SL 259

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Indicator Organisms: What Every Floridian May Want to Know about Microbiological Water Quality[1]

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Floridians rightfully brag about the bounty of water resources in our state. We enjoy beautiful beaches and springs. A lot of commercially bottled water is harvested right here in Central Florida. How do we know, however, that our tap and well water is safe to drink and our beaches are free from pollutants? Most of us can easily detect particulates, unpleasant odors or strange colors – all signs of poor quality. Detecting waterborne pathogens and toxins, however, is not that easy. To learn about the presence of microbes and toxins in water, one needs to consult the County Health Department or a private water-testing lab. These professional laboratories will test for heavy metals, some specific toxins and the presence of bacteria. This EDIS publication is designed to help you interpret the results of a microbiological water test.

There are three common misconceptions about testing microbiological safety of water. One is that pH and nitrates can predict the microbiological quality of water. Most labs test water pH and detect the presence of nitrates/nitrites, however numerous studies have demonstrated that, while these parameters certainly contribute to the overall water quality, they are not predictors of the microbiological safety of water.

Another common misconception is that water testing labs look for pathogens that may be present in drinking or recreational water. There are dozens of microbial species that are considered to be waterborne pathogens. Various viruses, bacteria and protozoa can cause diseases when ingested with drinking or recreational water. Most common waterborne pathogens are *Giardia*, *Cryptosporidium* (both protozoa), *Campylobacter*, *Salmonella*, *E. coli*, *Shigella*, Hepatitis A, and Norwalk-type viruses. Infections with *Plesiomonas* and *Vibrio* are less common in industrialized countries, but may occur after a major disruption in water disinfection or after hurricanes. All these waterborne pathogens initially cause similar symptoms: nausea, vomiting and diarrhea.

Unfortunatly detecting pathogens is not a simple process; only pathogenic protozoa (*Cryptosporidium*, *Giardia*) and the red tide organism are distinguishable under the microscope, viruses cannot be seen under a light microscope, and bacteria appear as either tiny spheres or rods. To identify pathogenic

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bacteria, they must either be cultured on specific growth media, characterized based on fingerprinting of their nucleic acids, or tested for their ability to cause immunological reaction. The latter two methods are commonly used to identify viruses. These tests are expensive and are typically conducted only during an outbreak or when an in-depth study is warranted. In most cases, water-testing labs survey samples for either total bacterial load or indicators of microbiological quality. Total bacterial load is often measured to see whether environmental conditions and nutrient availability are conducive to bacterial growth in a body of water. While high total bacterial counts (TBC) raise serious questions about the suitability of water for drinking, agricultural uses or recreation, high TBC does not necessarily mean that contamination with human or animal wastes has occurred.

To try to better estimate the likelihood of water contamination with human wastes, water-testing labs rely on indicator organisms. The World Health Organization (WHO) defines such an indicator as an organism that is typically absent from pristine waters, but present when contamination with human wastes is present. The indicator organism should be easy and safe to work with, thus minimizing health risks to the laboratory technicians. The indicator should be present in numbers that are correlated with, but higher than the numbers of pathogens. An indicator should not be able to multiply in the environment and should respond to the disinfection the same way that a pathogen would. These criteria were designed to learn whether a contamination with human waste might have occurred, although the presence of indicator organisms does not necessarily prove that pathogens may be present in a water source.

While criteria for the “ideal indicator” have been developed by WHO for decades, no such ideal candidate has been identified. Most water testing laboratories rely on fecal coliforms, *E. coli* *Enterococcus* or -- much more rarely -- on *Clostridium perfingens* or viruses of *E. coli* as indicators. The use of each of these organisms as indicators has advantages and disadvantages, discussed below.

Fecal coliforms are bacteria (*E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*) that are thought to have co-evolved with mammals, and have “learned” throughout evolution to utilize lactose and be resistant to bile acids. Microbiological media that contains these chemical is used to detect the presence of fecal coliforms in a water sample. Chemical supply companies sell inexpensive media for detection of fecal coliforms. Most commonly used media are McConkey Agar, Hektoen Enteric Agar or their various modifications. Fecal coliforms are always present in water contaminated with fecal material, they are easy to detect, are generally hardier than true pathogens, and will be found in greater numbers than the real pathogens. Absence of fecal coliforms is a good bacteriological indicator of safe water. For decades, coliform bacteria have been used as "indicator organisms" in the evaluation and monitoring of public and private drinking water sources and distribution systems.

While absence of coliforms is generally a good sign of microbiological safety of water, positive test results can be also be caused by the presence of environmental bacteria that are related to coliforms. The major drawback of relying on fecal coliforms or *E. coli* as indicators is that many of the same organisms have been found to be associated with plants or aquatic invertebrates. For example, *K. pneumoniae* 342 is a well-characterized beneficial bacterium that serves as a biological “fertilizer” for wheat and some other plants (Iniguez et al., 2004). While it is closely related to fecal coliforms and fits the detection criteria for fecal coliforms, presence of this plant-associated *Klebsiella* does not indicate contamination with human wastes. Plant-associated *Salmonella*, *E. coli*, *Enterobacter*, *Citrobacter* and *Klebsiella* are commonly isolated from plants in pristine environments (Wang et al., 2006; Winfield and Groisman, 2003). Nonetheless, positive coliform results should be regarded as an indicator of possible fecal contamination.

Because of the uncertainty associated with the detection of fecal coliforms in water, the positive sample is oftentimes re-tested specifically for presence of *E. coli* or other indicators. Total coliforms continue to be the basic microbiological standard in the United States because their absence generally suggests the absence of *E. coli*. Despite the limitations of the fecal coliform method which was developed early in the last century, detection of fecal coliforms is the cheapest and most commonly used technique for testing microbiological water quality. To try to compensate for some of the method s shortcomings, one should consider confirming the results using at least one other indicator.

The use of *Enterococcus* as an indicator of human fecal contamination has gained popularity in the past decades since the U.S. Environmental Protection Agency recommended it as an indicator of marine water quality (http://www.epa.gov/waterscience/beaches/files/rvsdman.pdf). Unlike *E. coli* or other coliforms, fecal enterococci generally appear to persist in seawater for longer periods of time and they are not as sensitive to solar radiation. Fecal enteroccoci (*E. feacalis* and *E. feacum*) are most commonly associated with humans or warm-blooded animals, which makes them more direct indicators of human activities. This has made fecal enterococci a reliable predictor of the microbiological quality of marine waters (Noble et al., 2003). Similarly to coliforms, enterococci have been reported to multiply in many natural environments, therefore negating their value as predictors of contamination with human wastes.

The third misconception about microbiological water testing is that private well water is always safe and only needs to be tested for nitrates and heavy metals. Microbiological safety of a private water well is not only the concern of the household served by the well, but also the households using other nearby water supplies and the aquifer that the water is drawn from. Compliance with public health laws is based on the presence or absence of total coliform. In public water systems, repeat samples are required for each coliform positive sample, including same tap, and upstream and downstream connections. In addition, each positive must be tested for the presence of *E. coli* or fecal coliforms. If the repeat sample is fecal coliform positive, or if the original fecal or *E. coli* positive is followed by a total coliform positive, state regulators must be notified on the same business day. Positive coliform results, with negative *E. coli* or fecal coliform results may not require any immediate action, as the maximum contaminant level is based on the number of coliform positives in relation to the number of samples taken. Based on the CDC estimates, in 1999-2000, contaminated private well water caused 26% of the drinking water outbreaks that made people sick.

In light of the disadvantages of using microbiological indicators to predict water quality, one may question the wisdom of relying on such a water testing method. Completely discounting microbiological indicators is, perhaps, a rash decision. A combination of factors should be considered before making a decision about water safety. For example, presence of coliforms in well water and surface-exposed waters should be interpreted differently. Fecal coliforms in surface waters could have originated from a number of sources, some of which may have little to do with human waste or farm run-offs. On the other hand, fecal coliforms in well water are most likely a direct indicator of a fairly recent contamination with human or animal waste.

While it is important to understand the limitations of current testing methods, there is no substitute for a water test!

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