Sensitive and rapid detection of microbial pathogens is a task that has always challenged microbiologists. Earliest efforts focused on parasitic pathogens, beginning in 1674, when Antony van Leeuwenhoek peered through his rudimentary microscope and found “animalcules” that later were identified as *Giardia*. Efforts to detect and identify bacterial pathogens began around 100 years ago with the inoculation and incubation of growth media in the laboratory. These efforts resulted in the development of an array of microbial cultivation techniques. Cultivation of bacteria in the presence of different energy sources, analysis of their macromolecular composition and their metabolic byproducts, and use of specific immunological reagents subsequently came to be used for bacterial classification and identification. Development of these methods opened up a previously unimagined world of microscopic life that largely remains uncharacterized, as less than one percent of bacterial types present in any ecosystem are culturable.
Microbial monitoring and identification is essential to identify sources of contamination and to establish the level of treatment necessary to ensure safe drinking water. The molecular era that emerged in the 1980s resulted in sequence-based molecular methods for detecting pathogens. Rapid alternative approaches were then developed to allow microbial identification directly from specimens.

**Bacterial Pathogens**

Conventional bacterial monitoring in water samples worldwide relies on a 100-milliliter grab sample. The sample is analyzed by standard membrane filtration techniques: the sample is filtered onto a membrane, the membrane is placed into a culture media where the bacteria grow into colonies, and the colonies are counted.

**Viral Pathogens**

Viral detection can be accomplished by cell culture assays, polymerase chain reaction (PCR), or serological tests such as immunofluorescence, enzyme-linked immunosorbent assay, and immunoelectromicroscopy. The conventional detection method is by filter-adsorption of a large volume of water (100 to 1,800 liters), eluting the viruses off the filter into a solution that is then concentrated, and then transferring a portion of the concentrate onto the host cell. If viruses are present, they will infect the host, and physiological changes in the host cells will be visible by light microscopy. While cell culture assay can detect infectious viruses in environmental samples, without additional tests the particular strain of virus present in a sample cannot be determined. Additionally, the length of time needed to detect infection in the cell culture can vary from a few days to several weeks, depending on the type and number of viruses present.

**Parasitic Pathogens**

Currently, routine detection of *Giardia* and *Cryptosporidium* in water relies primarily on microscopic observation of water concentrates using either phase-contrast microscopy or an immunofluorescent technique; neither method can distinguish viable from nonviable cysts. Viability detection methods include infecting animal models and use of cell culture techniques, both of which are costly, time-consuming, and lack sensitivity because they require large numbers of cysts for statistically accurate results. New methods, such as immunomagnetic separation of pathogens from polluted water and integrated cell culture-PCR, have been widely and successfully used in recent studies.

**Cell Culture Methods**

Conventional methodology for the detection of enteric viruses in the environment relies on a few established cell lines that are known hosts for the specific viruses, for example kidney cells in a particular type of monkey. Cell culture involves filtering large volumes of water sample (1,000 liters), eluting the viruses off the filter into a solution that is then concentrated, and then transferring a portion of the concentrate onto the host cell. If viruses are present, they will infect the host, and physiological changes in the host cells will be visible by light microscopy. While cell culture assay can detect infectious viruses in environmental samples, without additional tests the particular strain of virus present in a sample cannot be determined. Additionally, the length of time needed to detect infection in the cell culture can vary from a few days to several weeks, depending on the type and number of viruses present.

Cell culture has some limitations compared to more sophisticated methods. In cell culture, the minimum detection level of viruses in a sample is, by definition, one infectious “unit” per unit volume – a quantity that may range from just a few virus particles to many more – some of which must be infectious. In addition, when a sample tests positive for viral infectivity using cell culture, the infectious agent is not necessarily known. Cell culture protocols do not detect all human viruses present in the environment. Noroviruses, for instance, have not yet been successfully grown in cell cultures; therefore environmental samples cannot be assayed for this pathogen by this method. Finally, since each environmental sample is unique, little is known regarding possible sample components that may inhibit the viral infectivity in culture. Cell culture, however, offers the advantage of isolating an infectious viral pathogen, and is widely accepted as the standard method for viral detection in water.

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The Polymerase Chain Reaction

Since its invention, polymerase chain reaction (PCR) has become one of the most widely used biochemical assays. The speed, specificity, and low cost of the procedure have led to its use in such fields as criminal and pathological forensics, genetic mapping, disease diagnosis, systematics and evolutionary studies, and environmental science.

PCR can amplify to detectable levels nucleic acids associated with pathogens that may be present in low numbers in water samples. PCR assays detect viruses and other pathogens after concentration from large volumes (100 to 1,500 liters) of water. This usually is accomplished by a filter-adsorption and elution method, resulting in a concentrate containing microbes and organic and dissolved solids.

PCR is a process in which target DNA, polymerase enzyme, and the DNA subunits are combined in a test tube and subjected to temperature changes that spur DNA duplication. Through repetition of this process and under ideal conditions, millions of copies of a single DNA molecule can be generated in just 20 to 30 repetitions of the temperature cycle, each cycle requiring only a minute. The PCR assay can selectively amplify a portion of the target DNA that will allow the pathogen to be identified.

The advantages of PCR are numerous. Compared with techniques such as cell culture for the detection of viruses and parasites, the time required for the assay can be reduced from days or weeks to hours. Initial and recurring costs for PCR are much less than cell culture techniques and the technique is easily performed. Additionally, PCR can be used to identify specific pathogens found in water. Standard PCR, however, can only detect the presence or absence of pathogen-specific DNA or RNA, not the infectious state of an organism.

And in the Future...

While the technologies currently available to identify pathogens are state-of-the-art, sample processing techniques need further development. For example, to perform a PCR assay, a 1,000-liter sample is ultimately concentrated down to milliliters or microliters. If significant other material exists in the original sample, it will also be concentrated, with the result that the ability to perform PCR is impaired. Real-time detection is another area in which analytical developments are advancing.

Using these and other microbial detection methodologies, researchers have developed databases on bacterial, parasitic, and viral occurrence in source and treated waters of the United States and other countries. Despite some difficulties and costs associated with microbial monitoring, the protocols provide data and quantitative approaches that can be used to screen and identify water systems at risk of fecal contamination.

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