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## Human Fecal Virus ID™

### Detection of Human Fecal Viruses by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) DNA Analytical Technology

Submitter: XYZ Municipal Water Plant

Submitter #'s: YYYY, QQQQ, RRRR, AAAA

Source Molecular #'s: SM 0222, SM 0223, SM 0224, SM 0225

Samples Received: November 4, 2002

Date Reported: November 7, 2002

**SAMPLE**

SM #	Client #	DNA Analytical Results
SM 0222	YYYY	<b>Human Fecal Viruses Detected</b>
SM 0223	QQQQ	Negative
SM 0224	RRRR	Negative
SM 0225	AAAA	<b>Human Fecal Viruses Detected</b>

#### Laboratory Comments

Four 1-liter water samples were filtered and analyzed for the presence of human enteroviruses (i.e. human fecal viruses). These viruses are transmitted via the fecal-oral route; therefore the detection of their nucleic acid indicates likely human fecal contamination. Furthermore, these viruses are highly stable in water and have a low infectious dose; therefore, positive findings suggest a serious health risk, especially for children.

All reagents, chemicals and apparatuses were verified and inspected beforehand to ensure that no false negatives or positives could be generated. In that regard, positive and negative controls were run to attest the integrity of the analysis. All inspections and controls tested negative for possible extraneous contaminants, including PCR inhibitors.

Samples QQQQ (Our Ref: SM 0223) and RRRR (Our Ref: SM 0224) tested negative for the presence of human enteroviruses. It is important to note that a negative result does not mean that the sample does not have human fecal contamination. Negative samples should be analyzed further for the presence of other human enteric viruses such as adenoviruses, rotaviruses, Norwalk viruses, reoviruses and Hepatitis A viruses. Additional microbial source tracking methods may also be used to analyze for the presence of human fecal contamination, such as the Human Bacteroidetes ID™ and Human Enterococcus ID™ services.

Samples YYYY (Our Ref: SM 0222) and AAAA (Our Ref: SM 0225) tested positive for human enteroviruses suggesting human fecal contamination is present in these water samples. The analysis targeted and detected virus RNA, strongly indicating the presence of intact, encapsulated viruses since free RNA is quickly degraded in the environment. It is not known whether these viruses are in an infectious state, but it has been shown that infections can occur with as few as 1 virus particle. The client is encouraged to conduct a cultivable enterovirus test to determine conclusively whether the viruses in the water source are in an infectious stage.

## **DNA Analytical Method Explanation**

One-liter water samples were filtered through a negatively charged hydrophobic filter membrane (Millipore Inc.) at pH 3.5. Filters were collected and a total viral RNA extraction was performed directly from the filter using a commercially available viral RNA extraction kit according to manufacturer's instructions (Qiagen, Inc.).

Each filter concentrate sample was purified and concentrated to 60.0 µl by using spin-column chromatography (RNEasy Mini Kit; Qiagen, Santa Clarita, Calif.). Afterwards, 10 µl of the sample was used for reverse transcriptase PCR (RT-PCR) for the assayed viruses. Nested PCR reactions were employed to increase sensitivity. RT-PCR profiles and master mixes were used according to previously published literature (Chapron et al. 2000).<sup>1</sup> Positive and negative controls were used in each reaction where applicable.

## **DNA Analytical Theory Explanation**

Detection of human viruses in water samples can serve as an indicator of human contamination. Of particular concern are human enteric viruses. These viruses infect the gastrointestinal tract of humans and animals, and are excreted in feces. More than one hundred different enteric viruses may be excreted in human fecal material and as many as 1,000,000 plaque-forming units (pfu) of enteroviruses (a subgroup of enteric viruses) per gram may be present in the feces of a sick individual.

Enteroviruses can serve as a good indicator of human fecal and viral contamination.<sup>2,4</sup> Enteroviruses account for an estimated 10 to 15 million symptomatic infections in the United States each year. At present, 66 serotypes of enteroviruses are recognized, including three poliovirus serotypes. Enteroviruses are a major cause of gastrointestinal symptoms and they are recognized as an important factor in acute infections especially of the central nervous system, i.e., encephalitis and meningitis and in chronic infections of the cardiovascular system, i.e., myocarditis, pericarditis, and cardiomyopathy. They can also lead to post viral fatigue syndrome.

Enteroviruses are found worldwide. Infections occur by the fecal-oral route, and in most cases, treated surface water acts as the carrier of these pathogens.<sup>3</sup> Enteroviruses are highly stable in water and are not completely eliminated by sewage treatment plants. Thus, the increasing use of treated surface water for drinking purposes harbors a potential source of pathogens, which are often not screened in a satisfactory manner.

Enteroviruses are RNA viruses; therefore, a PCR (polymerase chain reaction) method called reverse transcriptase PCR (RT-PCR) must be used to transcribe the detected RNA back into DNA. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the viruses to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the virus or genome to be studied, then billions of copies of the DNA fragment will be available for detection by gel electrophoresis.

The gel electrophoresis apparatus uses an electrical field to distinguish different DNA fragments according to their molecular weights. Lighter DNA fragments will move farther along the gel than their heavier counterparts. At the end of the procedure different bands of accumulated DNA fragments will aggregate at different parts of the gel. It is this accumulation of DNA fragments that creates a band on the gel. Researchers use these bands to confirm and distinguish viral genomes.

Viruses cannot replicate themselves. They need a host organism to transcribe and replicate their genetic code. Viruses come in two genetic forms, either RNA or DNA based. Their genetic material is protected with a protein coat. Detection of virus RNA or DNA strongly indicates the presence of intact, encapsulated viruses, as free RNA or DNA quickly degrades in the environment.

It is not known whether detected enteroviruses by RT-PCR are in an infectious stage. Nonetheless, it has been shown that infections with enteroviruses can occur with as few as 1 virus particle. Therefore positive findings by RT-PCR could suggest a serious health risk. A cultivable enterovirus detection test should nonetheless be done to provide conclusive evidence of infectivity of the enteroviruses in the water source.

Human viral contamination is potentially the most serious health concern effecting water systems, yet it is also the least monitored. Enteric viruses that are spread through water such as Norwalk-type viruses, rotaviruses, enteroviruses, reoviruses, Hepatitis A viruses and adenoviruses are of a particular concern. Furthermore, certain viruses have been known to survive for months, and even years in water systems. Viability is maintained when high levels of suspended sediment in water provide substrates to which viruses can absorb. Sorbed viruses may remain nearly 100% viable for extended periods of time.

For the time being, no proven methods have been demonstrated that remove viable viruses entirely from water systems. Consequently, proper monitoring of viruses can at least prevent additional illnesses and possible deaths of immunocompromised individuals, such as HIV/AIDS or cancer patients. Should potentially hazardous viruses be confirmed in specific water systems, then more rigorous remediation steps can be undertaken to remove, or at least diminish the viral loads.

To strengthen the validity of the results, the Human Fecal Virus ID™ service should be combined with other DNA analytical services such as the Human Bacteroidetes ID™ and Human Enterococcus ID™ services. Negative results should be analyzed further for the presence of other human enteric viruses such as adenoviruses, rotaviruses, Norwalk viruses, reoviruses and Hepatitis A viruses.

<sup>1</sup> Chapron, Christopher D., Ballester, Nicola A., Fontaine, Justin H., Frades, Christine N., Margolin, Aaron B. **Detection of Astroviruses, Enteroviruses, and Adenovirus Types 40 and 41 in Surface Waters Collected and Evaluated by the Information Collection Rule and an Integrated Cell Culture-Nested PCR Procedure** Appl. Environ. Microbiol. 2000 66: 2520-2525.

<sup>2</sup> Gantzer, C., Maul, A., Audic, J. M., Schwartzbrod, L. **Detection of Infectious Enteroviruses, Enterovirus Genomes, Somatic Coliphages, and Bacteroides fragilis Phages in Treated Wastewater** Appl. Environ. Microbiol. 1998 64: 4307-4312.

<sup>3</sup> Gilgen, M, Wegmuller, B, Burkhalter, P, Buhler, HP, Muller, U, Luthy, J, Candrian, U **Reverse transcription PCR to detect enteroviruses in surface water** Appl. Environ. Microbiol. 1995 61: 1226-1231.

<sup>4</sup> Fong, Theng-Theng, Griffin, Dale W., Lipp, Erin K. **Molecular Assays for Targeting Human and Bovine Enteric Viruses in Coastal Waters and Their Application for Library-Independent Source Tracking** Appl. Environ. Microbiol. 2005 71: 2070-2078.

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