Summary: *New faster, more specific detection methods for discerning which virus may be contaminating your customer's water offer opportunities for water treatment dealers to deal much more quickly with problems encountered. And, along with these new methods comes increased accuracy and reduced costs associated with testing.*

New Methods for Human Virus Detection:

A New Approach for "Real Time" Monitoring

By Dr. Kelly A. Reynolds, Ph.D.

More than 140 different types of viruses are known to infect the human intestinal tract and are subsequently excreted in feces. These human pathogens find their way into the environment via municipal waste disposal, septic tank seepage, storm water runoff, wastewater reclamation practices and recreational bathers, just to name a few. Viruses present in the environment pose a public health risk because they are transmitted by the fecal-oral route through contaminated water, and low numbers are able to initiate infection in humans. In fact, the infectious dose may be as low as one culturable organism.[7] Therefore methods of virus monitoring and detection must be able to detect very low levels of viruses in very large water volumes.

Hazard of enteric viruses

Enteric viruses previously associated with waterborne outbreaks include the enterovirus group (poliovirus, coxsackievirus, and echovirus), hepatitis A virus, rotavirus, adenovirus and Norwalk virus. The viruses listed are responsible for a wide range of illnesses including meningitis, paralysis, myocarditis, hepatitis, encephalitis, diabetes, respiratory illness and perhaps the most commonly identified symptom, diarrhea. Because of the wide spectrum of enteric virus symptomology, viral waterborne outbreaks have been difficult to document and researchers believe we have only identified the tip of the iceberg concerning viral waterborne illnesses. Although no etiological source has been identified in nearly half of all waterborne outbreaks, viruses are known to be the causative agent in 15 percent of all documented waterborne outbreaks to date.[3] This percentage has increased in the last decade due in part to improved virus detection methods and to successful viral resistance to water treatment processes.

The average attack rate of certain enteric viruses has been documented as high as 40-to-50 percent. That is, nearly half of all persons exposed to a waterborne, human pathogenic virus will develop an infection. Furthermore, some enteric viruses exhibit a secondary attack rate as high as 30 percent, meaning an infected individual will pass on their illness to one-in-three subsequent individuals contacted. Immunocompromised populations (i.e., the elderly, infants, pregnant women, transplant and chemotherapy patients) are at greatest risk for waterborne disease. These individuals not only have a greater risk of becoming ill following a virus exposure but also have a greater chance of experiencing a more serious outcome such as hospitalization or death.[2]

Control of viruses in the environment

What attempts have been made to target and control viral pathogens in the environment? Current standards for evaluating the sanitary quality of drinking water are based on levels of coliform and fecal coliform bacteria. While coliform tests have greatly reduced the number of outbreaks associated with enteric bacteria, they are not effective indicators of enteric virus presence. Other potential indicators are being evaluated, but many scientists have opted for improving methods for direct pathogen detection.

In 1974, the Safe Drinking Water Act (SDWA) was enacted, giving the U.S. federal government authority for the protection of drinking water sources and regulation of water treatment techniques based on suggested maximum contaminant levels. Within the SDWA, versions of the Groundwater Disinfection Rule recommend unspecified levels of disinfection to protect groundwater supplies from contamination with human pathogens. Unfortunately, because of the widespread use of bacterial indicators and previous difficulties with virus detection methodologies, the database on virus presence in source waters is incomplete at best. Thus, the U.S. Environmental Protection Agency (USEPA) is currently heading studies under the Information Collection Rule (ICR) to determine background levels and occurrence of enteric viruses and other pathogens in public source water systems throughout the country. Results of this study are expected to provide information for a better understanding of risks due to waterborne pathogens and disinfection byproducts. Should the ICR studies indicate the need for routine monitoring of viruses

in drinking water, availability of rapid, specific and inexpensive detection techniques would be critical.

Methods for detecting enteric viruses in the environment

Concentration of viruses in water

Human viruses are typically present in very low numbers in the environment. Therefore, samples must be concentrated prior to analysis. Membrane or Viradel (virus-adsorption-elution) filtration is commonly used for the concentration of enteroviruses from hundreds of liters of water.[1] Viradel filters concentrate viruses based on opposing electrostatic charges. At neutral pH, enteroviruses are negatively charged and electropositively charged filters are used for virus catchment. At sample pH values below 3.5, enteroviruses reconfigure their surface proteins to display a positive charge, in which case electronegatively charged filters are used. Elution of viruses from charged filters is accomplished using a specially formulated beef extract solution. Up to 1,000 liters of water may be filtered followed by elution with one liter of beef extract. The beef extract proteins and viruses present are then precipitated from the solution by temporarily lowering the sample pH. The flocculant is then pelleted and resuspended to a final sample volume of less than 30 ml. At this point, several methods of virus detection may be used, each with their own advantages and disadvantages.

Cell culture methodology

Conventional methods for virus detection involve animal cell cultures. Monkey or human cells, supporting virus growth, are maintained in laboratory flasks. Water sample concentrates are added to cell culture flasks and observed for days to weeks for any signs of cell destruction indicating virus propagation. In any given virus population, a ratio exists between infectious and noninfectious particles. Cell culture, unlike other methods of detection, has the advantage of detecting only infectious viral units. Thus, a cell culture positive result indicates a potential public health risk. Another advantage to cell culture is its ability to examine large sample volumes. An entire 30 ml of concentrated water sample, equivalent to 400 liters or more of source water, is easily examined by cell culture.

Cultural methods of virus detection do, however, have some major disadvantages. Environmental strains of enteric viruses may require two weeks of growth time for a preliminary result. Confirmed results often require

Table 1
Comparison Of Methods For The Detection Of Enteric Viruses In Water Concentrates

Advantages	Direct PCR	Cell Culture	ICC/PCR
Sensitive	yes	yes	yes
Specific	yes	no	yes
Rapid	yes	no	yes
Able to examine large equivalent volumes	no	yes	yes
Minimizes inhibition/toxicity	no	no	yes
Detects noncytophathogenic viruses	yes	no	yes
Detects infectious viruses only	no	yes	yes

Table 2

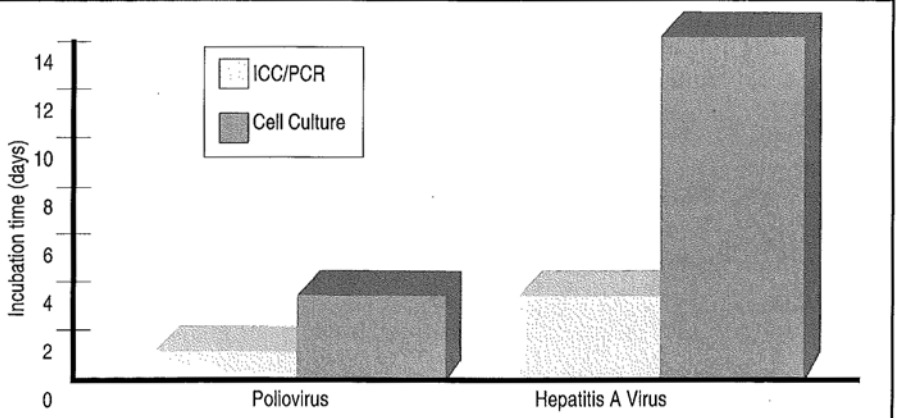

Table 3

Sample	Direct PCR: Original volume examined (L)	Direct PCR: Number of infectious virus/PCR	Direct PCR: Direct PCR results	ICC/PCF: Original volume examined (L)	ICC/PCF: Number of infectious virus/flask	ICC/PCF: ICC/PCR Results
1	0.003	2.9	+	0.78	870	+
2	0.120	0.025	0	36.0	7.5	+
3	0.116	0.001	0	34.8	0.3	+
4	0.082	0	0	24.6	0	0

The gray highlighted areas indicate the final results of direct PCR versus ICC/PCR. Note the potential for false negative results with direct PCR alone.,

incubation times of three weeks or more. Lengthy assay times add to the technical cost of analysis while certain strains of rotavirus and hepatitis A virus grow in cells, but do not produce any visual signs of cell destruction. These strains, known as noncytopathogenic viruses, would not be detected by conventional cell culture assays alone. Thus, cell culture is not the desired method for routine monitoring of viruses. In recent years, scientists have begun relying on molecular detection methods to address some of the problems of cell culture.

Molecular methodology

Molecular methods are used to detect the presence of a pathogen's genetic material (RNA or DNA). Different organisms have distinct nucleic acid sequences and may be differentiated at the genetic level. The polymerase chain reaction (PCR) is a molecular method commonly used for rapid detection of enteric viruses, requiring only 24 hours for definitive results. With PCR, specific primer sequences target complimentary genome sequences of specific viruses. PCR primers selectively attach to target viral sequences and, through enzyme reactions and temperature cycling, subsequently amplify the genomes present. After multiple amplification cycles, more than a million copies may be produced from a single viral genome originally present. Detection of a high concentration of PCR product is then relatively easy to achieve. PCR overcomes many of the disadvantages of conventional cell culture, providing a rapid, sensitive, specific and inexpensive system for virus detection. PCR is also capable of detecting noncytopathogenic virus strains. Unfortunately, molecular methodologies are often complicated by the presence of inhibitory compounds commonly present in environmental concentrates. PCR inhibitors decrease the detection sensitivity of the reaction, leading to false negative results.[6] Using PCR, only microliter volumes can be examined versus milliliter volumes with cell culture. Furthermore, PCR cannot distinguish between noninfectious and infectious virus particles, leaving one to wonder what a PCR positive result means with regard to public health. Due to the controversy over the interpretation of molecular results and the fact that cell culture is an impractical method to routinely sample for environmental viruses, a new method has been developed which promises to revolutionize virus monitoring.

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ICC/PCR

The laboratory at The University of Arizona has developed the newest and most promising method to routinely monitor for infectious enteric viruses.[4] This method, known as integrated cell culture/PCR (ICC/PCR) utilizes many of the advantages of both conventional cell culture and molecular approaches while eliminating many of their disadvantages (see *Table 1*). The first step in ICC/PCR is the addition of sample concentrates to cell culture flasks, followed by PCR on the cell culture medium. Lengthy incubation times are not needed since PCR can be used to detect low levels of virus growth. Thus, we can examine large sample volumes and still take advantage of PCR's specificity, speed and sensitivity. Since growth of the infectious virus present is a prerequisite for a PCR positive result, only viable viruses are detected. The bottom line is that ICC/PCR allows detection of infectious viruses in 24-to-48 hours compared to days to weeks with cell culture alone (see *Table 2*). Dilution of the concentrated environmental sample with the cell culture medium coupled with multiplication of infectious viruses overcomes the effect of PCR inhibitory compounds (see *Table 3*).[5] In addition, the integrated technology provides a means for detecting noncytopathogenic viruses, such as hepatitis A and rotavirus while greatly reducing the time involved for routine assay.

Future implications for water treatment

ICC/PCR allows for more rapid and sensitive detection of low levels of infectious enteric viruses in large volumes of water concentrates than any method previously described. This new methodology overcomes the traditional flaws of conventional cultural and molecular methods and provides a practical test for routine virus monitoring of water concentrates.

In addition, ICC/PCR will be useful for evaluating the effect of commonly used and developing methods of water treatment and disinfection, for the removal or inactivation of hu-

man enteric viruses. Although disinfection is known to render viruses as non-infectious, the question remains as to the effect disinfectants have on the nucleic acid sequences detected by PCR. Should direct RT-PCR exhibit increased detection over cell culture—presumably due to non-infectious viral particles—the integrated cell culture/PCR approach would be useful for rapidly evaluating the presence of infectious viruses only and the effectiveness of disinfection procedures.

Conclusion

In summary, the ease of this method will allow for increased data collection leading to more accurate assessments of public health risks while aiding in the prevention of drinking water outbreaks associated with human enteric viruses. And the reduced time that samples are contacted with cells greatly decreases assay costs.❑

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