Water Purity Tests*

Historically, most of our concern about water purity has been related to the transmission of disease. Therefore, tests have been developed to determine the safety of water; many of these tests are also applicable to foods.

It is not practical, however, to look only for pathogens in water supplies. For one thing, if we were to find the pathogens causing typhoid or cholera in the water system, the discovery would already be too late to prevent an outbreak of the disease. Moreover, such pathogens would probably be present only in small numbers and might not be included in tested samples.

The tests for water purity in use today are aimed instead at detecting particular **indicator organisms.** There are several criteria for an indicator organism. The most important criterion is that the microbe be consistently present in human feces in substantial numbers so that its detection is a good indication that human wastes are entering the water. The indicator organisms should also survive in the water at least as well as the pathogens would. The indicator organisms must be detectable by simple tests that can be carried out by people with relatively little training in microbiology.

In the United States, the usual indicator organisms in freshwater are the *coliform bacteria.*† **Coliforms** are defined as aerobic or facultatively anaerobic, gram-negative, non–endospore-forming, rod-shaped bacteria that ferment lactose to form gas within 48 hours of being placed in lactose broth at 35 C. Because some coliforms are not solely enteric bacteria but are more commonly found in plant and soil samples, many standards for food and water specify the identification of *fecal coliforms.* The predominant fecal coliform is *E. coli,* which constitutes a large proportion of the human intestinal population. There are specialized tests to distinguish fecal coliforms from nonfecal coliforms. Note that coliforms are not themselves pathogenic under normal conditions, although certain strains can cause diarrhea.

The methods for determining the presence of coliforms in water are based largely on the lactose-fermenting ability of coliform bacteria. The multiple-tube method can be used to estimate coliform numbers by the most probable number (MPN) method. The membrane filtration method is a more direct method of determining the presence and numbers of coliforms. This is possibly the most widely used method in North America and Europe. It makes use of a filtration apparatus. In this application, though, the bacteria collected on the surface of a removable membrane filter are placed on an appropriate medium and incubated. Coliform colonies have a distinctive appearance and are counted. This method is suitable for low-turbidity waters that do not clog the filter and have relatively few noncoliform bacteria that would mask the results.

A newer and more convenient method of detecting coliforms, specifically the fecal coliform *E. coli,* makes use of media containing the two substrates *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG). Coliforms produce the enzyme β-galactosidase, which acts on ONPG and forms a yellow color, indicating their presence in the sample. *E. coli* is unique among coliforms in almost always producing the enzyme β-glucuronidase, which acts on MUG to form a fluorescent compound that glows blue when illuminated by long-wave UV light (***Figure 1***). These simple tests, or variants of them, can detect the presence or absence of coliforms or *E. coli* and can be combined with the multiple-tube method to enumerate them. It can also be applied to solid media, such as in the membrane filtration method. The colonies fluoresce under UV light.

Coliforms have been very useful as indicator organisms in water sanitation, but they have limitations. One problem is the growth of coliform bacteria embedded in biofilms on the inner surfaces of water pipes. These coliforms do not, then, represent external fecal contamination of the water, and they are not considered a threat to public health. Standards governing the presence of coliforms in drinking water require that any positive water sample be reported, and occasionally these indigenous coliforms have been detected. This has led to unnecessary community orders to boil water.

A more serious problem is that some pathogens, especially viruses and protozoan cysts and oocysts, are more resistant than coliforms to chemical disinfection. Through the use of sophisticated methods of detecting viruses, it has been found that chemically disinfected water samples that are free of coliforms are often still contaminated with enteric viruses. The cysts of *Giardia lamblia* and oocysts of *Cryptosporidium* are so resistant to chlorination that completely eliminating them by this method is probably impractical; mechanical methods such as filtration are necessary. A general rule for chlorination is that viruses are more resistant to treatment than is *E. coli* and that the cysts of *Cryptosporidium* and *Giardia* are 100 times more resistant than viruses.

Questions

1. Which disease is more likely to be transmitted by polluted water, cholera or influenza?
2. Coliforms are the most common bacterial indicator of health threatening water pollution in the United States. Why is it usually necessary to specify the term *fecal coliform*?

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* G. Tortora, B. Funke, C. Case. 2010. *Microbiology: An Introduction*. San Francisco: Benjamin Cummings.

† The U.S. Environmental Protection Agency (EPA) recommends the use of *Enterococcus* bacteria as a safety indicator for waters in oceans and bays. Populations of the enterococci decrease more uniformly than coliforms in both freshwater and seawater.

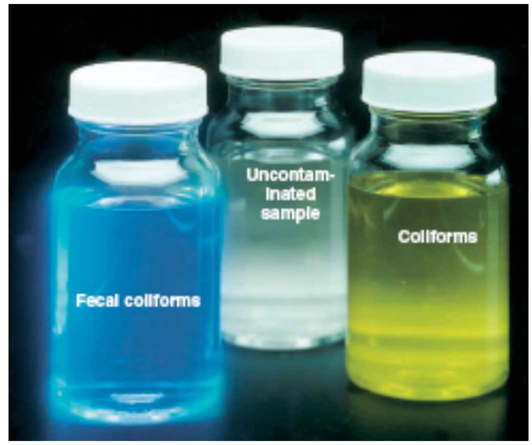

Figure 1. The ONPG and MUG coliform test. A yellow color (positive ONPG) indicates the presence of coliforms. Blue fluorescence (positive MUG) indicates the presence of the fecal coliform *E. coli*. The clear medium indicates an uncontaminated sample. *What causes the formation of the fluorescent compound in a positive MUG test?*