MACHEREY-NAGEL

NucleoMag® DNA/RNA Water

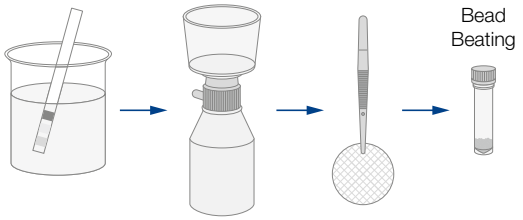
Viral nucleic acid isolation from wastewater samples

Introduction

Concentration of viral particles is essential for efficient nucleic acid isolation from water samples. However, due to their size virus particles cannot be efficiently concentrated on a membrane without a specific treatment. (Untreated or primary) wastewater sample volume usually varies between 50–250 mL or even higher volumes. Wastewater samples are subjected to different pretreatment methods (reviewed in Haramoto et al, Farkas et al 2020) to concentrate viral particles for subsequent nucleic acid extraction and detection.

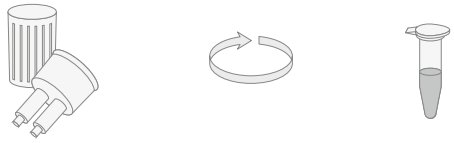
Sample concentration

1. Filtration (electronegative membrane)

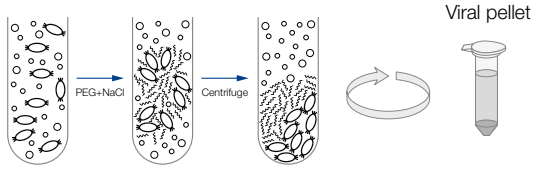


2. Ultrafiltration

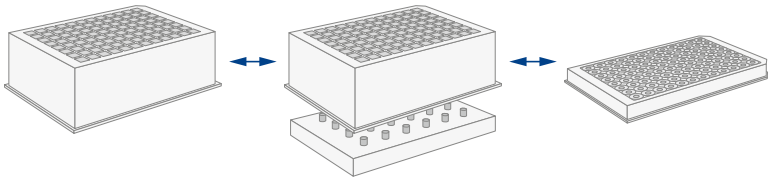
e.g. Centricon®



3. PEG Precipitation



Standard procedure NM Water

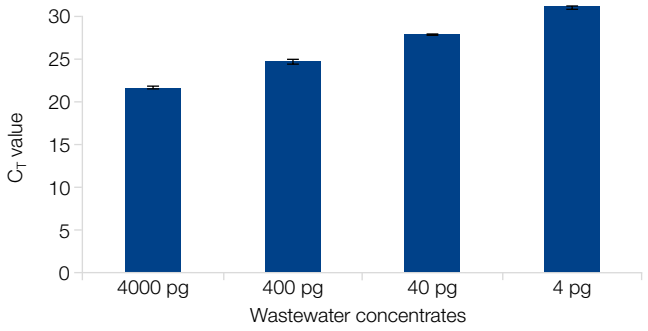


Transfer lysate | Bind/Wash/Elute | Pure nucleic acid

Ultrafiltration – Step by Step protocol recommendation for NucleoMag® DNA/RNA Water

The following step by step protocol describes the procedure for a 40 mL wastewater sample. The ultrafiltration method can be adapted to larger volumes. However, an additional pre-filtration step using a 3.5 µm filter is recommended. For larger samples volumes (> 1 Liter), tangential flow filtration is advantageous.

Ultrafiltration Procedure		
1	Pellet larger particles	Centrifuge wastewater at 4600 – 4700 x g for 30 min at 4 °C to pellet larger particles or debris. Transfer the supernatant to a fresh tube or container.
2	Filtration	Filter the cleared wastewater sample through 0.2 µm filter unit or syringe filter to remove residual cellular debris.
3	Concentration	Concentrate the cleared and filtered wastewater sample using a suitable ultracentrifugation unit (e.g. Centricon® Plus-70 centrifugal ultrafilter units 10 kDa, Millipore; Cat. No.: UFC701008) and recover the wastewater concentrate according to the manufacturer's instructions.
4	Lysis	Transfer 200 µL of the wastewater concentrate, add 200 µL MWA1 and 10 µL Liquid Proteinase K. Incubate at 56 °C for 10 min.
5	Binding	Add 25 µL NucleoMag B-Beads and 475 µL Binding Buffer MWA2 and proceed with step 3 of the standard protocol.



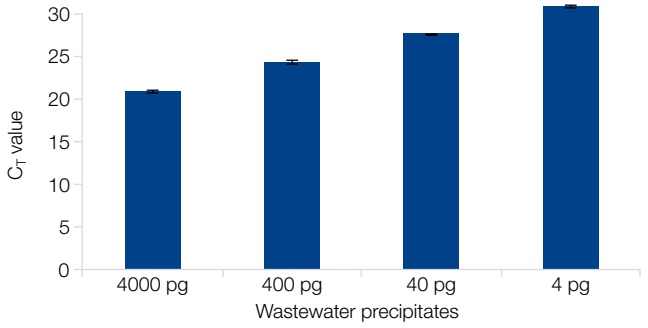
Detection of MS2 bacteriophage RNA in wastewater concentrates

Wastewater concentrates were generated from 40 mL of a substitute wastewater samples using ultrafiltration. MS2 bacteriophage RNA was spiked into the concentrates in a dilution series (n = 2 for each dilution) and isolated using the NucleoMag® DNA/RNA Water kit (see step-by-step protocol). qRT-PCR analysis was performed with a Taqman® probe for MS2 RNA using the SensiFast™ Probe One-Step Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System. MS2 bacteriophage RNA was detected consistently and reliably over a dilution series with excellent linearity (R^2 = 0.9999).

Precipitation – Step by Step protocol recommendation for NucleoMag® DNA/RNA Water

The following step by step protocol describes the procedure for a 40 mL wastewater sample. The precipitation method can be adapted to larger volumes.

Precipitation Procedure		
1	Remove particulates	Centrifuge wastewater at 4600 – 4700 x g for 30 min at 4 °C to pellet larger particles or debris. Transfer the supernatant to a fresh tube or container.
2	Filtration	Filter the cleared wastewater sample through 0.2 µm filter unit or syringe filter to remove residual cellular debris
3	Precipitate viral particles	Dissolve 4 g of PEG 8000 and 0.9 NaCl per 40 mL cleared wastewater sample by gently mixing.Centrifuge the sample at 12.000 x g for 120 min at 4 °C. Note: Use suitable centrifuge containers. Mark the side of the tube on which the pellet would be expected, as it may not be visible. The use of molecular biology grade chemicals is recommended.
4	Transfer the lysate	Remove the supernatant carefully without disturbing the pellet during aspiration. Perform a brief centrifugation step for 5 min at 4.000 x g and remove the residual liquid by pipetting.
5	Resuspension	Add 200 µL sterile H2O and 200 µL MWA1 to the pellet and resuspend by vortexing.
6	Lysis	Transfer the solution to a fresh reaction vessel, add 10 µL Liquid Proteinase K and incubate at 56 °C for 10 min.
7	Extraction	Add 25 µL NucleoMag B-Beads and 475 µL Binding Buffer MWA2 and proceed with step 3 of the standard protocol.



Detection of MS2 bacteriophage RNA in wastewater precipitates

Using PEG/NaCl-40 ml wastewater samples were precipitated using PEG/NaCl. MS2 bacteriophage RNA was spiked into the resuspended precipitates in a dilution series (n = 2 for each dilution) and isolated using the NucleoMag® DNA/RNA Water kit (see step-by-step protocol). qRT-PCR analysis was performed with a Taqman® probe for MS2 RNA using the SensiFast™ Probe One-Step Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System. MS2 bacteriophage RNA was detected consistently and reliably over a range of a dilution series with excellent linearity (R^2 = 0.9995).

Ordering Information

Product	Pack of	REF
NucleoMag® DNA/RNA Water	96 preps	744220.1
	384 preps	744220.4
Liquid Proteinase K	5 mL	740396
	30 mL	740396.30
MN Sterilizer CA	50 pieces	740401.50

Method	Reference
Ultrafiltration	Ahmed W, Angel N, Edson J, et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community [published online ahead of print, 2020 Apr 18]. Sci Total Environ. 2020;728:138764. doi:10.1016/j.scitotenv.2020.138764
Ultrafiltration	Medema G, Heijnen L, Elsinga G, Italiaander R, Brouwer A. Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. Environ Sci Technol Lett. 2020;acs.estlett.0c00357. Published 2020 May 20. doi:10.1021/acs.estlett.0c00357
Precipitation	Randazzo W, Truchado P, Cuevas-Ferrando E, Simón P, Allende A, Sánchez G. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area [published online ahead of print, 2020 May 16]. Water Res. 2020;181:115942. doi:10.1016/j.watres.2020.115942
Precipitation	Wu F., Xiao A., Zhang J., Gu X., Lee W.L., Kauffman K., Hanage W., Matus M., Ghaeli N., Endo N., Duvallet C., Moniz K., Erickson T., Chai P., Thompson J., Alm E. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. medRxiv. 2020 doi: 10.1101/2020.04.05.20051540. 2020.04.05.20051540
Review	Kata Farkas, Luke S. Hillary, Shelagh K. Malham, James E. McDonald, David L. Jones, Wastewater and public health: the potential of wastewater surveillance for monitoring COVID-19, Current Opinion in Environmental Science & Health, Volume 17, 2020, Pages 14–20, ISSN 2468-5844, https://doi.org/10.1016/j.coesh.2020.06.001.
Review	Eiji Haramoto, Masaaki Kitajima, Akihiko Hata, Jason R. Torrey, Yoshifumi Masago, Daisuke Sano, Hiroyuki Katayama, A review on recent progress in the detection methods and prevalence of human enteric viruses in water, Water Research, Volume 135, 2018, Pages 168–186, ISSN 0043-1354, https://doi.org/10.1016/j.watres.2018.02.004.

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