MACHEREY-NAGEL

SARS-CoV-2 extraction from wastewater concentrated with Nanotrap® Magnetic Virus Particles

Automatable concentration and purification of viral RNA from wastewater

Introduction

Many researchers, private companies, and public health agencies are utilizing wastewater testing to monitor COVID-19 infections in selected communities, campus dormitories, or health centers. Wastewater surveillance efforts can be used to look for early warning signs of an upcoming rise in infection rates, and the methods employed by these researchers can be used to estimate numbers of infected individuals who may or may not be symptomatic. Routine wastewater surveillance may prove to be an efficient, non-invasive tool to detect not only COVID-19 infections due to the SARS-CoV-2 virus, but other types of future viral outbreaks as well.

Viral RNA extraction from wastewater is a major challenge for laboratories due to the presence of so many substances that cause inhibition of downstream analysis, and because large volumes of wastewater are often needed for detecting the presence of the SARS-CoV-2 virus. Commonly used wastewater concentration methods tend to exacerbate the problem since they also usually concentrate the inhibitors as well. Many concentration techniques are also labor intensive and costly.

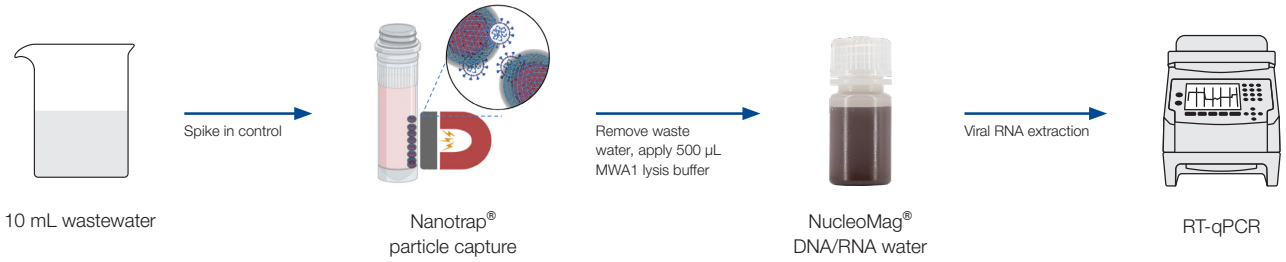
In this application note we show an efficient, cost-effective, and even automatable method to extract SARS-CoV-2 viral RNA from complex wastewater samples by pairing MACHEREY-NAGEL's NucleoMag® DNA/RNA Water kit for extraction with the Nanotrap® Magnetic Virus Particles from Ceres Nanosciences for wastewater concentration

Products at a glance

NucleoMag® DNA/RNA Water	
Technology	Magnetic bead technology
Sample material	150 µL Nanotrap® particles
Preparation time	40–120 min/96 preps (excl. lysis and sample concentration)
Fragment size	300 bp–approx. 50 kbp
Elution volume	50–250 µL

Nanotrap® Magnetic Virus Particles	
Technology	Hydrogel beads with viral capture technology
Sample Volume	Up to 40 mL of wastewater
Magnetized	For use with Dynamag® rack or KingFisher® Flex/Apex platform
Amount per sample	150 µL
Time/prep	Approximately 25–58 minutes/24 preps (incl. lysis and sample concentration)

Workflow Overview

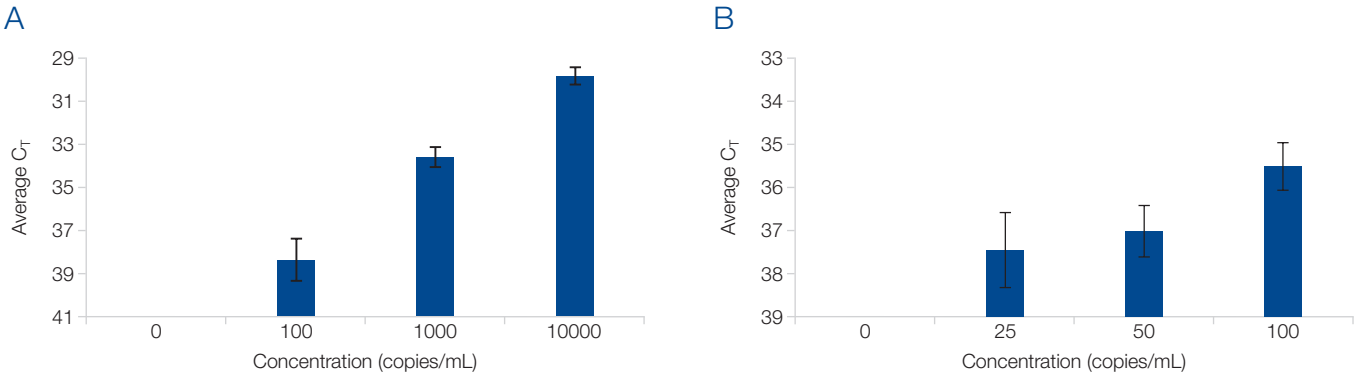


Material and Methods

The following step by step protocol describes the manual viral concentration and RNA extraction procedure for a 10 mL wastewater sample, though the procedure is scalable for alternative volumes.

Nanotrap® concentration procedure		
1	Capture and Concentrate Virus	Add 150 µL of Nanotrap® Magnetic Virus Particles to 10 mL sample. Invert sample 5 times to mix and incubate for 10 minutes at room temperature.
2	Collect Nanotrap® particles	Place samples on magnetic rack and allow the Nanotrap® particles to come out of solution.
3	Wash	Remove the supernatant and wash particles with 1 mL of 0.05 % Tween-20 in PCR-grade water. Transfer sample to fresh 1.5 mL tube.
4	Lysis	Pellet Nanotrap® particles. Remove the supernatant. Resuspend pellet in 500 µL MWA 1. Incubate at room temperature for 10 minutes.
5	Extraction	Pull Nanotrap® particles out of solution with a magnetic rack. Proceed with step 3 of the NucleoMag® DNA/RNA Water protocol using 450 µL of lysate.

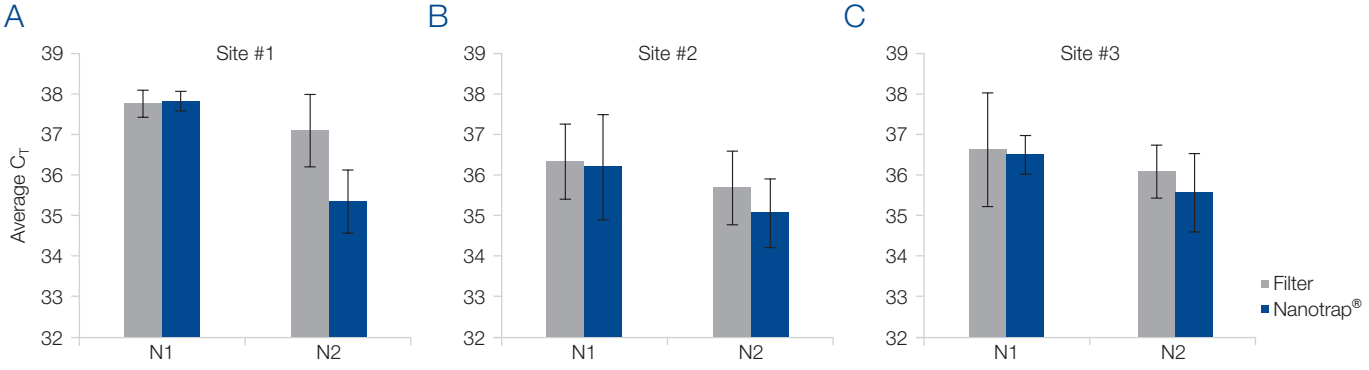
Application data



Limit of Detection (LoD) Range-Finding Study

Heat inactivated SARS-CoV-2 was spiked into wastewater that tested negative for SARS-CoV-2 at concentration ranges of 0–10,000 copies/mL (A) and subsequently 0–100 copies/mL (B).

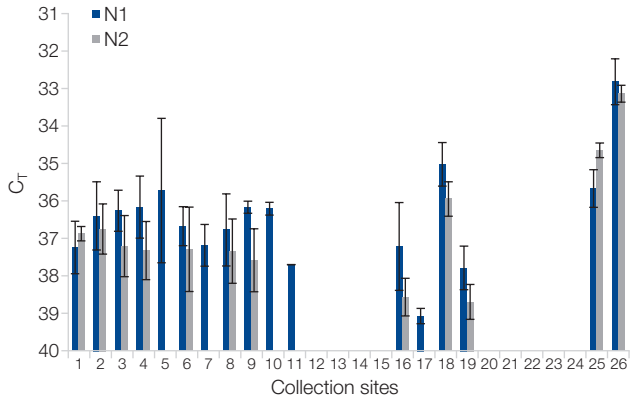
Samples were processed using Ceres Nanosciences' Nanotrap® particles with the MACHEREY-NAGEL NucleoMag® DNA/RNA Water kit on the Kingfisher Apex. Viral detection was achieved using the 2019-nCoV CDC EUA assay (A) or the Promega SARS-CoV-2 Wastewater RT-qPCR Kit (B) with a N1 primer/probe system. LoD was determined when 95 % of all biological replicates returned positive values. N=3 (A) and n=20 (B). LoD was determined to be 50 copies/mL. While detection was substantial at 25 copies/mL, < 95 % of biological replicates returned positives values therefore data was not statistically significant at this level.



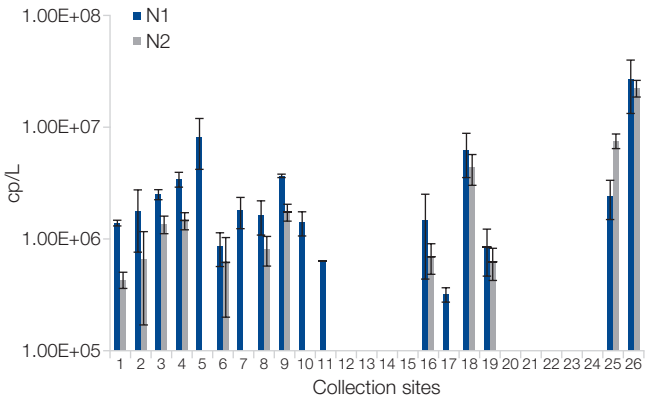
Comparison of viral concentration methods: HA Filter vs. Nanotrap® particles

10 mL of wastewater was processed using either Ceres Nanosciences' Nanotrap® particles or HA filtration. Viral RNA was then extracted and purified from all samples using the MACHEREY-NAGEL NucleoMag® DNA/RNA Water kit on the KingFisher Apex. Viral detection was achieved using the Promega SARS-CoV-2 Wastewater RT-qPCR Kit for both N1 and N2 primers. Samples were from two wastewater treatment plants in Seattle, WA (A, B) and another in Storrs, CT (C).

A



B



Detection of SARS-CoV-2 in wastewater samples in four geographic regions of the United States

10 mL of wastewater was concentrated using Ceres Nanosciences' Nanotrap® particles, and viral RNA was extracted using the MACHEREY-NAGEL NucleoMag® DNA/RNA Water kit on the KingFisher Apex. Viral detection was achieved using the Promega SARS-CoV-2 Wastewater RT-qPCR Kit for both N1 and N2 primers. Samples were from four geographic areas: Seattle, WA (1–9), San Bernardino, CA (10–15), Storrs, C_T (16–19), or Los Angeles, CA (20–26). Detection as C_T is seen in (A). In (B), the amount of virus in each sample was quantified and normalized to a BCoV spike-in control.

Automatable viral nucleic acid extraction from wastewater samples with convenient handling

Combine Ceres Nanosciences' convenient sample concentration tool, Nanotrap® Magnetic Virus Particles, with MACHEREY-NAGEL's proven nucleic acid extraction procedures for reliable and even automatable viral RNA concentration and purification from wastewater samples.

- Reliable performance with excellent removal of PCR inhibitors
- Workflow is automatable on a Kingfisher® Flex or Apex platform with a 24-well head.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® DNA/RNA Water	Magnetic bead-based isolation of DNA and RNA from water and air samples	96 preps 384 preps	744220.1 744220.4
NucleoMag® SEP Mini	Magnetic separator, for use with 12 x 1.5 mL or 2 mL reaction tubes	1 piece	744901
NucleoMag® SEP	Magnetic separator, for use with 96-well plates	1 piece	744900
Nanotrap® Magnetic Virus Particles *	Magnetically functionalized particles to capture and concentrate viruses	10 mL or 30 mL	44202

NucleoMag® is a registered trademark of MACHEREY-NAGEL; Nanotrap® is a trademark of Ceres Nanosciences *.
* For more detailed information, please visit https://www.ceresnano.com/