EPA 600/R-95/178 Enteric Virus Analysis

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Enteric viruses are a group of pathogens that include over 100 viral species that are shed with human feces. The health implications of these agents in humans include paralyzing polio, hepatitis, encephalitis, mild or sever gastroenteritis, myocarditis, and innocuous infections. Viruses are extremely small particles ranging from 20-85 nanometers in diameter. In comparison, a bacterial cell averages 1,000 nanometers in diameter. Each virus contains nucleic acid, either RNA or DNA, and is enclosed by a protein coat called a capsid. Viruses only replicate in a living host cell. Viruses are very specific as to what host cells they infect. Enteric viruses are typically ingested and transmitted along the fecal oral route. They have a very small infectious dose and high morbidity. Once ingested, they attach specifically to cells in the host and cause infection. Once attached, they enter the cell and take over the cell's machinery to produce more virus particles and ultimately cause cell death or cytopathy. In the case of enteric viruses, these particles are released along with the person's feces.

Enteric viruses are true human pathogens unlike coliform or fecal coliform bacteria that are considered an indicator. Compared to bacteria, many viruses are more resistant to commonly employed water and wastewater disinfection.

The process of the recovery of enteric viruses from environmental samples is tedious, labor intensive, and often lacks the sensitivity. Therefore large volumes of water are sampled. In surface and treated wastewater, culturable virus concentrations are typically low and less than 10 per liter. The number of viruses may in actuality be greater, but based upon the virus assay methods presently available, these are the orders of magnitude observed. Due to the low numbers and restrictions in assay procedure, virus concentration must be first conducted. Large sample volumes, as much as 1,500 Liters, are passed through specialized filters that allow the adsorption of viruses to the filter medium. Most often these are 10 inch electropositive charge modified filters through which the sample water is passed at a rate of approximately 1-2 gallons per minute. In the Laboratory, adsorbed viruses are then eluted from the collection filter and further concentrated into a small volume. The concentrate is assayed for the presence of culturable infectious viruses. The virus assay is performed inoculating the concentrate onto monolayers of mammalian tissue culture cells, the most common of which is a Buffalo Green Monkey Kidney (GBMK) cell line. If viruses are present, they will infect, multiply, and destroy the host cells (cytopathic effect (CPE)) usually within 28 days of incubation. Volumes of the sample concentrate are inoculated into separate flasks and the condition and cell monolayer sheet is observed by microscopy. A most probable number (MPN) method is then employed to estimate virus concentration in the original. This statistical method calculates concentration based on the number of flasks demonstrating CPE and the volumes inoculated into the flasks.

1. Sample: 100-1500 Liters recommended. Sample pH must be 6-8, have low salinity, and undetectable chlorine residual. Please contact lab with any questions.

2. Data Sheet: Name of Sampler; Source; Location; chlorine residual; Date; Collection Time; pH; Water Temp (C); Total Volume; Assay Requested; and Signature.

3. Collection: Use protective wear. Turn on the tap/pump to flush the system. Allow source water to flow until any debris that has accumulated has cleared and water physical parameters have equilibrated. The supplied, filter, housing, and tubing have already been decontaminated. Place supplied electropositive filter into provided housing. Label filter with site ID, initials, and date.

Connect the filtration set up to a pressurized unchlorinated water source via the provided tubing and connector. Alternatively, if a pressurized port with connections is not available a submersible pump with a variable flow valve can be used to pass the water through the filter. Connect other end of filter to the water flow meter and record initial meter (in Gallons) reading. Ensure that the filter is connected as per the correct orientation as the labels on filter cartridge. Turn on water slowly and **filter water at 1.0 gallons per minute.** *A head pressure of 0.5 bar (7.5 psi) is required for flow through the filter. The recommended pressure of 5 bar (75 psi) produces a flow rate of 1.0 gallon per minute per minute.* If sampling Chlorinated Effluent a dechlorination step is necessary prior to filtration. Dechlorination is achieved by injecting sodium thiosulfate solution into the water prior to filtration. The filter effluent must be monitored for chlorine residual throughout the sampling to ensure dechlorination. If chlorine residual be detected, immediately adjust parameters to neutralize disinfectant. Collect approximately 100 gallons (400 L) or more. Turn off water supply and record final meter reading. Detach filter and drain water from filter housing prior to placing into sterile sealable bag.

If collecting additional samples using the sample apparatus flush the system (after removing the filter for a minimum of ten minutes at high flow. Then insert a new filter (ensure the direction of the arrow on the filter is the same as the flow direction in the set-up) and repeat the above process.

Place filters in storage cooler with ice brix or in a refrigerator to chill prior to shipping. Do not allow to freeze. Ship samples to arrive within 48 hours of completion of sampling. Maximum holding time between sample collection initiation and processing of the filter by the laboratory is 72 hours. **Sample must arrive at 4°C ± 2°C. Do not freeze.**

Please note the EPA has made the following statement regarding shipping of the samples to a laboratory:

U.S. Department of Transportation (DOT) regulations (49 CFR172) prohibit interstate shipment of more than 4 L of solution known to contain infectious materials. State regulations may contain similar regulations for intrastate commerce. This method requires a minimum sample volume of 10 L. Unless the sample is known or suspected to contain Cryptosporidium or other infectious agents (e.g., during an outbreak), samples should be shipped as noninfectious and should not be marked as infectious. If a sample is known or suspected to be infectious, and the sample must be shipped to a laboratory by a transportation means affected by DOT or state regulations, it is recommended that the sample be filtered in the field, and that the filter be shipped to the laboratory to avoid violating transport regulations.