Sample collection and preparation

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Abstract

For Enumeration of virus particles in aquatic or sediment samples by epifluorescence microscopy protocol.

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Guidelines

Preservation of samples (SYBR only): If slides cannot be made in the field on freshly collected samples, preservation will be necessary. Many earlier estimates of viral abundance have been made on preserved samples; however, several studies have shown rapid and significant decay of viral particles in aldehyde-fixed samples (Brussaard 2004; Danovaro et al. 2001; Wen et al. 2004), although there is evidence that the decay is lessened when larger-volume samples are preserved (Patel et al. 2007). Samples preserved in the field should be flash-frozen in liquid nitrogen. Even freezing with dry ice (frozen CO_2) or immediately freezing in a –87°C freezer is not adequate for preservation, and to our knowledge, freezing in a dry ice/ethanol slurry (–78°C) has not been tested. Samples to be preserved should be fixed in 0.5% (final concentration) EM-grade glutaraldehyde and flash-frozen in liquid N_2. For relatively productive samples with virus abundances $\geq 10^6$ mL^{-1}, add 30 µL of 25% EM-grade glutaraldehyde into prelabeled 2-mL cryovials, add 1470 µL of the sample to be counted, and mix. These volumes can be halved for samples with virus abundances $\geq 10^7$ mL^{-1} and increased to 4 mL in 5-mL cryovials for very oligotrophic samples. Allow the samples to stand for at least 15 min but no longer than 30 min, and then freeze immediately in liquid N_2. To facilitate easy retrieval, vials can be placed in women's nylon stockings before freezing. (We find black sheer ones the most aesthetically pleasing.) Once frozen, the vials can be transferred to –80°C for long-term storage, until the slides can be prepared. For counting, the frozen samples are thawed in a 37°C water bath and immediately stained with SYBR Green I or SYBR Gold, as outlined.

In the field, if it is impossible to make slides or preserve in liquid N, then it probably is best to collect the samples in as large volumes as is practical and maintain them under in situ conditions for as short a time as is possible until slides can be made.

Protocol

Aqueous samples

Step 1.
Collect the samples in sterile containers that are rinsed three times with the sample water.

NOTES
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Polypropylene centrifuge tubes or bottles work well as sample containers.

Aqueous samples

Step 2.

The range of viral abundances suitable for enumeration is 10^5 to 10^7 mL^{-1}; hence, dilution may be necessary for very productive natural samples or cultures.

Aqueous samples

Step 3.

If necessary, dilute the sample with 0.02-µm filtered water, ideally prepared from the same or very similar water from which the sample was obtained.

Sediment samples

Step 4.

Undisturbed sediment samples should be collected with a piston corer.

NOTES

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The sediment–water interface can be sampled with a wide-bore serological pipette to minimize disruption to the sediment surface.

Sediment samples

Step 5.

Samples from deeper within the core can be obtained by carefully pushing the core up from the bottom of the core barrel and slicing the sediment at the desired depth.

NOTES

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The sediment should be sampled from the center of the core, leaving a well-defined area of sediment around the periphery to ensure the sample is not contaminated with sediment smeared along the side of the core barrel.

Sediment samples

Step 6.

Remove a 0.5-cm^3 subsample of sediment from the center of the core slice and transfer it into 4 mL of 0.02-µm filtered seawater and 1.0 mL pyrophosphate (10 mM final concentration).

Sediment samples

Step 7.

Sonicate the mixture for 3 min.

DURATION

00:03:00

NOTES

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(Ortmann and Suttle 2009)

Sediment samples

Step 8.

Centrifuge at 800g for 1 min.

DURATION

00:01:00

NOTES

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(Ortmann and Suttle 2009)

Sediment samples

Step 9.

The supernatant can then be diluted and the slides prepared as outlined.

Sediment samples

Step 10.

For different types of sediments and soils, the amount of pyrophosphate and length of sonication may need to be tested and optimized.

NOTES

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Potassium citrate has been reported to be superior to sodium pyrophosphate for extracting phage from soils (Williamson et al. 2003) and may be an alternative for aquatic sediments, as well.

Preservation of samples (SYBR only)

Step 11.

For preservation of samples (SYBR only), see guidelines.