Viral nucleic acid isolation from water samples

Methods for sample concentration

Concentration of viral particles are 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. Therefore, we summarize the three most common sample pretreatment recommendations for viral particle concentration from water samples prior nucleic acid isolation.

Filtration and concentration of viral particles using electronegative membranes

Methods for concentrating viral particles on filter membranes have been described in **Ahmed et al. 2020** and other publications. In our previous recommendations we describe in great detail how to extract nucleic acids from those filters (*https://www.mn-net.com/media/pdf/44/7b/8d/SP-Recommendation-viral-nucleic-acid-isolation-from-water.pdf*) using either **NucleoSpin® RNA Stool, NucleoBond® RNA Soil Midi** or **Nucleo-Mag® DNA/RNA Water.** For filter membranes we recommend a bead-based mechanical lysis for optimal results.

Ultrafiltration

This approach for viral concentration has been described by **Ahmed et al. 2020** and **Medema et al. 2020** utilizing ultrafiltration and 100 – 200 mL wastewater samples. Larger particles such as debris or bacteria are removed by centrifugation (e.g. 4600 – 4700 x g, 30 min). It is also possible to use standard filtration methods to remove debris. The supernatant (centrifugation) or cleared sample (standard filtration) is subjected to ultrafiltration (e.g. Centricon® Plus-70 centrifugal ultrafilter units 10 kDa, Merck; 1500 – 3500 x g for 15 min). Subsequently, nucleic acids can be isolated from the recovered, liquid concentrate using either **NucleoSpin® RNA Stool** or **NucleoMag® DNA/RNA Water** following the standard protocols. A mechanical sample lysis can be omitted. Because of the high concentration of PCR inhibitors after ultracentrifugation we don't recommend to use **NucleoSpin® RNA Virus**.

Precipitation

Different methods can be used for precipitating viral particles. R**andazzo et al. 2020** describe an aluminum hydroxide adsorption-precipitation basedmethod, **Wu et al. 2020** a PEG-precipitation based approach. Precipitation methods result in a pellet that can be used for further extraction of nucleic acids. To ensure efficient removal of PCR inhibitors we recommend **NucleoSpin® RNA Stool** or **NucleoMag® DNA/RNA Water** for nucleic acid extraction although **NucleoSpin® RNA Virus Kit** has been successfully used in combination with precipitation by **Randazzo et al. 2020**.

Ordering information

Kit	REF	Pack of
NucleoSpin® RNA Stool	740130.50	50 preps
NucleoMag® DNA/RNA Water (standard protocol)	744220.1	96 preps
	744220.4	384 preps

Method	Reference
Electronegative membrane 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

Management System EN ISO 13485:2016 ISO 9001:2015
TÜVRheinland CERTIFIED
www.tuv.com ID 9000008338

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11
52355 Düren · Germany

DE Tel.: +49 24 21 969-0 info@mn-net.com
CH Tel.: +41 62 388 55 00 sales-ch@mn-net.com
FR Tel.: +33 388 68 22 68 sales-fr@mn-net.com
US Tel.: +1 888 321 62 24 sales-us@mn-net.com