Elution and concentration of viruses from sediments

Kata Farkas[1], James E McDonald[2], Shelagh K Malham[3], Davey L Jones[1]

[1]*School of Environment, Natural Resources and Geography, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK*
[2] *School of Biological Sciences, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK*
[3] *School of Ocean Sciences, Bangor University, Menai Bridge, Anglesey, LL59 5AB, UK*

A B S T R A C T

This method describes the recovery of viruses from sediment using a beef extract-based elution followed by precipitation with polyethylene glycol. The method is suitable for the recovery of a wide range of viruses and preserving the integrity of the viral particles. Hence, the concentrate may be subject to molecular detection, targeting the viral genome or capsid proteins, or infectivity studies. For quality control, the addition of a process control virus, e.g. mengovirus, is recommended. The 10-100% recovery of the mengovirus indicates that the viral recovery is efficient.

Introduction

Viruses have been shown to readily adsorbed to organic and inorganic matter, and hence able to accumulate in marine and freshwater sediments (Rao et al., 1986), often in a higher concentration than in the overlaying water body (Duhamel and Jacquet, 2006). These viruses may be derived from wastewater discharge and hence are harmful for humans and animals. It has been shown that the association of viral particles with sediment may increase the persistence of viruses (LaBelle and Gerba, 1980). Furthermore, viruses may be released from sediment to water due to weather changes (Haramoto et al., 2009) and can be ingested by crustacea and shellfish destined for human consumption (Oliveira et al., 2011). These events can result in public health threats far from the source of contamination.

The viral recovery method described here consists of an initial elution of the virus particles from sediment using beef extract, followed by a precipitation using polyethylene glycol (PEG). The method has been shown to be suitable for the quantification of human pathogenic viruses in freshwater and estuarine sediment. The achieved recoveries using this method were 97% for hepatitis A virus, 78% for human rotavirus and 83-100% for human norovirus GII (Farkas et al., 2017; Lewis and Metcalf, 1988). The method has been successfully utilised for the 1-year surveillance of enteric viruses, including norovirus GI and GII, human adenovirus and polyomavirus, in the sediment of the Conwy River and estuary, North Wales, UK (Farkas et al., 2018). Furthermore, the method was shown to be suitable for the description of viral ecology in sediment using metagenomics approaches (Adriaenssens et al., 2018). The viral concentrate is suitable for subsequent viral nucleic acid extraction and quantification, viral capsid integrity assays and infectivity assays.

However, the method has been validated using different sediments, it may not be suitable for all sediment types. Therefore, the quality control of the procedure is necessary. Here we recommend the use of mengovirus as a process control. Mengovirus has very similar structure to hepatitis A virus and Caliciviruses and hence often used to estimate the recovery of these viruses. Mengovirus is commercially available and easy to culture in vitro and hence a suitable control for RNA quantification and infectivity studies. A minimum of 10% process control recovery is recommended to establish the efficiency of the procedure.

Equipment

- Laboratory shaker or equivalent
- Balance (range: 0.5-5 g)
- Refrigerated centrifuge with fixed angle rotor for 50 ml centrifuge tubes, or equivalent
- Pocket sized pH meter (Ishiro S2K992 or equivalent)
- Pipettes, size 0.1 ml, 1 ml

Materials

- 30 ml sterile beef extract – sodium nitrate ($NaNO_3$) solution, pH 5.5 (Appendix 1)
- 5 g polyethylene glycol 6000 (PEG6000)
- 0.6 g sodium chloride (NaCl)
- 1 M sodium hydroxide (NaOH) solution
- 50 ml sterile centrifuge tubes, 2pcs
- Sterile spatula
- 50 ml sterile measuring cylinder or equivalent
- Ice
- 0.1 ml sterile filtered pipette tip, 1 pc
- 1 ml sterile filtered pipette tip, 2 pcs
- Phosphate buffered saline solution (PBS), pH 7.45 (Appendix 1)
- 30 µl mengovirus strain VMC0 (prepared according to ISO/TS150216-1:2013) solution with approx. 10^6 mengovirus particles or equivalent

Method details

1. Measure the appropriate amount of sediment (e.g. 10g) into a sterile 50 ml centrifuge tube using a sterile spatula.
2. Add 10 µl mengovirus solution to the sample. Save the rest of the mengovirus sample for control measurements.
3. Add 3x volume beef extract-$NaNO_3$ solution to the sediment sample (10 g sediment + 30 ml beef extract -$NaNO_3$).
4. Mix at 50-100 rpm on ice for 30 min using a laboratory shaker.
5. Centrifuge at 2,500xg for 10 min, and decant the clear supernatant to new 50 ml centrifuge tube. Discard pellet.
6. Adjust pH of supernatant to 7.5 with approx. 100 µl 1 M NaOH. For pH measurement place a small aliquot (approx. 50 µl) of the solution on the pH meter electrode. Do not submerge the pH electrode to the solution to avoid contamination.
7. Add PEG6000 and NaCl to reach final concentration of 15% and 2% respectively (5 g PEG6000 and 0.6 g NaCl to 30 mL supernatant).
8. Mix to dissolve PEG6000. Adjust the pH to 7.0-7.5. For pH measurement place a small aliquot (approx. 50 µl) of the solution on the pH meter electrode. Do not submerge the pH electrode to the solution to avoid contamination.
9. Incubate at 4°C for 12-24 hours.
10. Centrifuge at 10,000xg for 10 min at 4°C. Discard supernatant. Remove the residual supernatant using a pipette.
11. Dissolve pellet in 0.5-2 ml PBS for infectivity/integrity studies or extract nucleic acids directly from pellet.
12. The viral concentrate can be stored at 4°C for up to 24 hours. For long term storage, -80°C is recommended.

Additional information

Quality control

For assessing viral recovery, mengovirus should be quantified in the spiked sample and in the control by RNA quantification or culturing. At least 10% recovery is recommended to conclude that the viral recovery was efficient. When <10% mengovirus recovery is estimated the concentrate should be diluted and retested.

Health and safety

Standard microbiology safety precautions should be applied. Laboratories should perform a full risk assessment before performing this procedure.

Appendices

Appendix 1 Reagents and solutions

Beef extract – sodium nitrate ($NaNO_3$) solution, pH 5.5

- 450 ml distilled water
- 85 g $NaNO_3$
- 15 g beef extract (#10443833, Lab-Lemco, Oxoid)
- Adjust pH to 5.5 (approx. 4 ml 0.5 N HCl)
- Bring to final volume of 500 ml

Phosphate buffered saline solution (PBS), pH 7.45

The use of the Gibco PBS tablets is recommended (Life Technologies, #18912-014). Alternatively, the following recipe can be followed:

- 400 ml distilled water
- 4 g NaCl

- 0.1 g KCl
- 0.72 g Na_2HPO_4
- 0.12 g KH_2PO_4
- Adjust the pH to 7.45 with HCl
- Bring to final volume of 500 ml

References

Adriaenssens, E., Farkas, K., Harrison, C., Jones, D., Allison, H.E., McCarthy, A.J., 2018. Viromic analysis of wastewater input to a river catchment reveals a diverse assemblage of RNA viruses. bioRxiv. doi:10.1128/mSystems.00025-18

Duhamel, S., Jacquet, S., 2006. Flow cytometric analysis of bacteria- and virus-like particles in lake sediments. J. Microbiol. Methods 64, 316–332. doi:10.1016/j.mimet.2005.05.008

Farkas, K., Cooper, D.M., McDonald, J.E., Malham, S.K., de Rougemont, A., Jones, D.L., 2018. Seasonal and spatial dynamics of enteric viruses in wastewater and in riverine and estuarine receiving waters. Sci. Total Environ. 634, 1174–1183. doi:10.1016/j.scitotenv.2018.04.038

Farkas, K., Hassard, F., McDonald, J.E., Malham, S.K., Jones, D.L., 2017. Evaluation of molecular methods for the detection and quantification of pathogen-derived nucleic acids in sediment. Front. Microbiol. 8, 53. doi:10.3389/fmicb.2017.00053

Haramoto, E., Kitajima, M., Katayama, H., Asami, M., Akiba, M., Kunikane, S., 2009. Application of real-time PCR assays to genotyping of F-specific phages in river water and sediments in Japan. Water Res. 43, 3759–3764. doi:http://0-dx.doi.org.unicat.bangor.ac.uk/10.1016/j.watres.2009.05.043

LaBelle, R.L., Gerba, C.P., 1980. Influence of estuarine sediment on virus survival under field conditions. Appl. Environ. Microbiol. 39, 749–755.

Lewis, G.D., Metcalf, T.G., 1988. Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. Appl. Environ. Microbiol. 54, 1983–1988.

Oliveira, J., Cunha, A., Castilho, F., Romalde, J.L., Pereira, M.J., 2011. Microbial contamination and purification of bivalve shellfish: Crucial aspects in monitoring and future perspectives – A mini-review. Food Control 22, 805–816. doi:10.1016/j.foodcont.2010.11.032

Rao, V.C., Metcalf, T.G., Melnick, J.L., 1986. Human viruses in sediments, sludges, and soils. Bull. World Health Organ. 64, 1–13.