the use of 10 to 15 test tubes, this method also takes up quite a bit more incubator space; and

(3) the MPN test does not yield a direct bacteria count.

It does have one advantage, however; suspended sediments within a water sample do not affect the MPN process, whereas the MF method involves the use of filters that can become clogged with sediments and/or algae.[17] This is something to consider if your water sample is from a lake or waterbody with high algal counts or turbidity problems (i.e., lots of suspended solids).

### Plate Counts

Plate count methods are traditionally used for monitoring drinking water. The reference book *Standard Methods for the Examination of Water and Wastewater*[18] describes three variations:

♦ **Pour plate method** –(a.k.a., the standard plate method) involves pouring liquefied agar medium into petri dishes and then adding a measured amount of sample water. Once the sample is mixed with the medium, the plates are left to sit so the contents can solidify. They are then inverted and placed in an incubator. Once the samples are incubated, bacteria colonies are counted and reported as "colony-forming units" per milliliter of water sample (CFUs/mL).

♦ **Spread plate method**– is different from the pour plate method in that agar is poured onto the plate and allowed to solidify before it is exposed to the sample. Once the agar is solidified, the sample is spread onto the plate surface with a sterilized bent glass rod and allowed to be absorbed into the agar medium before it is incubated. Colonies, that appear after a period of incubation, are identified and counted.

♦ **Membrane Filtration technique** – see Membrane Filtration on page 25.

### Presence/Absence (P/A)

This method is just what its name implies. Similar to the plate methods, it is mostly used for monitoring drinking water. The theory holds that as long as there are zero coliform organisms within a large number of samples, actual bacteria counts are not necessary. In other words, testing is done simply for the presence or absence of organisms.

While there may be problems with this approach as it relates to the use of recreational waters, it can be an effective screening tool. For example, if there are no total and/or fecal coliforms found in a sample, there is a high probability that there is not a bacteria problem. Conversely, if total and/or fecal coliforms are present, one can then go ahead and do more specialized testing for identifying specific bacterial organisms or groups such as fecal coliforms, *Pseudomonas aeruginosa*, *Enterococcus spp*., etc.—whatever one's budget will allow.

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**16** *These tables are developed and used in bacteria laboratories to calculate statistical probabilities.*

**17** *The Volunteer Monitor. Fall '98. Bacteria Testing Part 1. Methods Primer. Page 9.*

**18** *Often abbreviated as* ***Standard Methods****, this book is considered to be the foremost authority on the science of water analysis.*

## Which Laboratory Method Does Florida LAKEWATCH Use?

**In the year 2000, Florida LAKEWATCH began a preliminary statewide survey collecting bacteria data on more than 80 lakes. The objectives of the survey are two-fold. Using data from the survey, researchers are:**

1 Trying to determine if there are patterns in the abundance of total coliform bacteria and the fecal coliform known as *Escherichia coli* (*E. coli*) in Florida lakes and waterbodies.

***Note:* E. coli *are a subgroup of fecal coliform bacteria. Both fall under the larger category known as total coliforms. See page 20 for more information.***

2 Looking for relationships that can be drawn between coliform abundance and other environmental factors such as changes in water temperature, rainfall, aquatic plant abundance, algae blooms, etc.

Florida LAKEWATCH uses the membrane filtration technique for fast, simultaneous detection of total coliforms and the fecal coliform known as *E. coli*. Test kits are purchased from the HACH Company and are identified as **Method 10029 m-ColiBlue24 Broth**.

Most Florida LAKEWATCH bacteria samples have been collected one time only from each of the lakes involved in the survey and therefore should only be considered as a description of the bacteria concentrations for that day; data posted in the annual LAKEWATCH Data Summary book 1986-2001 are not intended for use in making public safety decisions. However, they are helpful in looking at patterns among bacteria counts and other environmental factors.

Preliminary analyses of the data shows that 15% of the (approximately) 1,000 samples collected had total coliform counts that would exceed Florida's state criteria for total coliforms. However, less than 0.01% of the samples had *E. coli* counts that would have exceeded Florida's state criteria for fecal coliforms.

Joe Richard

### What does this mean?

High total coliform counts, as found in 15% of the samples, are generally associated with abundant plant material, and may indicate the potential for a variety of infections, including skin rashes and/or external ear infections in swimmers. The presence of abundant plant material also introduces the possibility of other water-related illnesses such as swimmer's itch.

The low *E. coli* counts suggest that there is not a major problem with fecal contamination in this sampling of Florida lakes (i.e., even though the data are preliminary).

Details related to bacteria detection techniques are sure to change in the coming years as improvements continue to be made, on an almost daily basis, in the microbiology field. For this reason, we are reluctant to get too specific about the many techniques, agars, broths, incubation temperatures, etc. For a historical perspective we recommend that you refer to a copy of ***Standard Methods for the Examination of Water and Wastewater***. For the latest up-to-date information, we suggest you contact any of the laboratory suppliers listed in the back of this booklet.

**ATTENTION: Water quality standards for bacteria vary from state to state; residents living outside of Florida should consult with their own state public health agencies for more information about bacteria standards.**

Joe Richard

# Part 6
# Criteria Used for Assessing Coliform Contamination in Florida Waters

Like many other states, Florida has established numerical bacteria counts that are used as the legal standard for determining the presence of total and/or fecal coliform contamination in Class III waters.[19] The criteria are the same for both fresh and marine waters:

**Florida's <u>total coliform</u> standard ***

Less than or equal to 1,000 as a monthly average; nor to exceed 1,000 in more than 20% of the samples examined during any month; less than 2,400 at any time. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30-day period using either the MPN or MF counts.[20]

**Florida's <u>fecal coliform</u> standard ***

MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on 10 samples taken over a 30-day period.

* *Florida Department of Environmental Protection; Chapter 62-302.530, Florida Administrative Code.*

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**19** *Class III waters are defined as "waters designated for the purpose of recreation and the propagation and maintenance of healthy, well-balanced populations of fish and wildlife."*

**20** *The abbreviation **MPN** stands for "Most Probable Number." The MPN technique refers to a specific method, used in laboratories, to estimate the number of bacteria colonies in a measured amount of water, usually 100 ml.*

*The abbreviation **MF** stands for "Membrane Filter." The MF technique refers to a specific method used to count the number of bacteria colony forming units (or CFUs) on a membrane filter after 100 milliliters of sample water have been poured through it.*

In August 2000, the Florida Department of Health initiated the Florida Healthy Beaches Program. The program was designed to assess the bacteriological quality of coastal beaches in 34 counties. To do this, the Department of Health measures both fecal coliforms and enterococci.

**The following criteria for fecal coliforms, were established by the Florida DEP and are based on the legal criteria established by Florida law:**

**<u>Fecal coliforms:</u>**

**Good** = 0-199 fecal coliforms per 100 milliliters (mL) of marine water

**Moderate** = 200-799 fecal coliforms per 100 mL

**Poor** = 800 or greater fecal coliforms per 100 mL

***Note:*** *If a fecal coliform count is observed to exceed 800 colonies per 100 milliliters of beach water and a re-sampling result also exceeds this value, then a health warning would be issued for the sampling site.*

**The following criteria for enterococci bacteria have been recommended by the U.S. EPA as a saltwater quality indicator. As of 2002, they have not been established as legal criteria.**

**<u>Enterococci:</u>**

**Good** = 0-34 enterococci per 100 milliliters (mL) of marine water

**Moderate** = 35-103 enterococci per 100 mL

**Poor** = 104 or greater enterococci per 100 mL

***Note:*** *If Enterococci results are observed to equal or exceed 104 colonies per 100 milliliters of beach water sampled and a re-sampling result also exceeds this value, an "Advisory" (not as strong as a "Warning") would be issued for the sampling site.*

Amy Richard

**With careful thought and good detective work,**
**it is possible to locate the source**(s) **of bacterial contamination.**

# Part 7

# A Four Step Process for Identifying and Locating Bacterial Contamination

Unless there is a catastrophic failure of a major sewage collection line, finding the source of bacterial contamination in a lake or waterbody can be difficult, time consuming and, depending on which testing regimen you choose, it can also be expensive. In fact, lack of funding is often the largest hurdle that citizens run into when sampling is proposed. Because of this, public agencies are often hard-pressed to conduct bacteria testing in a timely or consistent manner. If they have the resources to sample at all, it's often limited to one sampling event, which usually tells little.

**Re-sample!**

**Re-sample!**

**Re-sample!**

**Re-sample!**

### The Good News

With careful thought and some good detective work, it is possible to locate the source(s) of bacterial contamination. Working together, lakeside communities can raise money to pay for sampling by private laboratories or they can do it themselves. Regardless of which approach you take, Florida LAKEWATCH's Four Step process—described on the following pages—provides a simple framework and relatively inexpensive testing strategy to follow. It was developed by LAKEWATCH staff in response to hundreds of inquiries and a pilot study that was conducted in several counties.

When reading through the steps, you may notice that re-sampling often, and in many locations, is a major component of our plan. It's been our experience that a willingness to do this will help assess risks related to water usage and, if there is evidence that bacterial contamination may be present, it can help pinpoint possible sources. While there is no guarantee that these efforts will find every source, there is a high probability that most of the important ones can be identified.

Also, the LAKEWATCH approach is based largely on the use of total and fecal coliform counts. These methods are recommended because they are generally easier and less expensive to process than other tests currently available and are therefore, more accessible to the average citizen or monitoring group. In fact, more and more volunteer groups are investing in basic laboratory equipment (i.e., incubator and test kits) and are doing the testing themselves. Now, thanks to the development of several new culturing mediums, *E. coli* counts are just as easy as to process as total and fecal coliform counts.

**See page 27 for more on the *E. coli* count testing method used by LAKEWATCH.**

One more thing: the fecal coliform count "criteria" in Steps 2 and 3 are roughly based on Florida's state regulatory codes. This is not a coincidence. However, the importance of the following four step plan goes well beyond state regulatory codes, which are apt to change in the near future. Our main goal in presenting this information is to: (1) enable you to determine if there is a problem to begin with; (2) enable you to spot patterns in your test results; and (3) help you locate the source of contamination.

## Step 1
### Collect Water Samples from Multiple Sites

If contamination is suspected, sampling at a single site will not provide sufficient information to make an accurate assessment of the problem. Therefore, it's recommended that water samples be collected from approximately 12 sites, spaced as uniformly as possible around the lake or waterbody. Nine of the sampling sites should be located near shore.

It's also recommended that three sites be sampled offshore in open water, as this will permit a judgment of the magnitude of contamination.

If the waterbody has a large amount of aquatic plants, try to sample just away from the plants. If there are not a lot of aquatic plants, the samples should be collected in water less than three feet deep, as this offers the best possible chance of detecting contamination.

All samples should then be analyzed for fecal coliform counts. This can be done in a professional laboratory or even with the help of some home-monitoring kits that are now on the market.

**See List of Suppliers in the back of this circular.**

David Watson

**While disposable Whirl-Pak baggies are popular for the collection of bacteria water samples, plastic Nalgene bottles can also be used. The only drawback is that they need to be sterilized each time.**

## Step 2
### Identify Sites with Elevated Fecal Coliform Counts

Once fecal coliform counts have been obtained from various sampling sites on your lake, classify them using the three categories listed below. Generally speaking, if the sample results fall within Low Risk category, the waterbody would generally be considered "safe," as they are within Florida's criteria for Class III waters. However, if samples fall into the Potential Risk or High Risk categories, they may not meet Florida's Class III water standards and re-sampling is strongly recommended.

### Low Risk Category
**Sampling sites with fecal coliform counts of less than 200 (CFUs per 100 mL)**

As a general rule, sampling sites with fecal coliform counts of 0 -199 colonies per 100 milliliters of water (CFUs per 100 mL) are most likely not a problem and for all practical purposes, the bacteriological quality of these sites can be ranked as good. Furthermore, if all the sites in the waterbody have results in this category, there is a strong possibility that the waterbody is not being contaminated.

***Note:*** *It must always be recognized that even a count of zero bacteria does not absolutely preclude the possibility of contamination.*

### Potential Risk Category
**Sampling sites with fecal coliform counts ranging between 200 and 799 (CFUs per 100 mL)**

Sampling sites with fecal coliform counts ranging between 200 and 799 CFUs per 100 mL of water represent sites with potential fecal contamination. One of the first things to do when such results are obtained, is to examine the sampler's field notes and any comments from local residents regarding possible reasons as to why the counts would be high at the sites. Move to Step 3.

***Note:*** *There is really no need to re-sample areas that meet Florida's fecal coliform criteria [see Chapter 62-302.530, Florida Administrative Code]*

**High Risk Category**
**Sampling sites with fecal coliform counts of 800 or greater (CFUs per 100 mL)**

When a fecal coliform result is observed to exceed 800 CFU/100 mL of water sampled, consideration should be given to issuing a health warning for the sampling site until re-sampling can be done. State rules indicate that health warnings should only be issued after additional samples provide high counts. This is considered the best approach to prevent undue public concern, but the public should at least be notified that additional testing is planned. Move to Step 3.

## Step 3
### Test for *E. coli* and Look for False Positives

Once you have determined (from Step 2) that fecal coliform counts are high, the objective at this stage should be:

♦ To determine whether or not there are *E. coli* organisms present in the fecal coliform tests; and

♦ To rule out the possibility of "false positives" that may have occurred due to large concentrations of birds (i.e., bird waste) and/or the presence of the naturally occurring soil bacterium known as *Klebsiella*.

**Test for** *E. coli* – It is recommended that you have a private laboratory do the testing for *E. coli*. If it is demonstrated that the vast majority of detected fecal coliforms belong to the *E. coli* bacteria group, there is a high probability that the contamination source is human and continued re-sampling at the water body is warranted until the source of contamination is found and eliminated. It is also recommended that you contact your local public health agency and move on to Step 4.

**Rule out false positives** – If *E. coli* are not detected, yet the fecal coliform counts remain high, two possibilities should be considered: Contamination from birds (ducks, geese, sea gulls, etc.) or false positives from soil borne bacteria.

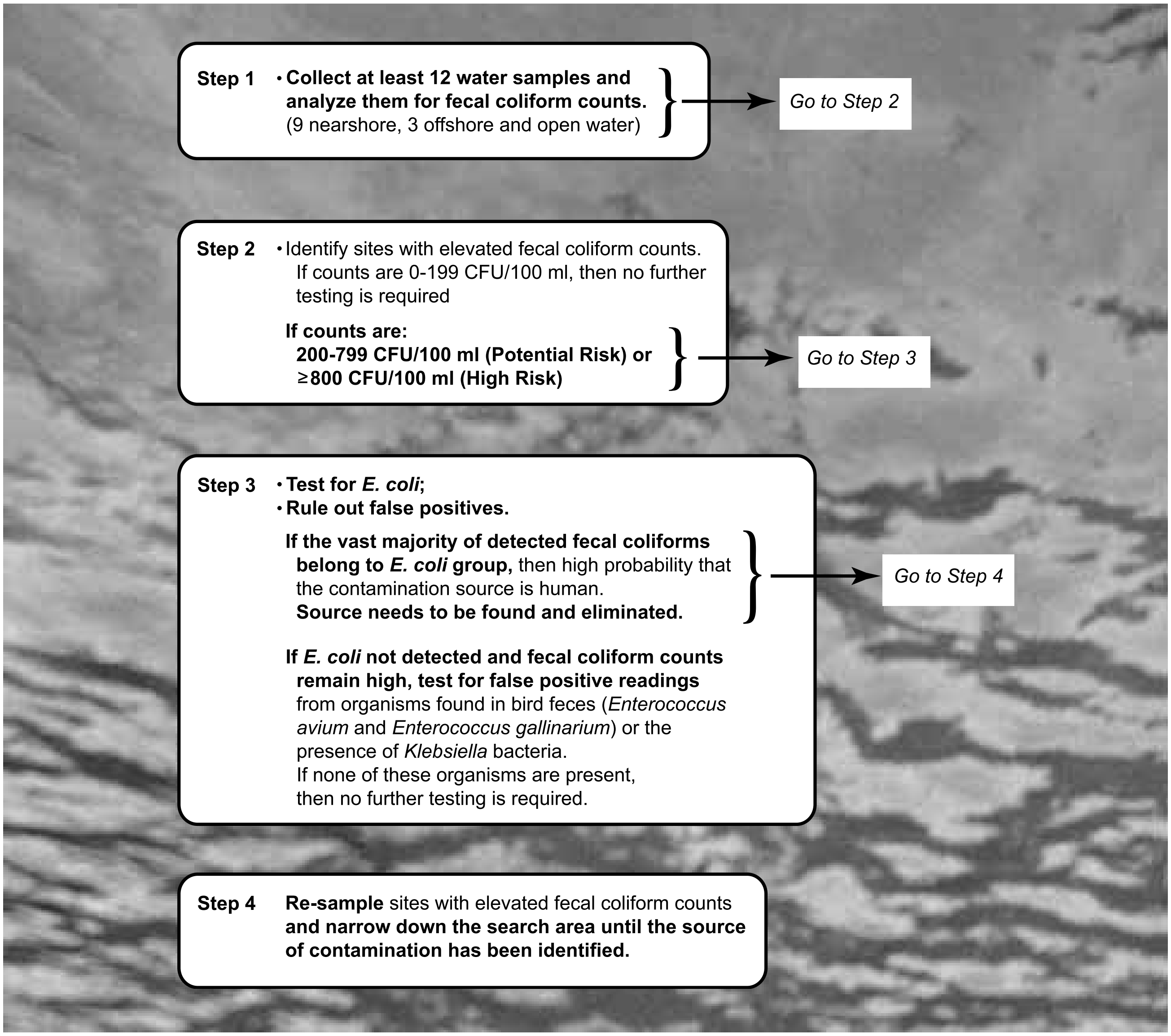
Joe Richard

When testing for false positives, the primary focus should begin with birds. Are birds residing in these areas on a regular basis or being routinely fed at these sites? If the answer is yes to either of these questions, birds may be the most logical explanation rather than septic tanks or sewage lines. If you want to be sure that birds are the culprit, you can test for two specific organisms that may be showing up in your fecal coliform testing regimen, *Enterococcus avium* and *Enterococcus gallinarium*. To do this, you will need to contract with a private laboratory.

If it is indeed a bird-induced problem, various management strategies can be tried. Steps can be taken to encourage the birds to leave and/or discourage them from roosting in the area. Hunting (when legal) and noise devices can be effective, but you'll need to check with your local wildlife agency before implementing either of these approaches. The good news is, this usually means you have eliminated the need for further testing.

If high fecal counts are not related to birds, the next thing you should do is test specifically for the *Klebsiella* bacteria. The presence of these organisms in a fecal coliform test can also give false positives—especially in Florida. If tests indicate that high fecal coliform counts are due to *Klebsiella*, then there is no serious health risk and the need for further testing can most likely be eliminated.[21] If tests indicate that high fecal counts are not related to *Klebsiella*, then it is time to move to Step 4.

**21** *There is no evidence to suggest that* Klebsiella *bacteria have caused any healthy individuals to experience illnesses due to exposure in the natural environment.*




## Step 4
### Re-sample Sites with Elevated Fecal Coliform Counts

Because false positives have been ruled out by now, the remainder of your bacteria testing can be done using the less expensive fecal coliform test. The objective of this sampling regimen is to identify the location of a contamination source. Sites that have been identified as potential sources should be re-sampled as soon as possible. With these results, it may be possible to narrow the search down to a smaller area.

Once re-sampling is initiated, new sites should be chosen near previously identified sites (i.e., those with high fecal coliform counts). Attention should be given to the locations of inflowing streams, ditches, stormwater pipes, and/or water currents, with additional consideration given to larger point sources such as farms.

If fecal coliforms are higher in one area, you will have narrowed down your search area and greatly improved the chances of identifying the source.

**See page 35 for Locations to Consider When Identifying and Locating Possible Sources of Contamination.**

If the source for contamination is not readily found, the assistance of local property owners should be enlisted in the effort. Specifically, they should be asked to examine their

# Locations to Consider When Tracking Possible Sources of Contamination

**When tracking down suspected sources of bacterial contamination, special consideration should be given to the following locations:**

♦ **In-flowing pipes, ditches or streams** – If you know of a place where water comes into a lake or waterbody, it's a good idea to follow (i.e., on foot) the pipe or stream and identify any other locations where water may be entering the system. Make an effort to determine what water source the pipe is linked to. It may be coming from a previously unidentified source.

♦ **Water currents** – For example, look at the way submersed aquatic vegetation (i.e., aquatic plants) may be bending or leaning, as it could be an indication of the direction that water is flowing into a lake. Follow that source and collect water samples at evenly spaced intervals.

♦ **Potential point sources** – Identify areas with high densities of animals that might represent potential problems (i.e., farms, ranches, pet parks, etc.). Keep in mind that even if these areas are not visible from the shoreline or are located away from the waterbody, stormwater runoff could still drain from them. However, it must also be said that larger "point sources" such as farms sometimes attract misdirected attention when it comes to suspected bacterial contamination. Nothing should be assumed until the actual source is confirmed with additional sampling. Quite often, the real culprit turns out to be something other than the original suspected source, such as a leaking sewer pipe between the farm and the lake.

♦ **Septic systems** – When a contamination source is not readily found, the assistance of local property owners can be enlisted to help find the source. Specifically, they should be asked to examine their septic drainage field for possible breach sites. If a sewage smell is detectable, it may be an indication that additional sampling is needed in the area.

City of Orlando Streets, Drainage and Stormwater Utility

Joe Richard

Joe Richard

Joe Richard

septic drainage field for possible breach sites. They should also be encouraged to use the "smell" test. If they can smell a sewage odor, this alone is evidence for supporting additional sampling.

Once the source of contamination is identified and it is determined that the problem cannot be easily remedied by the private homeowner or community, report it to your public health agency. If the source happens to be on public land, you may have to recruit assistance to raise funds to solve the problem or to exert pressure on governmental agencies to fix it.

## Conclusion

Franklin Delano Roosevelt once stated, "We have nothing to fear, but fear itself!" Life itself is not without risk, and aquatic activities are certainly not exempt. It is impossible to guarantee with 100% confidence that an individual will not become ill upon contact with water. However, with the technology and information now available, bacterial contamination of water is much less of a problem than it used to be. As they say, "the cup is at least half full." Rather than being fearful, citizens are encouraged to remain vigilant and solve problems as they emerge.

As far as the future is concerned, we all need to pay close attention to the developing technology for measuring bacterial contamination. Things are changing almost daily and better techniques are becoming available. For the time being, however, the use of total coliform, fecal coliform and *E. coli* counts continues to do the job in most instances—especially when combined with the Four Step approach described in this circular.

So for now, one can take comfort in knowing that if total and fecal coliform counts are below the legal state-established criteria, there is a strong probability that the water is safe for recreation. With that knowledge, we hope you'll enjoy Florida's wealth of unique and refreshing aquatic environments—in good health!

# Suppliers

**Hach Company**
Loveland, CO
Phone: (800) 227-4224
Website: http://www.hach.com

**Millipore Corporation**
Bedford, MA
Phone: (800) MILLIPORE
Phone: (800) 221-1975 or (800) 645-5476
Website: http://www.millipore.com

**Micrology Laboratories** (for Coliscan products)
Goshen, IN
Phone: (888) 327-9435
Website: http://www.micrologylabs.com

**IDEXX Laboratories** (for Colilert products)
Westbrook, ME
Phone: (207) 856-0300
Website: http://www.environmental-center.com/technology/idexx/idexx.htm

# Selected References

## Books

American Public Health Association. 1998. STANDARD METHODS for the Examination of Water and Wastewater. 20th Edition. American Public Health Association. Waldorf, Maryland.

Canadian Council of Ministers of the Environment. 1999. Canadian Water Quality Guidelines. Eco-Health Branch. Ecosystem Sciences and Evaluation Directorate. Environment Canada. Ottawa, Canada.

## Publications

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Hach Company. 2000. Microbiological Laboratory Start-up Guide. See Microbiological Testing Section, pages 135-182.

Hach Company. 2000. Analytical Procedures. Coliforms: Membrane Filtration (simultaneous detection) for the detection of total coliforms and *E. coli* from potable water, non-potable water, wastewater.

Hach Company. 1997. Products for Analysis Catalog (Literature #3226).

The Volunteer Monitor. 1998. Issue Topic: Monitoring Estuaries. Special Section: Bacteria Testing, Part 1. Volume 10, No. 2. Fall. Pages 8-15.

***Note:*** *This publication is a must for individuals or groups interested in doing their own bacteria testing. Numerous testing methods are discussed and compared and information is even provided on how to build an incubator.*

Toranzos, G. A. 1991. Current and possible alternate indicators of fecal contamination in tropical waters: a short review. Environmental Toxicology and Water Quality 6: 121-130.

U.S. EPA. 2000. Non-point Source News-Notes. Special Focus: On-site Wastewater Treatment. December. Number 63.

***Note:*** *News-Notes are accessible on EPA's website:* ***www.epa.gov/OWOW/info/NewsNotes/index.html***