An Improved Method for Detecting Viruses in Water

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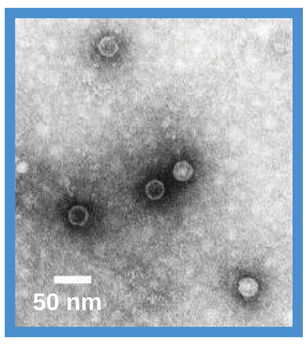
Background

Enteroviruses, such as echovirus and coxsackievirus, have been implicated in numerous waterborne disease outbreaks of gastroenteritis worldwide. However, comprehensive occurrence studies of enteroviruses in drinking water matrices are limited, in part because of the lack of available methods that are rapid, sensitive, and able to detect live infectious virus. To address this issue, the US EPA has included echoviruses and coxsackieviruses on the microbial contaminant candidate list (CCL). To provide the research needed to support regulation decisions for these CCL viruses, our efforts have focused on developing methods for the rapid detection of live virus. To achieve this goal we have developed a technique which integrates cell culture and molecular assays into one method. This integrated cell culture/polymerase chain reaction method (ICC/PCR) approach uses molecular tools to rapidly detect infectious enterovirus.



An electron micrograph of an enterovirus

Current Detection Methods for Enterovirus in Water:

Molecular-Based Assays

- Rapid
- Sensitive
- Does not determine if the virus present is infectious
- Often the assay is inhibited by environmental matrices

Culture-Based Assays

- Detects any viable virus that can grow in cell culture
- Requires several days to weeks
- No one cell line grows all viruses
- Not all virus growth can be visualized by cell culture alone

Method

Integrated Cell Culture & Molecular Method

Sample Collection & Elution

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Concentration

↓

Tissue Culture

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Detection

Step 1: Sample Collection and Elution

The method begins by filtering 200 liters of surface water through a 10-inch 1MDS filter. Any virus present in the sample will be adsorbed by electrostatic interactions with the filter. These viruses are then eluted from the filter using 1.6 liters of 1.5% w/v beef extract at pH 9.5.

Step 2: Concentration

The viral particles are extracted from the eluate by adding celite. The celite/virus complex is subsequently collected by filtration and the virus is eluted from the celite using sodium phosphate at a high pH. The sample is further concentrated by an ultra-centrifugation step, and the virus containing pellet is resuspended in 0.5ml of PBS + 0.2% BSA.

Step 3: Tissue Culture

The entire concentrated sample is then inoculated into two culture tubes containing BGM cell monolayers, and incubated for 1-7 days at 37°C. At daily time points, one of the two infected cell culture tubes undergoes a quick freeze/thaw to release the virus from the cells, and cell lysate aliquots are taken to test for viral nucleic acid. The remaining cell culture lysate is used to inoculate a new BGM monolayer to allow for additional growth.

Step 4: Detection

Aliquots of the cell lysate are tested for the presence of viral nucleic acid using both conventional reverse transcription-polymerase chain reaction (RT-PCR) and quantitative real-time RT-PCR (qRT-PCR). Identical pan-enterovirus primer sets were used for both molecular assays and a pan-enterovirus specific TaqMan probe was used for the qRT-PCR. Conventional PCR products are resolved in an agarose gel and hybridization with a viral specific probe in order to determine the specificity of the amplicon; in contrast, the qPCR assay gives an immediate measure of the amount and specificity of the PCR product.

Results and Conclusions

Table 1. Tests of the integrated cell culture/PCR method using spiked reagent water samples

	Cell Culture Alone	ICC/PCR method	
PFU/tube*	CPE†	qRT-PCR	RT-PCR
50	3 days	24 hours	24 hours
20	4 days	24 hours	48 hours
10	3-4 days	24 hours	24 hours
5	3-6 days	48 hours	72 hours
2	4-8 days	48 hours	72 hours

* Each tube spiked with plaque forming units (PFU) of poliovirus

† Cytopathic effect (CPE) refers to cell lysis by viral replication

Table 2. Tests of the ICC/PCR method using surface water samples

	Cell Culture Alone	ICC/PCR method
PFU/tube*	CPE†	qRT-PCR
400 (2)	2 days	24 hours
200 (4)	2 days	24 hours
100 (4)	2-3 days	24 hours
50 (4)	3 days	24 hours
20 (4)	3-4 days	24 hours
10 (4)	4-6 days	24 hours
6 (4)	4-6 days	24-48 hours

* Each tube spiked with plaque forming units (PFU) of poliovirus and number of trials is in parentheses

† Cytopathic effect (CPE) refers to cell lysis by viral replication

- Using the integrated cell culture/PCR method allows for detection of 6-10 infectious virus units in 24-48 hours verses the 4-6 days required for detection by cell culture alone.
- Similar results were observed for poliovirus and other enteroviruses (echovirus and coxsackievirus A9, data not shown).
- qRT-PCR is more sensitive than conventional RT-PCR, also qRT-PCR is faster and less labor intensive than conventional RT-PCR
- Cytotoxicity and PCR inhibition has not been found to be deleterious to this method and can be addressed easily without large decreases in sensitivity and speed
- Our results demonstrate that incorporating a qRT-PCR step allows the detection of live infectious virus before infection is visible in cell culture alone.
- The sensitivity of the method is comparable to cell culture and molecular assays alone
- Serial passage of the sample during cell culture allows for early detection without loss of any of the concentrated water sample

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