Capsid integrity assay for the detection and quantification of norovirus particles in environmental samples

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 a rapid and simple approach to distinguish intact and degraded, hence non-infectious norovirus particles using magnetic beads coated with porcine gastric mucin (PGM). The PGM has high affinity to norovirus capsid proteins and hence using PGM-coated magnetic beads, intact norovirus particles can be extracted from a sample, whereas the degraded norovirus particles and free viral nucleic acids will remain in the solution. The assay is suitable for the purification of concentrated environmental samples (river, estuarine water, wastewater and sediment) to eliminate organic matter (e.g. humic substances) that may inhibit the detection and quantification of viruses. The assay is also suitable for laboratory experiments aiming to study the survival of norovirus under different conditions.

Introduction

Noroviruses are frequently found in environmental waters due to wastewater discharge. They are highly persistent in environmental waters and are able to travel long distances, threatening recreational waters, drinking water sources and shellfish harvesting areas (Campos et al., 2015).
Even though noroviruses often associated with waterborne and foodborne illnesses and outbreaks, little is known about their fate and behaviour in the environment, primary due to the lack of methods for detection (Bosch et al., 2011; Rodríguez-Lázaro et al., 2012). There is no routine infectivity assay for human noroviruses that can be used for environmental samples, hence, molecular methods (e.g. quantitative polymerase chain reaction; qPCR) are used for their quantitation. However, these methods detect not only intact and hence infectious noroviruses, but degraded particles and free viral nucleic acids, which are not infectious. Furthermore, these methods are often inhibited by the organic matter (e.g. humic substances) often found in environmental samples resulting in false negative results (Meschke and Sobsey, 1998; Rock et al., 2010). To overcome these limitations, capsid integrity assays can be used.
The porcine gastric mucin (PGM) assay is a simple and rapid method to assess norovirus capsid integrity. The PGM has high affinity to norovirus capsid proteins. Hence, when PGM-coated magnetic beads (PGM-MBs) are mixed with a sample the beads bind to norovirus particles and using magnetic forces those particles can be separated from free viral nucleic acids and damaged, non-infectious norovirus particles. Furthermore, the assay helps the elimination of qPCR inhibitors, and hence the subsequent quantification gives accurate results (Tian et al., 2008).
The PGM assay described here has been successfully used to assess norovirus degradation in wastewater, river and estuarine water and sediment samples (Farkas et al., 2018). The PGM-MBs are widely used to estimate the risks associated with shellfish (Ye et al., 2014) and fresh produce (Bartsch et al., 2016; Tian et al., 2012).

Equipment

- Laboratory shaker or equivalent
- Analytical balance
- Spectrophotometer
- Magnetic separation rack
- Pipettes, size 0.1 ml, 1 ml

Materials

- Porcine Gastric Mucin (PGM) #M1778-10G (Sigma)
- Carboxyl derivatized magnetic beads #21353 (Thermo Scientific)
- EDC [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] #22980 (Thermo Scientific)
- MES buffer: 0.1 M MES (N-morpholinoethane sulfonic acid), 0.9% NaCl; pH 4.7 (BupH™ MES Buffered Saline Packs; #28390, Thermo Scientific)
- Sulfo-NHS (N-hydroxysulfosuccinimide) #24510 (Thermo Scientific)
- Phosphate buffered saline solution (PBS), pH 7.45 (Appendix 1)
- Bradford reagent
- Cuvettes for spectrophotometer
- 1.5 ml sterile centrifuge tubes
- 0.1 ml sterile filtered pipette tips
- 1 ml sterile filtered pipette tips
- Molecular-grade water

Method details

Coupling PGM to magnetic beads

1. Create coupling buffer:
   Add 40 mg EDC and 40 mg sulfo-NHS to 1 ml MES buffer. Prepare the coupling buffer fresh.

2. Wash 1 ml of beads three times with 1 ml of PBS.
    a. Mix MagnaBind Beads thoroughly.
    b. Magnetically separate and aspirate beads. Perform magnetic separation using, magnetic rack, perpendicular to gravity.
    c. Repeat washing twice.
    d. Save last wash for protein test (label: 'Wash 0').
    e. After last wash resuspend beads in 0.5 ml water.
3. Add 0.5 ml coupling buffer to beads and mix thoroughly. Incubate for 15-30 min at room temperature.
4. Separate beads on magnetic rack and discard buffer.
5. Prepare protein solution:
    a. Prepare 5 ml 10 mg/ml PGM solution by mixing 50 mg PGM with 5 ml molecular grade water.
    b. Add 0.5 ml 10 mg/ml PGM to 0.5 ml MES buffer.
6. Resuspend beads in 1 ml protein solution and gently agitate at room temperature for 14-16 hours. Do not exceed 16 hours.
7. Wash beads three times with 1 ml PBS. Mix sample by pipetting the solution after each wash. Save wash solutions for protein test (label: 'Wash 1-3').
8. Resuspend beads in 1 ml PBS (label: 'PGM-MB').
9. Perform protein test to test coupling efficiency:
    a. Prepare a dilution series of PGM solutions with five points in the concentration range of 0.01 – 5 mg/ml.
    b. Mix the PGM-MBs, wash solutions ('Wash 0-3') and standards with the Bradford reagent according to manufacturer's instructions.
    c. Measure adsorbence at 595 nm.
    d. Create a standard curve (**Figure 1**) using the absorbance of the standards and determine sample concentration using the curve equision.
    e. Calculate the coupling efficiency according to the following equision:

$$Efficiency\% = \frac{5mg - \left(\frac{cWash1}{1ml} + \frac{cWash2}{1ml} + \frac{cWash3}{1ml}\right)}{5mg} * 100\%$$

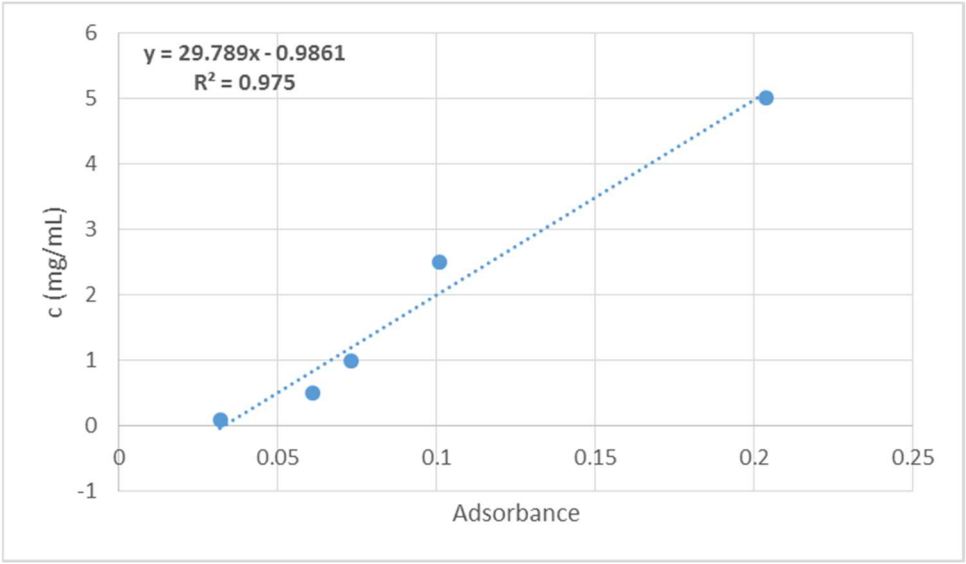

Figure 1. Example of the standard curve used to determine coupling efficiency.

PGM assay

1. Add 30 µl of coated magnetic beads to 100-500 µl of sample solution.

2. Incubate samples for 30 mins with gentle agitation (20 rpm on lab shaker) at room temperature.
3. Use magnetic rack to separate PGM-MBs.
4. Keep supernatant (label: 'Wash A').
5. Resuspend the magnetic beads in 0.5 ml PBS and pipette up and down to mix.
6. Use magnetic rack to separate PGM-MBs.
7. Keep supernatant (label: 'Wash B').
8. Resuspend the magnetic beads in 0.2-0.5 ml PBS and pipette up and down to mix.
9. Heat release the sample by heating at 95°C for 5 mins.
10. Use magnetic rack to separate PGM-MBs.
11. Use the final supernatant for nucleic acid extraction (label: 'Sample'). This solution contains presumably intact norovirus particles, whereas the wash solutions contain free RNA.
12. RNA should be extracted from the samples immediately after the PGM assay using extraction method suitable for envirnmental samples.

Additional information

Quality control

The results of the protein test using Bradford reagent indicates the efficiency of PGM coupling. The 'Wash 0' solution should be negative. The last wash solution ('Wash 3') should be negative suggesting the PGM has been covalently bound to the MBs. The coupling efficiency should be 50-80%.

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

Phosphate buffered saline solution (PBS), pH 7.45

The use of the Gibco PBS tablets is recommended (#18912-014, Life Technologies). 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

Bartsch, C., Szabo, K., Dinh-Thanh, M., Schrader, C., Trojnar, E., Johne, R., 2016. Comparison and optimization of detection methods for noroviruses in frozen strawberries containing different amounts of RT-PCR inhibitors. Food Microbiol. 60, 124–130. doi:10.1016/j.fm.2016.07.005

Bosch, A., Sánchez, G., Abbaszadegan, M., Carducci, A., Guix, S., Le Guyader, F.S., Netshikweta, R., Pintó, R.M., van der Poel, W.H.M., Rutjes, S., Sano, D., Taylor, M.B., van Zyl, W.B., Rodríguez-Lázaro, D., Kovač, K., Sellwood, J., 2011. Analytical Methods for Virus Detection in Water and Food. Food Anal. Methods 4, 4–12. doi:10.1007/s12161-010-9161-5

Campos, C.J.A., Avant, J., Gustar, N., Lowther, J., Powell, A., Stockley, L., Lees, D.N., 2015. Fate of Human Noroviruses in Shellfish and Water Impacted by Frequent Sewage Pollution Events. Environ. Sci. Technol. 49, 8377–8385. doi:10.1021/acs.est.5b01268

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

Meschke, J.S., Sobsey, M.D., 1998. Comparative adsorption of Norwalk virus, poliovirus 1 and F RNA

coliphage MS2 to soils suspended in treated wastewater. Water Sci. Technol. 38, 187–189.
Rock, C., Alum, A., Abbaszadegan, M., 2010. PCR inhibitor levels in concentrates of biosolid samples predicted by a new method based on excitation-emission matrix spectroscopy. Appl. Environ. Microbiol. 76, 8102–8109. doi:10.1128/AEM.02339-09 [doi]
Rodríguez-Lázaro, D., Cook, N., Ruggeri, F.M., Sellwood, J., Nasser, A., Nascimento, M.S.J., D'Agostino, M., Santos, R., Saiz, J.C., Rzezutka, A., Bosch, A., Gironés, R., Carducci, A., Muscillo, M., Kovač, K., Diez-Valcarce, M., Vantarakis, A., von Bonsdorff, C.H., de Roda Husman, A.M., Hernández, M., van der Poel, W.H.M., 2012. Virus hazards from food, water and other contaminated environments. FEMS Microbiol. Rev. 36, 786–814. doi:10.1111/j.1574-6976.2011.00306.x
Tian, P., Engelbrektson, A., Mandrell, R., 2008. Two-log increase in sensitivity for detection of norovirus in complex samples by concentration with porcine gastric mucin conjugated to magnetic beads. Appl. Environ. Microbiol. 74, 4271–4276. doi:10.1128/AEM.00539-08
Tian, P., Yang, D., Pan, L., Mandrell, R., 2012. Application of a receptor-binding capture quantitative reverse transcription-pcr assay to concentrate human norovirus from sewage and to study the distribution and stability of the virus. Appl. Environ. Microbiol. 78, 429–436. doi:10.1128/AEM.06875-11
Ye, M., Li, X., Kingsley, D.H., Jiang, X., Chen, H., 2014. Inactivation of human norovirus in contaminated oysters and clams by high hydrostatic pressure. Appl. Environ. Microbiol. 80, 2248–2253. doi:10.1128/AEM.04260-13