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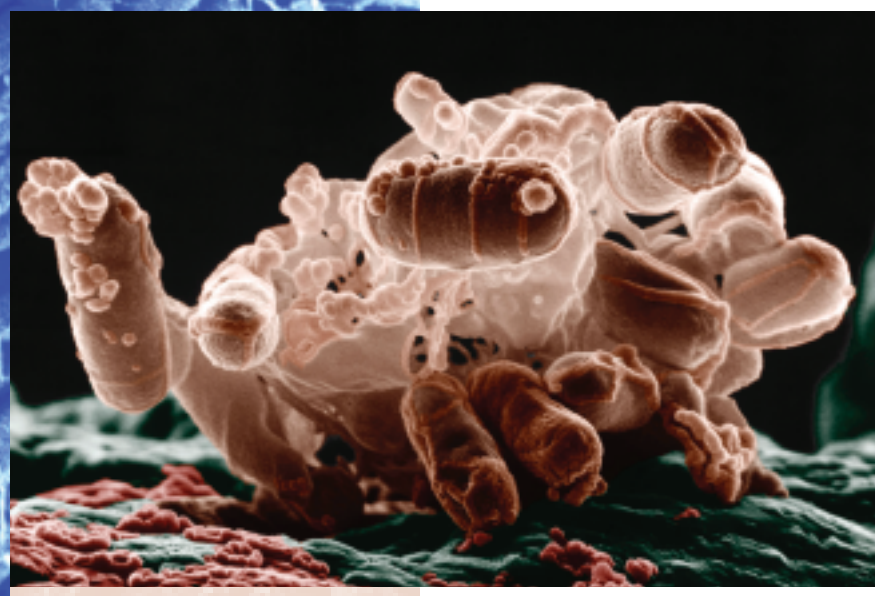

Above: Colorized low-temperature electron micrograph of a cluster of *E. coli* bacteria. Individual bacteria in this photo are oblong and colored brown. *Photo by Sandra Silvers.*

Background: As bacteria accumulate on surfaces, they exude a complex matrix of fibrils that connect cells, and many bacteria align side to side. *Photo by Eric Erbe. Colorization by Christopher Pooley.*

Photos courtesy of USDA's Agricultural Research Service

Testing for Bacteria

Abundant and everywhere, these small creatures can wreak havoc in water systems. High-quality testing offers assurance to protect customers against these dangerous bacteria.

Bacteria are the most abundant organisms on the planet. It is estimated that they have been in existence 3.5 billion years. These fascinating little creatures are responsible for many of the functions that allow Earth to sustain other forms of life. The health of our planet ultimately depends on their activities as they dwell everywhere throughout the planet such as in the soil, in food, on plants, in our bodies, in the waters and way deep within the Earth.

Background and Characteristics

A Dutch scientist, Anthony van Leeuwenhoek, first discovered bacteria in 1676. During his initial observations with a crude microscope, he referred to them as "tiny little 'animalcules' very prettily moving about." Later, these 'animalcules' would become known as bacteria. Bacteria are single-celled organisms classified as prokaryotes. A prokaryote is an organism that lacks a true nucleus to contain the DNA (genetic material) leaving it to 'free-float' within the cell. Only visible through a microscope, bacteria average in size from 0.1 µm (micrometer) to greater than 10 µm in diameter.

Bacteria usually are grouped together based on their like characteristics such as shape, need for oxygen, the way they obtain food and stain differential. There are three common shapes that bacteria come in called bacilli (rod shaped), cocci (spherical or spheroid) and spirilla (spiral or corkscrew). Single cocci may be found in colonies, which are further defined by their arrangement: diplococcus (paired), streptococcus (chains), tetracoccus (four cells arranged in a square) and staphylococcus (grape-like clusters). Bacilli also may be found in pairs and chains.

Bacteria may be grouped by their need of oxygen to survive and are known as aerobic or anaerobic. Aerobic organisms are those that thrive on and require oxygen to survive while the anaerobic organisms cannot tolerate oxygen. Many of the bacteria that can cause food poisoning are of the anaerobic variety. Bacteria also can be defined as autotrophs, those organisms that are capable of making their own food, or heterotrophs that derive their food from other nutrients or hosts.

Reproduction

One of the most amazing occurrences to view in a live culture of bacteria is the way in which they reproduce. Bacteria reproduce asexually by a process called binary fission. During binary fission, a single cell divides to form two new cells called daughter cells. In this process, the chromosomes will replicate and a cell wall will form between the chromosome and its duplicate, therefore each daughter cell contains an exact copy of the genetic information contained in the parent cell. This process continues with each daughter cell, allowing the bacteria population to increase by geometric progression. Generation time is the time required for a given population to double in size and some generation times can be as short as 20 minutes.

As populations grow they experience different phases of growth. Initially, they are acclimating to their surroundings and experiencing slow growth or generation times called the lag phase. After the lag phase they enter into the log phase where generation times are shorter and growth occurs exponentially. As the population multiplies at such great rates, food sources tend to dwindle as competition ensues and they enter a stationary phase. As food sources are depleted and toxins build, the last stage of growth is entered and the population declines in the death phase. This cycle will repeat as conditions improve and decline.

Bacteria and Drinking Water

Since 1989, all water systems are required to test for coliform and *E.Coli* bacteria based on U.S. Environmental Protection Agency (EPA) Safe Drinking Water Act regulations, specifically the Total Coliform Rule. The reason the EPA has established a guideline for these organisms is because they are a good indicator species that can be tested in an economical and relatively easy manner. An indicator species means that in optimum conditions for coliform to flourish, it also is an excellent possibility that other infectious disease organisms may exist, as they tend to flourish in like conditions. *E.Coli*, a subset of fecal coliform bacteria, most commonly grow and flourish in the intestines of warm-blooded animals. *E.Coli* presence also is tested as an indicator, as it generally suggests that the water source has been in contact with waste contamination. Waste contamination sources may include septic system infiltration, storm runoff or direct human or animal waste deposits. The total coliform rule states that no more than 5 percent of samples can be positive in a month period for coliform bacteria, and no *E.Coli* detections are allowed in any sample. The number of samples that a system has to test for is determined by the population size that the system serves. It generally is assumed and accepted that in the absence of these organisms, that the water is considered safe for consumption. This cannot be considered a 100 percent rule, however, because the presence or absence of these organisms doesn't always correlate with the presence or absence of some viral or protozoan pathogens.

Testing for Coliform and *E.Coli*

There are numerous methodologies available for the testing of coliform and *E.Coli* bacteria in drinking water. This article briefly explains how two of the more popular methods are performed and what the results mean.

About the Author
Tami E. D'Amico, technical support and accounts manager with National Testing Laboratories, Ltd. (NTL) since 1997, works with companies such as water treatment equipment manufacturers and dealers, well drillers, contractors, engineers and consultants in the water industry. D'Amico has degrees in biology and general science studies. She serves on various Water Quality Association committees and has authored several articles for industry publications. NTL has served the water industry with quality analysis for informational and compliance testing requirements for more than 18 years. With headquarters in Cleveland, NTL has laboratory facilities in Michigan, Florida and Virginia. D'Amico can be contacted at 800-458-3330, ext. 222; tdamico@ntllabs.com.

One of the most familiar methods is the **chromogenic substrate test,** also known as the presence/absence method due to the type of result that is obtained. This method is very accurate in determining the presence or absence of both coliform and *E.Coli* bacteria. Special enzymatic substrates are added to the samples and the bottles are incubated for 24 hours. After 24 hours, the samples are examined for any color change in the sampling bottle. Coliform bacteria in this technique are defined as the bacteria possessing the enzyme β-D-galactosidase. This enzyme cleaves the chromogenic substrate used and results in release of the chromogen, causing a distinct color change in the sample. *E.Coli* bacteria are identified because they have an enzyme that results in the release of a fluorogen in the presence of the fluorogenic substrate. The fluorogen can be viewed when observed under long-wavelength ultraviolet light. One advantage to performing this type of test is that there is no interference from other types of non-coliform bacteria. There often is interference observed in some other analytical methods that yield a count of bacteria.

Having only briefly touched on the subject, the following web links are highly recommended for additional information.

- **EPA** website for the total coliform rule—www.epa.gov/safewater/tcr/tcr.html
- **Center for Biofilm Engineering,** information on biofilms—www.erc.montana.edu
- **General information** and more bacteria links specifically related to well water—www.wellowner.org/awaterquality/coliformindex.shtml

One popular count method is known as the **standard total coliform membrane filter procedure.** This process is slightly more complex and can take additional time for processing if confirmations of presence results are necessary. A sample is initially filtered through a semi-permeable membrane, which has a pore size small enough that coliforms, if present, will not filter through. Once the sample is filtered, the membrane is transferred to a special nutrient dish, inverted and incubated for 22–24 hours. The definition of coliforms that pertain to this test is those bacteria that produce a metallic (golden) sheen within the incubation period. To determine specific colony counts, the analyst uses a specialized microscope.

The typical coliform colony has a pink to dark-red color with a metallic surface sheen. With coliforms confirmed, to determine *E.Coli* counts, the membrane is transferred to a different medium, incubated for four additional hours and then re-examined under long wavelength ultraviolet light for a fluorescence that often appears as a halo around the perimeter of the colony. Colonies that lack the coliform sheen may be pink, red, white or colorless and are considered non-coliform colonies. If there is a colony in question, it goes through the "confirmation" process. To describe the confirmation process in simple terms, colonies are taken from the sample and transferred to a different broth or medium that after an additional incubation period causes a color change in the presence of coliforms. The main problem with this method is that if there is a large presence of bacterial colonies only a "too numerous to count" result can be reported. Sometimes it may not be known if there are coliform colonies on the plate that were "masked out" or unable to be seen. This test usually is only recommended if an actual count of colonies must be obtained such as for regulatory requirements.

Having a basic understanding of bacteria and how they function is important in knowing how to deal with them when encountered in the field. WQP

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