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Viral Analyses

ASI Technical Document #107

Introduction

Outbreaks of foodborne and waterborne illness have been associated with viruses[1,2,3]. The viruses that are typically transmitted by water (via the fecal-oral route) belong to one of the largest groups of viruses called the enteric viruses. At least 140 human enteric virus types are known, including 72 serotypes of enteroviruses, adenoviruses, reoviruses, rotaviruses, norwalk virus, and others[2]. Most of the enteric viruses are responsible for causing gastroenteritis or hepatitis, but some can affect the central nervous system, heart muscle, and respiratory system.

In conjunction with its affiliated laboratory at the University of New Hampshire (UNH), Analytical Services, Inc. was one of ten microbiology laboratories approved by the United States Environmental Protection Agency (EPA) to participate in virus sample analysis for the Information Collection Rule (ICR). ASI's Senior Scientist, Dr. Aaron Margolin, Associate Professor of Microbiology at UNH, was awarded the EPA contract to develop and test the protocol for preparation of the ICR virus Performance Evaluation (PE) samples.

Cell Culture

Viruses which are pathogenic to humans have traditionally been detected by cell culture methods. These methods involve culturing host cells as stationary monolayers in sterile petri dishes and infecting the cells with a viral inoculum. Cytopathic effects or morphological changes are visualized in the infected cells. Cell culture media components are expensive, the technique is relatively difficult to perform, and results are often not available for up to six weeks after commencing the assay. In addition, viruses are host specific and require different cell lines for successful infection and replication. Lastly, not all viruses exhibit CPE.

Polymerase Chain Reaction (PCR)

Another method of detection ASI/UNH offers is polymerase chain reaction (PCR). PCR is a molecular method that amplifies viral nucleic acid without the need of culture techniques[5,6]. ASI/UNH routinely analyzes environmental samples for enteroviruses (poliovirus, coxsackie virus, and echovirus types), hepatitis A virus, rotavirus, and noroviruses using PCR. PCR does not indicate whether or not the viral particles are infectious, but does

indicate the presence or absence of viral nucleic acid. There are several advantages to using PCR including its sensitivity of viral detection, rapid generation of results, and cost effectiveness. These qualities support its use as a screening tool.

PCR detects specific targeted genomic material rather than relying on visually observed secondary effects as in the ICR or cell culture method; that is, PCR detects the specific virus, not just evidence of its presence.

ASI is the first environmental microbiology laboratory licensed by PE Biosystems, Inc. to perform commercial PCR analyses on environmental samples and report site-specific results.

Integrated Cell Culture/ Polymerase Chain Reaction (ICC/PCR)

Combining traditional and molecular methods, ASI/UNH offers ICC/PCR for the detection of enteric viruses. This method involves inoculation of cell monolayers with virus, incubation of the culture flasks for up to seven days, and performing PCR (or Reverse Transcriptase-RT-PCR) on the cell lysate and supernate. Unlike standard PCR, ICC/PCR allows for confirmation of infectivity of the viruses detected. Relative to traditional techniques, more major groups of viruses that have been directly linked to waterborne outbreaks, or suspected of causing outbreaks, can be detected more quickly, less expensively and with a high degree of specificity.

Dr. Margolin's research has involved ICC/PCR methods applied to biosolids and also in analysis of archived ICR (source and finished water) samples. (*Applied & Environmental Microbiology*, (AEM), June 2000). Abstracts for the initial phase of these studies are available. The EPA expressed interest in this work and has funded the analysis of additional ICR samples to expand their database regarding viral occurrence. Since Dr. Margolin's initial research, ASI/UNH has completed validation and optimization work and now offers ICC/PCR for the detection of enteroviruses (as a group), adenovirus, astrovirus, rotavirus, noroviruses and Hepatitis A Virus (HAV).

With ICC/PCR, ASI/UNH is able to confirm infectivity of any recovered viruses with additional testing. ICC/PCR provides a qualitative result (presence/absence).

The sensitivity and specificity of molecular techniques provide a related benefit in terms of sample collection. We require collection of a grab sample (typically 5L) for raw water or treated wastewater whereas the ICR method requires pumping large volumes of water through a cartridge filter and then shipment of the whole sampling unit to the lab.

Bacteriophages (Please see ASI Technical Document # 108).

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