EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

EDVO-Kit #

952

Water Quality Testing II:

PCR-based Testing for Water Bacterial Contaminants

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is to use PCR to determine the quality of water and to detect and monitor the presence of bacterial contaminants in environmental bodies of water.

This experiment is designed for DNA staining with InstaStain® Ethidium Bromide.

EDVOTEK, Inc. • 1-800-EDVOTEK • www.edvotek.com

EVT 004177K

Water Contamination

Water pollution is a major worldwide health problem and thus an essential subject area for incorporation into health education (specifically biology and ecology courses). According to the World Health Organization (WHO), over nine million deaths annually are attributed to waterborne diseases, occurring mostly in developing countries. It is projected that 38% of those deaths occur in children afflicted with diarrhea. Furthermore, the WHO estimates that 80% of all worldwide infectious disease is due to unsafe water sanitation. Typical infections are cholera, giardiasis, hepatitis, shigellosis, typhoid, and acute gastrointestinal illness (AGI).

In the United States and other developed countries, water treatment systems have been in place for nearly 150 years. Chlorination was first incorporated into urban water treatment in the early 1900s, eliminating most infectious pathogens. Even today, however, most rural areas rely on private, untreated wells for drinking water, supplying an estimated 42 million Americans. The U.S. Department of Agriculture projects that 2.5 million of these rural citizens have a critical need for safe drinking water, with one million lacking piped-in water.

Many concerns still remain in U.S. urban areas supplied with water treatment. Corrosion in aging water supply pipelines can increase biofilm formation, resulting in increased contamination of drinking water. A 1999 EPA study revealed 14 states having more than 11% of their community water systems violating maximum contaminant levels. From 1997-1998, the Centers for Disease Control reported 17 waterborne disease outbreaks from 13 states, resulting in over 2000 illnesses. In the past 15 years, outbreaks caused by the parasite *Cryptosporidium parvum* have been increasing. In 1993, an epidemic of *cryptosporidiosis* in Milwaukee, Wisconsin afflicted over 400,000 persons, with 4000 hospitalizations and more than 50 deaths. Chlorine resistance and the small size of *C. parvum* allow this organism to escape some water treatment programs. HIV and other immunocompromising diseases increase the risk of contraction of cryptosporidiosis.

A second dangerous water contaminant is the bacteria *Escherichia coli* (*E. coli* strain 0157). Certain strains of *E. Coli* are relatively harmless and actually live in the large intestines of humans where they aid in digestion. *E. Coli* 0157, however, produces a potent toxin that can cause bloody diarrhea and, in severe infections, kidney failure and death. Infections from this organism are commonly due to inadequate cooking of meat; however it is also estimated that 11,000 *E. Coli* 0157 infections occur in the U.S. each year due to waterborne transmission.

Water Contamination

Background Information

Another waterborne parasite, *Giardia*, forms cysts that can be highly resistant to chlorination. Although rarely life-threatening, *Giardia* infections cause diarrhea and result in over 4,600 U.S. hospital visits per year, mostly due to dehydration. Additionally, *Giardia* is very common in farm animals and household pets. The infection can be transmitted to humans from cysts that are shed from the animals.

In addition to drinking water, illnesses caused by unsafe swimming water (recreational water illnesses, or RWI) appear to be increasing. This is likely due, in part, to increased crowding of public pools and increased pollution of recreational lakes and streams. From 1997-1998, the CDC reported a total of 32 RWI outbreaks in 18 states, 2100 afflicted persons, with 4 deaths from meningitis. As with drinking water, common RWI causative agents are *E. Coli* O157 and *C. Parvum*, which can survive in chlorine for nearly seven days. Infections due to microbial contamination of spas and hot tubs also appear to be increasing.

Education in water public health issues requires an understanding of the processes that lead to water contamination. One process that significantly contributes to waterborne infectious disease is eutrophication, the over-enrichment of nutrients. Eutrophication leads to the destruction of animals and plants via a domino effect by eliminating specific organisms required in the food chain. For example, increased nitrogen and phosphate in water results in algal blooms that lower oxygen levels in water. This process leads to the destruction of animals and plants higher on the food chain. Since most instances of eutrophication are man-made, public awareness can have dramatic impacts on preventing the destruction of aquatic environments.

It is now realized that pollution of water at a distant site, otherwise known as non-point source pollution, is often a fundamental source of eutrophication. Efforts to curb water pollution focus on public awareness of the impact of distant land-based activities that interconnect with other types of water bodies, including underground lakes and rivers. Some examples of non-point pollution include: 1) animal farm runoff water that contains large amounts of nitrogen and high levels of toxic bacteria; 2) sewage; and 3) runoff from lands with high use of fertilizer. Public awareness can lead to reductions in water pollution by the use of low phosphate or phosphate-free detergents, limited use of fertilizer in landscaping and gardening, and water conservation measures.

Water Contamination

The importance of monitoring water quality is vitally important to public health. Assessment of water contamination and environmental control decisions requires quantitative data necessary for both the location and the identification of early water pollution sources. Effective methods for monitoring water constituents and sediments include:

1) measurements of dissolved oxygen and levels of suspended sediment nutrients;
2) seasonal biological measurements of the abundance and the variety of aquatic plants and animals;
3) general water conditions, such as pH, temperature and color;
4) molecular biology techniques, including culturing and plating of possible water contaminants;
5) the use of the polymerase chain reaction (PCR) for sensitive detection of microorganisms.

PCR, a technique for amplifying specific DNA sequences, has revolutionized all facets of biology. It is now used routinely in medicine and for human identification. PCR was invented in 1984. Dr. Kary Mullis of the Cetus Corporation, earned the Nobel Prize only nine years later in 1993. The basis for the PCR reaction is the thermal stability of a DNA replicating enzyme, known as *Taq* polymerase, at high temperatures.

In the first step of the PCR reaction, known as denaturation, the DNA is heated to 95°C to separate the complementary DNA strands of the organism under study. Even at 95°C, the Taq polymerase remains stable In addition to the polymerase and the test DNA, the reaction mixture contains the four deoxynucleotides (A, C, G, and T) and synthetic, single-stranded DNA molecules known as primers. In the second step of the PCR reaction, known as annealing, the reaction temperature is lowered to a temperature between 50°C and 70°C to allow the primers to bind (hybridize) to the separated DNA strands. The primers are synthesized to be complementary to the ends of the sequence to be amplified.

For detection of waterborne microorganisms, the primers are designed to be specific to a bacterial gene found exclusively in the organism under study. In the third step, known as extension, the temperature is raised to 72°C to allow the *Taq* polymerase to extend the new DNA strand from the primers and complete the synthesis, using the four deoxynucleotides (A, C, G, and T). These three steps, denaturation, annealing, and extension, constitute one PCR cycle (see Figure 1). The process is typically repeated for 20-40 cycles, doubling the number of target sequence DNA molecules after each successive cycle.

Following PCR, the amplified DNA from the positive and negative controls are subjected to gel electrophoresis. Distilled sterile water will be used as the negative control. If the water samples collected from the environment show PCR results identical to the control, the presence of the organism is confirmed in the water sample.

The Experiment

Water Contamination