Molecular Detection of Infectious Viruses in Water

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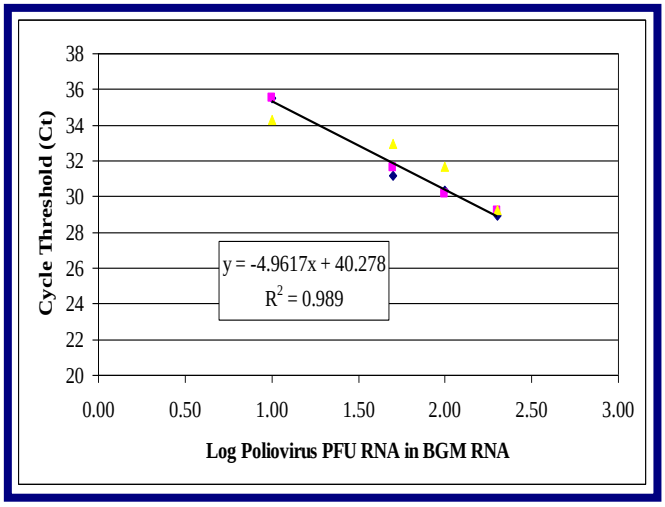

Poliovirus host cell capture quantitative polymerase chain reaction (HCC-qPCR) standard curve used to quantify viruses in water samples

BACKGROUND

More than 140 different enteric viruses are known to infect humans causing a wide range of diseases, from diarrhea to heart infections. Enteric viruses have been found in raw surface water and wastewater. Compared to bacteria, enteric viruses are resistant to conventional water and wastewater treatment. It is suspected that many waterborne disease outbreaks for which no cause was identified (about 47% of all reported outbreaks) may be due to viruses. Viruses are frequently not identified as the cause of outbreaks due to the limitations of current detection methodology and the failure to examine clinical specimens for viruses. The conventional detection method uses growth of the viruses in cell culture (animal tissues grown in the laboratory). However, this method is costly and may take weeks to complete. In addition, some viruses, such as hepatitis A and noroviruses, are difficult to grow in cell culture. Although sensitive, molecular methods alone cannot distinguish between infectious and noninfectious viruses. Methodologies that are both quantitative and address infectivity are needed to assess the public health risks associated with exposure to viruses in water.

OBJECTIVES

- Develop analytical methods for the rapid and quantitative detection of infectious viruses in water. Our approach uses brief contact of purified water samples with cell culture (animal or human cells) to "capture" the viruses in the samples, followed by a molecular assay to detect the captures viruses. This assay is referred to as host cell capture quantitative sequence detection (HCC-QSD).
- Evaluate different host cell lines and virus combinations to identify the most effective cell lines for the host cell capture of human enteric viruses. Poliovirus and Buffalo Green Monkey kidney cells, and reovirus and Vero cells will be used as model systems.
- Evaluate the ability of HCC-QSD to distinguish potentially infectious viruses from chlorine and ultraviolet light disinfected viruses.

FINDINGS AND BENEFITS

- Using the model virus and cell line systems, HCC-QSD method was refined and a new protocol developed. As anticipated, ultraviolet light inactivation of virus was difficult for HCC-QSD to detect due to the mechanism of inactivation. However, chlorine disinfection trials using poliovirus demonstrated equivalent results between the conventional cell culture assay and HCC-QSD in quantifying virus inactivation.
- This research will aid in the development of a standard method for routine monitoring of infectious viruses in water by providing a technological foundation for the water industry and EPA.

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