General protocol for the concentration of virioplankton from a large volume (>20 L) water sample

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Abstract

This protocol outlines and evaluates the filtration steps needed for the preparation of samples (viral concentrates) containing a density of viral particles concentrated from large volumes of natural water samples. (Fig. 2A and B)

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Guidelines

I. List of materials
- A. Large-scale concentration.
  - i. Tubing: silicone, various sizes
  - ii. Tubing: PharMed for pump heads only
  - iii. Connectors to tubing and filters
  - iv. Diaphragm pump (Jabsco Industrial Diaphragm pump #31801, 12 L per min capacity)
  - v. Two 50-L plastic carboys vi. 25-L carboy
  - vii. 25-µm wound polypropylene sediment filter (pool filter) and housing
  - viii. Large peristaltic pump with 2 pump heads
  - ix. 0.22-µm TFF filter x. 30- to 50-kD TFF filter
  - xi. 0.22-µm syringe filters
  - xii. 60-mL syringes
  - xiii. 50-mL conical tubes
- B. Small-scale concentration.
  - i. Tubing: silicone, various sizes
  - ii. Tubing: PharMed for pump heads only
  - iii. Connectors to tubing and filters
  - iv. Small, reversible peristaltic pump with 1 pump head
  - v. Two 2-L polycarbonate bottles
  - vi. 500-mL polycarbonate bottle
  - vii. 30- to 50-kD compact spiral filter
- C. Postconcentration.
  - i. Ultracentrifuge
  - ii. Swing bucket rotor
  - iii. Polyallomer tubes
  - iv. Balance
  - v. Waste container
  - vi. Final collection tubes of needed size

vii. 200-µL pipettman and tips

II. List of reagents

A. Sterile 60% glycerol

Concentration of virioplankton (Small-Scale)

The steps involved in the concentration of viruses from smaller water samples (i.e., <2 L) are similar to those for large samples. However, the TFF filters used for concentration of viruses from small-scale samples are usually 10-fold smaller in filter area. The smaller size of these filters, and the tubing connected to them, results in a coordinately smaller minimum hold-up volume. In our experience, small-scale TFF filtration results in viral concentrates of ca. 250 mL in volume. Small-scale concentration of viruses from ambient water samples will require prefiltration to remove cells and particulates larger than 0.22 µm in size. If small-scale TFF filtration is used as a second step viral concentration following a large-scale (50 L to 2 L) procedure no prefiltration is required. After this two step process, theoretical viral concentration ratios of 200 fold (50 L to 0.25 L) can be achieved. Actual viral concentration ratio will depend on overall filtration efficiency. Oftentimes, large- and smallscale TFF concentration of viruses is performed in the field, and the final 250 mL concentrate is frozen (preferably snap frozen in LN_2) for transport back to the lab. Some investigators have reported better viral preservation by adding glycerol to a final concentration of 10% prior to snap freezing (Glass and Williamson pers. comm.).

Postconcentration procedures

Oftentimes, it is desirable to reduce the volume of viral concentrates below the ca. 250 mL minimum hold-up volume of most small-scale TFF apparatus. In particular, fingerprinting of viral assemblages by pulsed-field gel electrophoresis (Steward 2001; Wommack et al. 1999) or preparation of viral concentrates for metagenomic sequencing (Bench et al. 2007; Breitbart et al. 2002) requires viral particle densities of $\geq 10^9$ mL^{-1}. By and large, investigators have produced highdensity viral concentrates using either spin filters, which rely on centrifugal force to push water through a 30- to 50-kD molecular sieve, or an ultracentrifuge to pellet viruses followed by resuspension in a smaller volume of UF or buffer. Disposable spin filters are provided by a number of manufacturers and require only a benchtop swinging bucket rotor for filtration (Bench et al. 2007). Pelleting of viruses can only be done in an ultracentrifuge at centrifugal forces exceeding 100,000g, a requirement that can be cost prohibitive or unavailable at smaller research facilities (Wommack et al. 1992). Recently, Colombet and coworkers (2007) adapted polyethylene glycol (PEG) precipitation of viruses, for postconcentration of viruses within 1 L viral concentrates derived from 20-L water samples. The PEG protocol showed a greater than 2-fold increase in the recovery efficiency of virus particles as compared with the ultracentrifugation procedure. The lower cost of this procedure is welcomed; however, we have found that postconcentration procedures can have a dramatic effect on the quality of PFGE virioplankton fingerprints. In contrast to the reported improvement in fingerprints after PEG precipitation (Colombet et al. 2007), we found that postconcentration of viral concentrates by ultrafiltration consistently produces the best resolved PFGE fingerprints (Fig. 3). The source of loss in PFGE band clarity and sharpness for samples processed by PEG precipitation or spin filtration is not known and did not appear to be attributable to sample loading as the PEG and Centricon samples were loaded with 2-fold less and 3-fold more viruses, respectively, than the ultracentrifuged sample.

Before start

All filters should be cleaned and rinsed according to manufacturer's recommendations before use in any application during the concentration procedures.

Protocol

Prefiltration

Step 1.

Prefilter ambient water sample with a 25-µm wound polypropylene sediment filter before any concentration using a peristaltic or diaphragm pump. (Fig. 2A).

NOTES

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This step removes large objects and organisms, detritus and sediment before it can interfer with later pumping and filtration steps.

Prefiltration

Step 2.

Rinse the concentration container three times with prefiltered water before filling with prefiltered sample water.

Tangential flow microfiltration to remove particles and cells >0.22 µm

Step 3.

Once the concentration container is full of 25 µm prefiltered ambient water, arrange the tubing for tangential flow filtration (TFF). See Fig. 2B

1. Attach the 0.22-µm tangential flow filtration (TFF) filter feed and retentate tubing to the TFF filter housing inlet and outlet ports, respectively.
2. Attach the permeate tubing to the permeate port of the TFF filter housing and direct into a separate container to catch virus-containing water.
3. Run the feed tubing through a large peristaltic pump head.

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This step separates viruses from >0.22 micron organisms (bacteria and plankton) creating a solution containing viruses in the permeat and a concentrated solution containing bacteria and plankton >0.22 micron, but <25 microns in size.

Tangential flow microfiltration to remove particles and cells >0.22 µm

Step 4.

Insure that the permeate port is closed, then begin priming the system as follows.

Tangential flow microfiltration to remove particles and cells >0.22 µm

Step 5.

If the peristaltic pump head has an occlusion setting, set it to maximal occlusion to help prime the 0.22-µm TFF filter and remove all air.

Tangential flow microfiltration to remove particles and cells >0.22 µm

Step 6.

Turn the peristatic pump on and increase the peristaltic pump speed to 20% to prime the tubing and remove excess air.

Tangential flow microfiltration to remove particles and cells >0.22 µm

Step 7.

Once the tubing and filter are completely filled with water, adjust the occlusion knob to a looser setting to prevent excessive tubing wear.

Tangential flow microfiltration to remove particles and cells >0.22 µm

Step 8.

Slowly increase the pump speed to 45%.

Tangential flow microfiltration to remove particles and cells >0.22 µm

Step 9.

Once the system is running smoothly, partially open the permeate valve on the 0.22-µm TFF filter to the second tick mark (20° open).

Tangential flow microfiltration to remove particles and cells >0.22 µm

Step 10.

Collect the <0.22 µm permeate into an appropriately sized carboy. (Fig. 2B)

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Cells and particulates between 25 µm and 0.22 µm will concentrate within the 50-L carboy as TFF through the 0.22-µm filter proceeds.

Concentration of virioplankton (Large-Scale)

Step 11.

When the permeate carboy is more than half full with <0.22 µm permeate, prepare the 30-kD TFF filter for viral concentration.

Concentration of virioplankton (Large-Scale)

Step 12.

Slow the large peristaltic pump speed to 10%.

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Do not stop the pump as this encourages adherence of viruses to filter matrix.

Concentration of virioplankton (Large-Scale)

Step 13.

Attach the feed and retentate tubing to the appropriate ports on the 30-kD TFF. See Fig. 2B

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Make sure that the backpressure knob on the 30-kD TFF filter is completely open (counter-clockwise).

Concentration of virioplankton (Large-Scale)

Step 14.

Direct the permeate tubing from the 30-kD TFF filter into a clean carboy to collect ultrafiltrate (UF; virus-free water).

Concentration of virioplankton (Large-Scale)

Step 15.

Carefully work the feed tubing into the second pump head of the large peristaltic pump.

Concentration of virioplankton (Large-Scale)

Step 16.

Increase the large peristaltic pump speed to 20%.

Concentration of virioplankton (Large-Scale)

Step 17.

Again, tighten the occlusion knob on the pump head to prime the 30-kD TFF filter and remove all air.

Concentration of virioplankton (Large-Scale)

Step 18.

Once the filter and lines are fully primed loosen the pump head occlusion knob.

Concentration of virioplankton (Large-Scale)

Step 19.

Slowly increase the pump speed to 45%.

Concentration of virioplankton (Large-Scale)

Step 20.

Slowly close the backpressure knob on the 30-kD TFF filter until permeate begins to flow.

Concentration of virioplankton (Large-Scale)

Step 21.

Monitor the level of <0.22 µm water in the <0.22 µm permeate carboy. The level should be maintained at half full until the prefiltered ambient water is nearly gone.

Concentration of virioplankton (Large-Scale)

Step 22.

Collect 1 L of ultrafiltrate (30 kD TFF permeate) in a 2-L bottle for rinsing of the 30-kD TFF filter.

Concentration of virioplankton (Large-Scale)

Step 23.

When 5 L of 25 µm filtered ambient water remains in the large carboy, release the pump head and remove the 0.22-µm TFF filter feed tubing from the large peristaltic pump.

Concentration of virioplankton (Large-Scale)

Step 24.

Continue to run the 30-kD TFF filter until 1 L of <0.22 µm water remains in the carboy.

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Avoid entry of air into the feed line as the level of retentate water nears this minimum.

Concentration of virioplankton (Large-Scale)

Step 25.

When 1L or less of <0.22 µm water remains, stop the large peristaltic pump and drain the 30-kD TFF filter into the <0.22 µm water carboy.

Concentration of virioplankton (Large-Scale)

Step 26.

Slowly prime the 30-kD TFF filter from the 1 L of collected UF from step 22 and then recirculate (feed, retentate and permeate lines of the 30-kD filter in the 1L of UF) the UF at 30% pump speed for 5 min.

DURATION

00:05:00

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Be careful to avoid air bubbles.

Concentration of virioplankton (Large-Scale)

Step 27.

Stop the pump, release the pump head, and drain all tubing into the 2-L UF bottle.

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Note: Recirculation of UF after primary viral concentration has been reported by the manufacturer to substantially improve recovery of retained molecules and viruses [Millipore 2003].

Concentration of virioplankton (Large-Scale)

Step 28.

Pool the recirculated UF with the 1 L of retentate from the primary viral concentration through the 30-kD TFF filter.

Concentration of virioplankton (Large-Scale)

Step 29.

The volume of the 2 L viral concentrate can be further reduced by using a small-scale TFF ultrafilter.

Concentration of virioplankton (Large-Scale)

Step 30.

To avoid excessive degradation of viruses, it is advisable to store the first stage VC at 4°C and perform a second stage small-scale concentration a soon as possible (i.e., within no more than 1 d).

NOTES

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See guidelines for Concentration of virioplankton (Small-Scale) and Postconcentration procedures.

Warnings

Cleaning solutions recommended by the manufacturer are corrosive.
Final viral concentrates should be enumerated, extracted, or frozen in liquid nitrogen as soon as possible after completion of concentration to avoid degradation.