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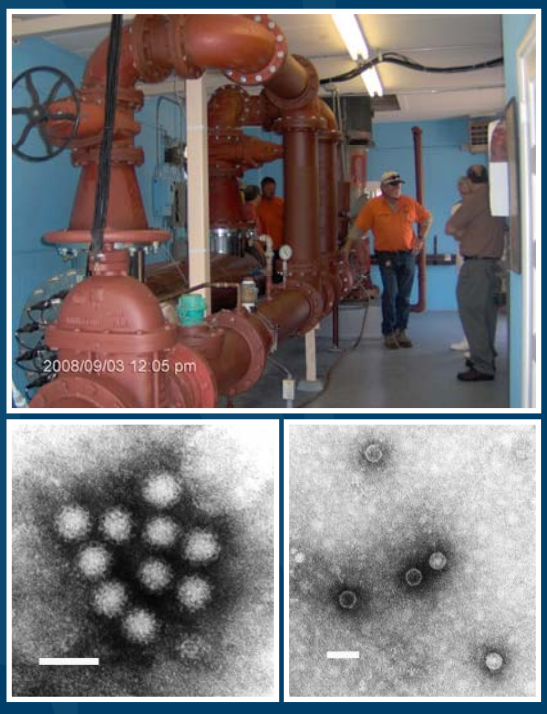
United States
Environmental Protection
Agency

Method 1615

Measurement of Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR



Office of Research and Development
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8.4.4.2. Add 1.0 mL of a QC sample (see item 7.1.2) to the water.

8.4.4.3. Place a magnet into the vessel or container and stir for 10 minutes at a speed sufficient to create a vortex.

8.4.4.4. Pass the water through a sterile standard apparatus containing a sterile electropositive filter using a flow rate of approximately 10 L/minute.

8.4.4.5. Process and analyze the filter using the Elution (step 10), Organic Flocculation (step 11), Total Culturable Virus Assay (step 12) and Enterovirus and Norovirus Molecular (step 13) procedures.

8.4.5. QC sample stocks (item 7.1.2) are also to be used for the Positive Assay Control (see item 7.3.3 and step 12.1.2.3.2).

8.5. PE SAMPLES

8.5.1. PE samples will be sent to analysts in a randomized fashion and may contain no, low, medium, or high levels of Sabin poliovirus type 3 on the filter type (e.g., 1MDS or NanoCeram) used for sampling.

8.5.2. Process and analyze the PE filter using the Elution (section 10), Organic Flocculation (section 11), Total Culturable Virus Assay (section 12) and Enterovirus and Norovirus Molecular (section 13) procedures and according to any additional requirements supplied with the samples.

8.5.3. PE sample results should meet the method performance characteristics defined in section 14.

8.6. MATRIX SPIKE

Matrix spike should be run for every sample location initially and then after every 20th sample from the same location. Matrix spikes duplicates are performed by collecting two samples at the sampling location as described in section 9, except that the sampling volume of the second sample is reduced by 10 L. The last 10L is collected in a 10 L cubitainer (item 6.2.3), shipped back to the laboratory, seeded with 1 mL of the matrix spike (item 7.1.3), passed through the duplicate filter, and analyzed by the method procedures (steps 10 through 12.2.11). The results of the analysis of the matrix spike must meet the performance measures in section 14.

8.7. RECORD MAINTENANCE

Laboratories shall maintain all records related to data quality. This shall include a record of the analyst name, date, and results of all QA controls performed, records of equipment calibration and maintenance, and reagent and material catalog and lot numbers used for all analytical procedures.

9. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

9.1. SAMPLE COLLECTION

9.1.1. Filter sampling apparatus sterilization

9.1.1.1. Before each use, analytical laboratories must wash, and then sterilize the intake and cartridge housing modules, any necessary injector modules, and pumps as described in section 15.2.4.

9.1.1.2. Cover the apparatus module ends and the injector port(s) with sterile aluminum foil.

9.1.1.3. Place the injector module and tubing into a sterile bag or wrapping in such a way that they may be removed without contaminating them.

9.1.1.4. Ship the filter sampling apparatus components to the individuals who will be collecting water samples.

9.1.2. Preparation for sample collection

9.1.3. Note: Individuals collecting water samples for virus analysis must wear surgical gloves and avoid conditions that can contaminate a sample with virus. Gloves should be changed after touching human skin or handling components that may be contaminated (e.g., water taps, other environmental surfaces). Care must be taken to ensure that cartridge filters are properly seated in the housings. Housings with properly seated filters must not leak. Filters should be checked for proper seating upon opening the housing at the analytical laboratory by examining the gaskets for depressions that do not extend beyond the edge of the filter.]

9.1.3.1. Purge the water tap to be sampled before connecting the filter apparatus. Continue purging for 2-3 minutes or until any debris that has settled in the line has cleared.

9.1.3.2. Remove the foil from the backflow regulator. Loosen the swivel female insert slightly to allow it to turn freely and connect the backflow regulator to the tap. Retighten the swivel female insert. Disconnect the cartridge housing module at the quick connect, if connected, and cover the open end with sterile foil.

9.1.3.3. Remove the foil from the ends of the discharge module and connect it to the regulator module. Place the end of the regulator module or the tubing connected to the outlet of the regulator module into a 1 L plastic bottle.

9.1.3.4. Slowly turn on the tap and adjust the globe valve until the flow meter/totalizer reads 10 L/min. If the tap is incapable of reaching this flow rate, adjust the valve to achieve the maximum flow rate. Slower flow rates will result in longer sampling times.

9.1.3.5. Flush the apparatus assembly with at least 75 L of the water to be sampled. While the system is being flushed, measure the

chlorine residual, pH, temperature, and the turbidity of the water collecting in and overflowing from the 1 L plastic bottle.

9.1.3.6. Record the pH, temperature, and turbidity values onto a sample data sheet.

9.1.3.7. Turn off the water at the tap.

9.1.4. Injector module adjustment (Note: If a NanoCeram filter is being used and if the water pH is 9.0 or less and if it does not contain a disinfectant, skip to section 9.1.5. If disinfected waters above pH 8.0 are being used with a 1MDS cartridge filter, substitute a double injector module for the single injector module in the following steps. With 1MDS filters under these conditions, use a second metering pump connected to the second connection on the double injector module to add 0.12 M HCl at a rate, which brings the pH of the water exiting the discharge module to 6.5 to 7.5.)

9.1.4.1. If the sample contains a disinfectant, turn off the water at the tap and disconnect the discharge module from the regulator module.

9.1.4.2. Remove the foil from the ends of an injector module and connect the module to the quick connect of the regulator module.

9.1.4.3. Turn on the metering pump. Set the pump to deliver 2.4 or 6±0.2 mL/minute for flow rates of 4 or 10 L/minute, respectively (see Table 2). Measure the flow exiting the injector module for several minutes to ensure that the flow rate is correct. Measure the chlorine residual and if present, re-adjust the flow rate until no residual is present. Re-mark the setting, if necessary. Turn off the metering pump.

9.1.5. Virus collection

9.1.5.1. If connected, remove the discharge module. Remove the foil from the cartridge housing module and connect it to the end of the regulator module, or if used, the injector module. Connect the discharge module to the outlet of the cartridge housing module.

9.1.5.2. If the water sample has turbidity greater than 75 NTU, remove the foil from each end of the prefilter module and connect the prefilter module between the regulator module or, if used, the injector module and the cartridge housing module.

9.1.5.3. Record the unique sample number, location, date, time of day and initial totalizer reading onto a sample data sheet (section 17.1).

9.1.5.4. If an injector module is being used, turn on the metering pump.

9.1.5.5. With the filter housing placed in an upright position, slowly open the water tap until it is completely open (Note: If the cartridge housing has a vent button, press it while opening the tap to expel air from the housing. When the air is totally expelled from the housing, release the button, and open the sample tap completely. If the housing does not have a vent button, allow the housing to fill with water before completely opening the tap).

9.1.5.5.1. After the tap is opened completely, check the flow rate and readjust to the recommended rate from Table 2, if necessary.

9.1.5.5.2. Check and readjust the metering pump rate, if necessary.

9.1.5.6. Using the totalizer readings, pass a volume of water through the apparatus that equals the volume specified in Table 2.

9.1.5.7. Turn off the flow of water at the sample tap at the end of the sampling period and record the date, time of day, and totalizer reading onto a sample data sheet (see section 17.1). Although the totalizer reading may be affected by the addition of thiosulfate, the effect is insignificant and may be ignored.

9.1.5.8. Loosen the swivel female insert on the regulator module and disconnect the backflow regulator from the tap. Disconnect the cartridge housing module and the prefilter housing module, if used from the other modules.

9.1.5.9. Turn the filter housing(s) upside down and allow excess water to flow out. Turn the housing(s) upright and cover the quick connects on each end of the modules with sterile aluminum foil.

9.2. SHIPMENT OF SAMPLES

9.2.1. Pack the cartridge housing module(s) into an insulated shipping box.

9.2.2. Add 6-8 small ice packs (prefrozen at -20°C) or double bagged ice cubes around the cartridge housings to keep the sample cool in transit (the number of ice packs or bags may have to be adjusted based upon experience to ensure that the samples remain cold, but not frozen).

9.2.2.1. Add an iButton (or other temperature recording devise) to a location in the shipping box where it will not come in direct contact with the ice packs or bags.

9.2.2.2. The temperature during shipment must be in the range of 1-10°C.

9.2.3. Drain and add the regulator and injector modules used.

9.2.4. Place the sample data sheet (protected with a closable plastic bag) in with the sample.

9.2.5. Drain and then cover the ends of the discharge module with foil. The discharge module may remain at the sampling site, if samples will be taken on a routine basis. If not, pack the module into the shipping box.

9.2.6. Close the insulated portion of the shipping box and tape to prevent any leakage of water. Close and label.

9.2.7. If the shipping box cannot be directly transported to the laboratory for virus analysis by close of business on the day collected or by the next morning, ship it to the laboratory by overnight courier.

9.3. LABORATORY HOLDING TIME AND TEMPERATURE

9.3.1. Record the date of arrival and the arrival condition on the sample data sheet packed with the sample. Print out the transit temperature reading from the iButton.

9.3.1.1. Attach the readout of the iButton or other temperature-recording device for recording the temperature during shipment to the sample data sheet.

9.3.1.2. Warning: The cartridge filters must arrive from the utility or other sampling site in a refrigerated, but not frozen, condition. The temperature during shipment must be in the range of 1-10°C.

9.3.1.3. Brief transient temperatures outside the acceptable range associated with the initial packing and closing of the shipping box and its opening at the analytical laboratory may be ignored.

9.3.2. Filters must be refrigerated immediately upon arrival. Ideally, viruses should be eluted from filters within 24 h of the start of the sample collection, but all filters must be eluted within 72 h of the start of the sample collection.

10. FILTER ELUTION PROCEDURE

10.1. ELUTION EQUIPMENT SETUP

10.1.1. Attach sections of braided tubing to the inlet and outlet ports of the cartridge housing containing the cartridge filter (see Figure 4). Note: If a prefilter or more than one electropositive filter was used to collect a sample, each filter must be eluted and analyzed separately using the procedures below.

10.1.2. Place the sterile end of the tubing connected to the outlet of the cartridge housing into a sterile 2 L glass or polypropylene beaker.

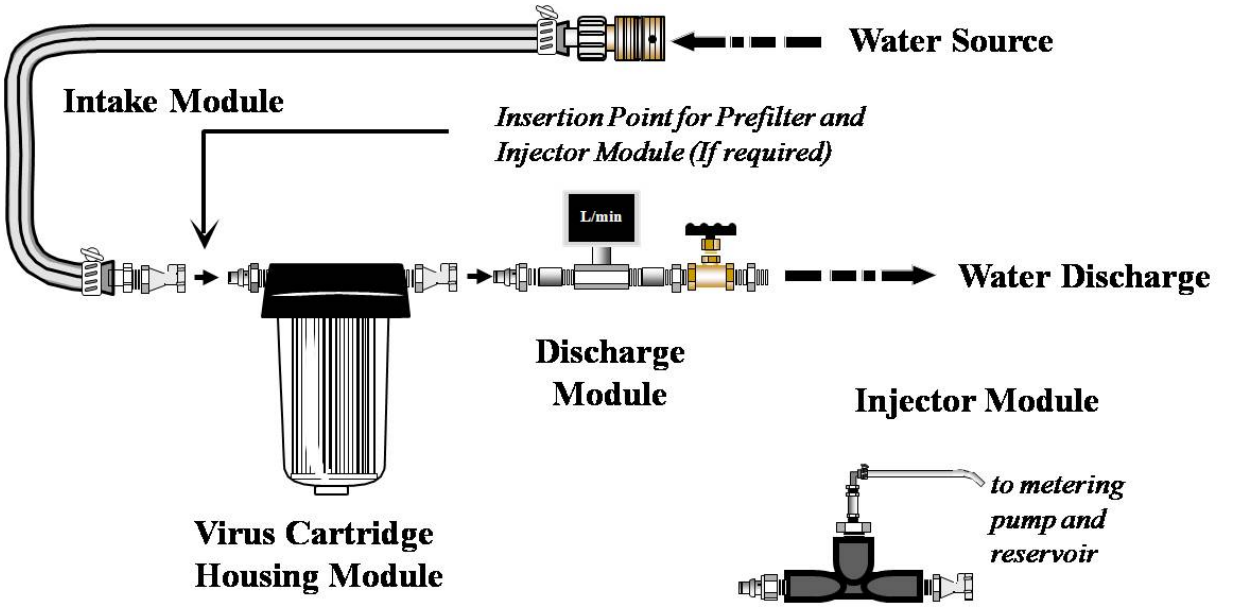

Figure 3. Sample Filtration Apparatus

Figure 4. Elution of an Electropositive Filter with Beef Extract