MACHEREY-NAGEL

COVID-19 Wastewater Surveillance:
Solutions for Successful SARS-CoV-2 Viral RNA Extraction

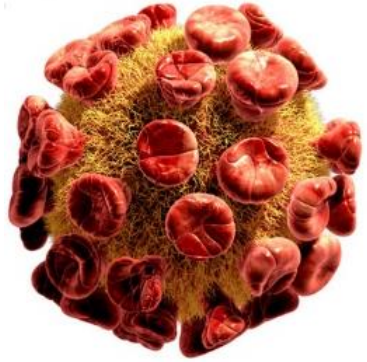
NEMC 2021 – SARS CoV-2 Wastewater Testing - State of the Science

Cynthia Ripoll, PhD, 8/11/2021

MACHEREY-NAGEL - Bioanalysis

MACHEREY-NAGEL (MN) Mission

- Make your nucleic acid purification easy, fast, affordable and reliable





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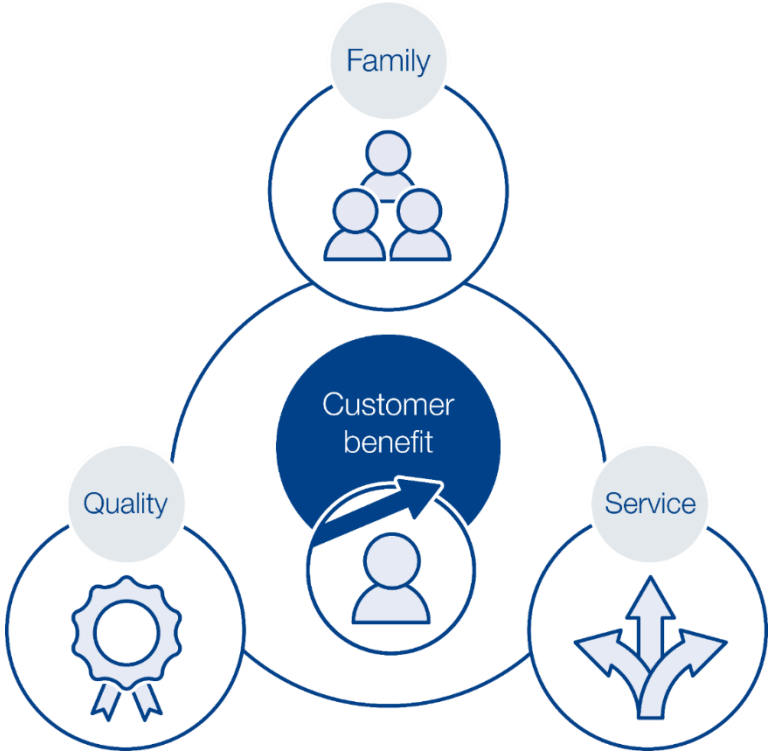
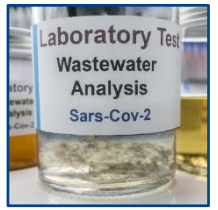


MACHEREY-NAGEL - Bioanalysis

MN Advantage

- Certified quality: products made in Germany
- Thousands of customers worldwide trust in our solutions
- Over 70,000 research publications citing our products
- Highly skilled team with advanced degrees in various biological backgrounds to support our customers

Wastewater Surveillance for SARS-CoV-2



nature sustainability

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Review Article | Published: 19 August 2020

Rethinking wastewater risks and monitoring in light of the COVID-19 pandemic

Anne Bogler, Aaron Packman, [...]Edo Bar-Zeev

Nature Sustainability **3**, 981–990 (2020) | Cite this article

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Letter | Published: 18 September 2020

Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics

Jordan Peccia, Alessandro Zulli, Doug E. Brackney, Nathan D. Grubaugh, Edward H. Kaplan, Arnau Casanovas-Massana, Albert I. Ko, Amyn A. Malik, Dennis Wang, Mike Wang, Joshua L. Warren, Daniel M. Weinberger, Wyatt Arnold & Saad B. Omer

Nature Biotechnology **38**, 1164–1167 (2020) | Cite this article

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NEWS | 10 May 2021

The myriad ways sewage surveillance is helping fight COVID around the world

Wastewater tracking was used before the pandemic to monitor for polio and illicit drug use, but interest in the field and its applications has now ballooned.

scientific reports

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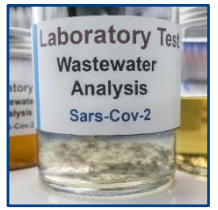
Article | Open Access | Published: 08 March 2021

Long-term monitoring of SARS-CoV-2 RNA in wastewater of the Frankfurt metropolitan area in Southern Germany

Shelesh Agrawal, Laura Orschler & Susanne Lackner

Scientific Reports **11**, Article number: 5372 (2021) | Cite this article

Wastewater Surveillance for SARS-CoV-2



COVID19Poops Dashboard

https://www.covid19wbec.org/covidpoops19

Dashboards
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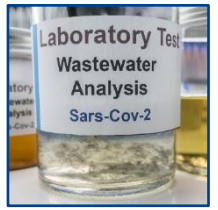
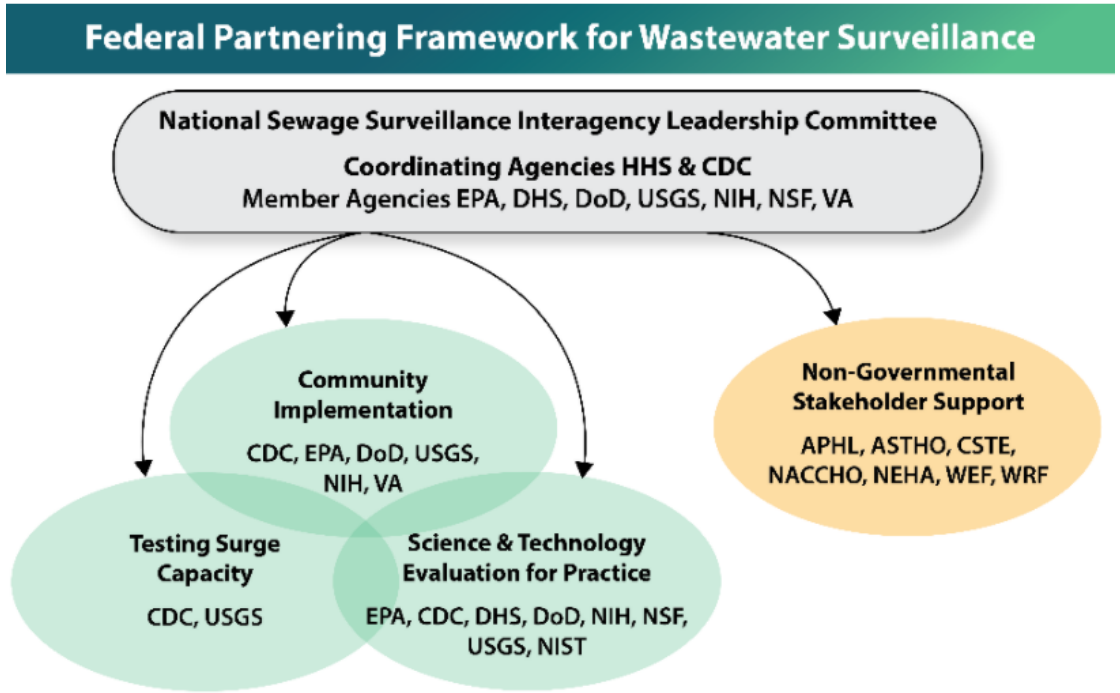
Wastewater Surveillance for SARS-CoV-2



- CDC partners with multiple agencies for National Wastewater Surveillance
  - Dept of Health & Human Services
  - Environmental Protection Agency
  - Dept of Defense
  - National Institutes of Health
  - National Science Foundation
  - US Geological Society
  - Non-governmental agencies



https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/federal-coordination-partnering-wastewater-surveillance.html

- Goal - collect, analyze, and integrate wastewater-based COVID-19 data with COVID-19 case data to assist state and local partners making response decisions.

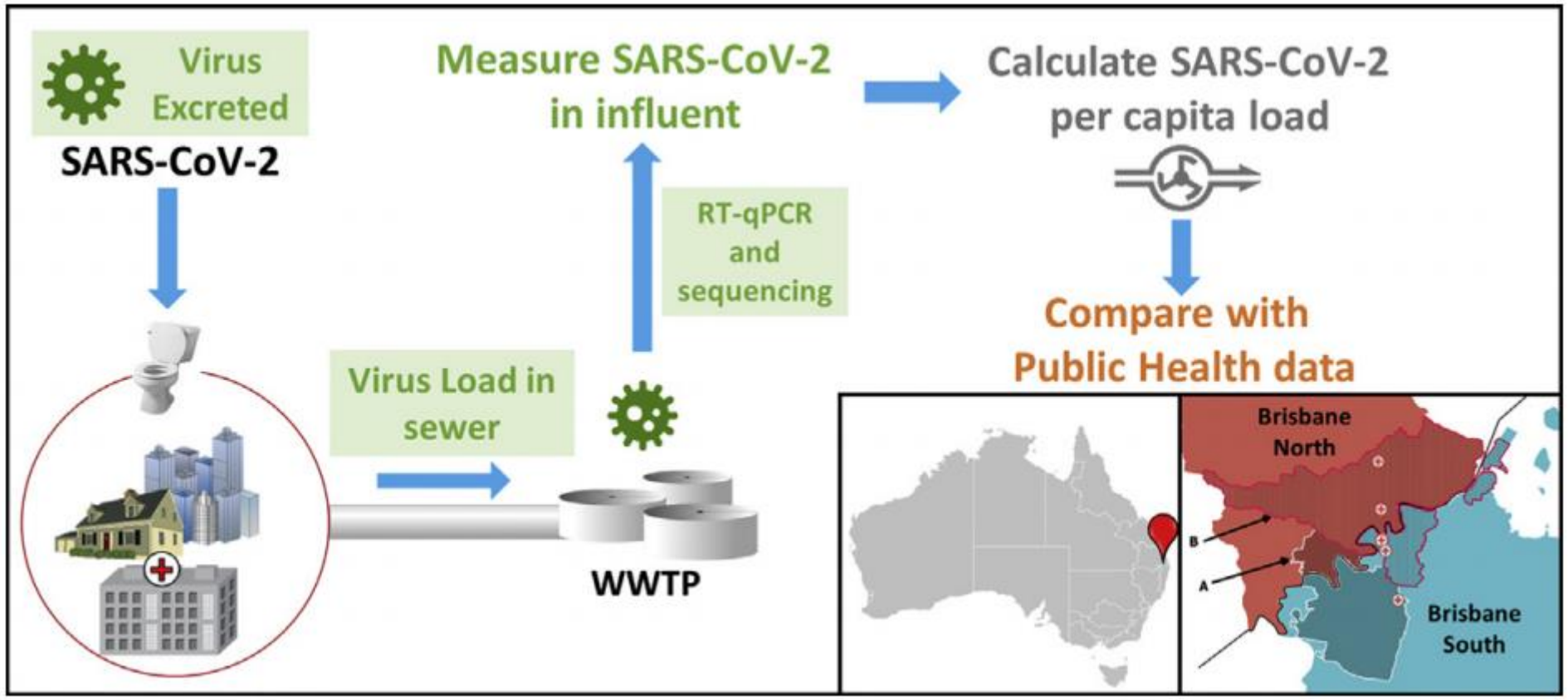
Detection of SARS-CoV-2 in wastewater samples

Why test wastewater for SARS-CoV-2?

- Time and cost saving compared to testing individuals
- Early warning sign for new disease outbreaks
- Estimation of infection numbers in communities where not all individuals can be tested
- Wastewater testing can account for people with mild or no symptoms that are not tested
- Established method to non-invasively monitor Norovirus, poliovirus, or antibiotic resistance

Detection of SARS-CoV-2 in wastewater samples

Basic principle



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

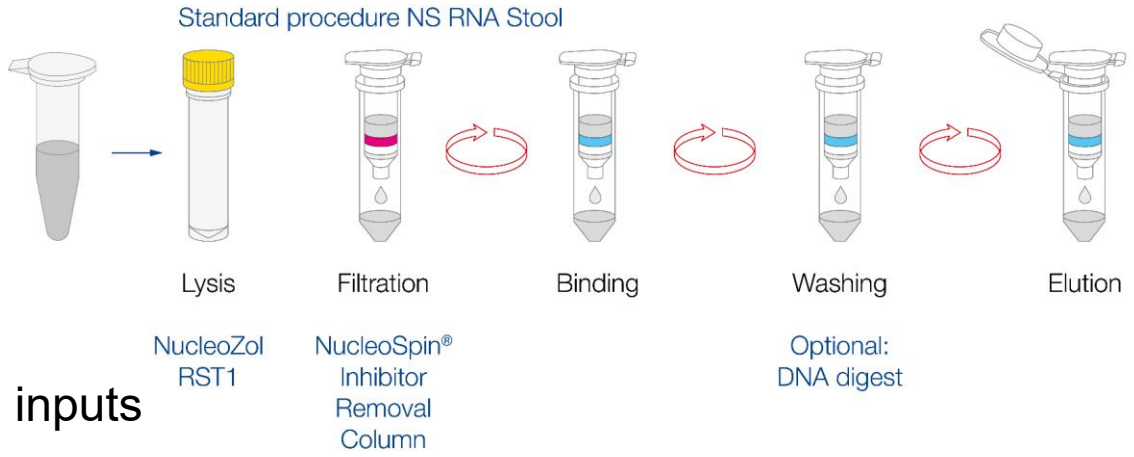
Detection of SARS-CoV-2 in wastewater samples

Challenges

SARS-CoV-2 viral RNA extraction methods

Silica Membrane Technology

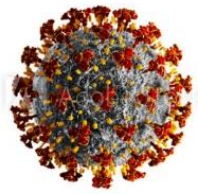
- NucleoSpin RNA Stool
  - Wastewater concentrates or sludge
  - Inhibitor Removal Technology
- NucleoSpin RNA Virus
  - Standard viral RNA extraction from “clean” inputs



SARS-CoV-2 related publications from wastewater

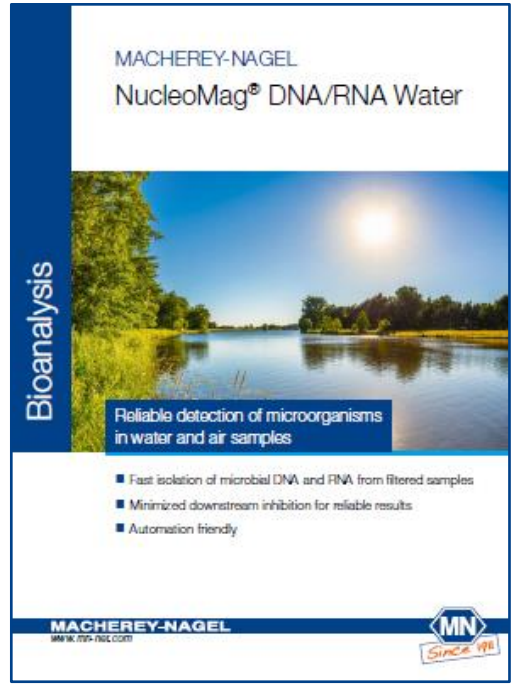
Featured Product	Application	Author	Article	Year	Journal
NucleoSpin® RNA Virus	Viral RNA isolation from wastewater	Yaniv et al.	City-level SARS-CoV-2 sewage surveillance	2020	MedRxiv
	Viral RNA isolation from wastewater	Alygizakis et al	Analytical methodologies for the detection of SARS-CoV-2 in wastewater: Protocols and future perspectives	2020	TrAC Trends in Analytical Chemistry
	Viral RNA isolation from wastewater	Cuevas-Ferrando et al.	Recovering coronavirus from large volumes of water	2020	Science of the total environment
	Viral RNA isolation from wastewater	Ali et al.	Tracking SARS-CoV-2 RNA through the wastewater treatment process	2020	MedRxiv
	Viral RNA isolation from wastewater	Patel et al.	Coronavirus (SARS-CoV-2) in the environment: Occurrence, persistence, analysis in aquatic systems and possible management	2020	Science of the total environment
	Viral RNA isolation from wastewater	Colosi et al.	Development of wastewater pooled surveillance of SARS-CoV-2 from congregate living settings	2020	MedRxiv
	Viral RNA isolation from wastewater	Westhaus et al.	Detection of SARS-CoV-2 in raw and treated wastewater in Germany- suitable for COVID-19 surveillance and potential transmission risk	2020	Science of the Total Environment
	Viral RNA isolation from wastewater	Randazzo et al.	SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area	2020	Water Research
	Viral RNA isolation from wastewater	Kumar et al.	First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2	2020	Science of the Total Environment
	Viral RNA isolation from wastewater	Trottier et al.	Post-lockdown detection of SARS-CoV-2 RNA in the wastewater of Montpellier, France	2020	One Health

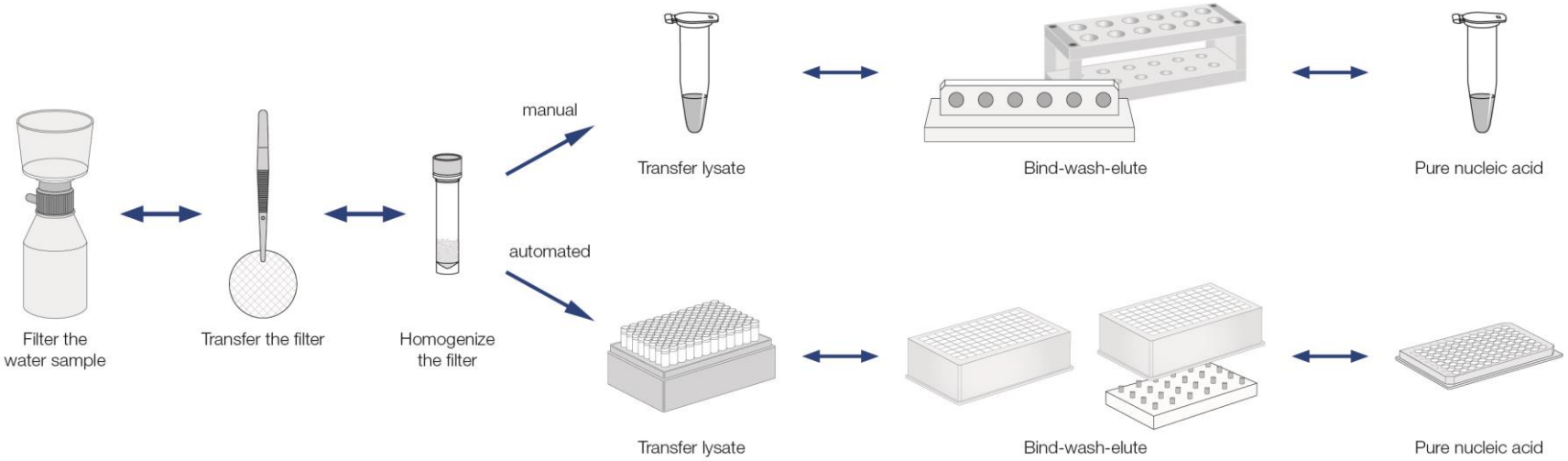
SARS-CoV-2 viral RNA extraction methods



Magnetic Bead Technology

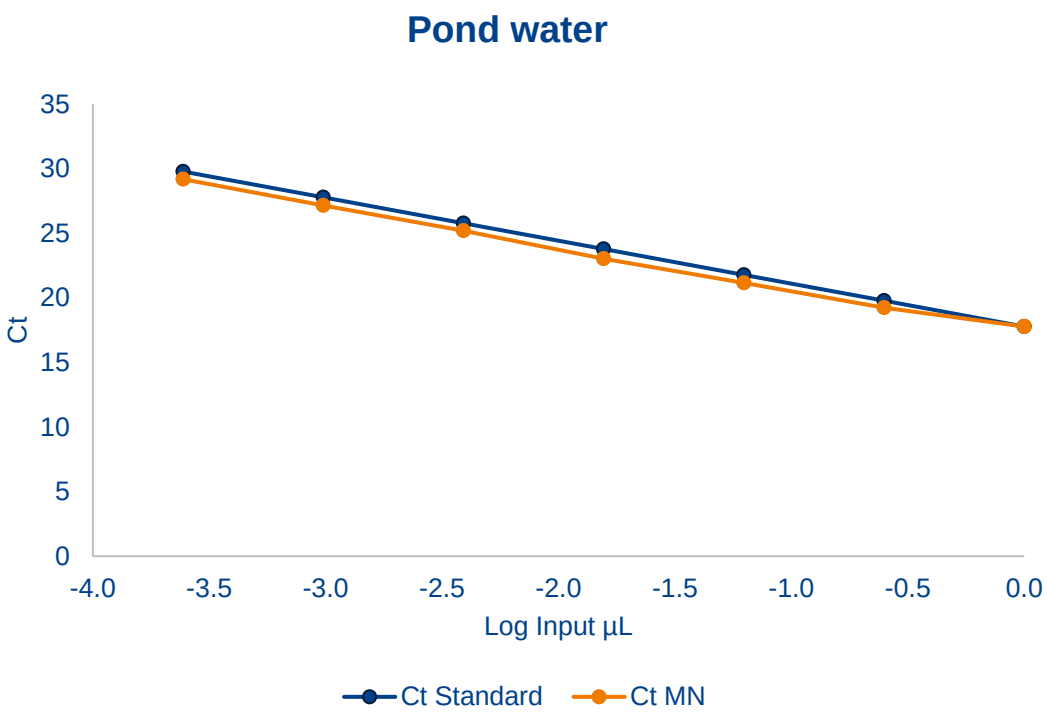
- NucleoMag® DNA/RNA Water
  - Manual or automated extraction of DNA and/or RNA from water or air (filtered)
  - Scalable, flexible, no column clogging
  - Inhibitor removal technology
  - Compatible with multiple wastewater concentration techniques

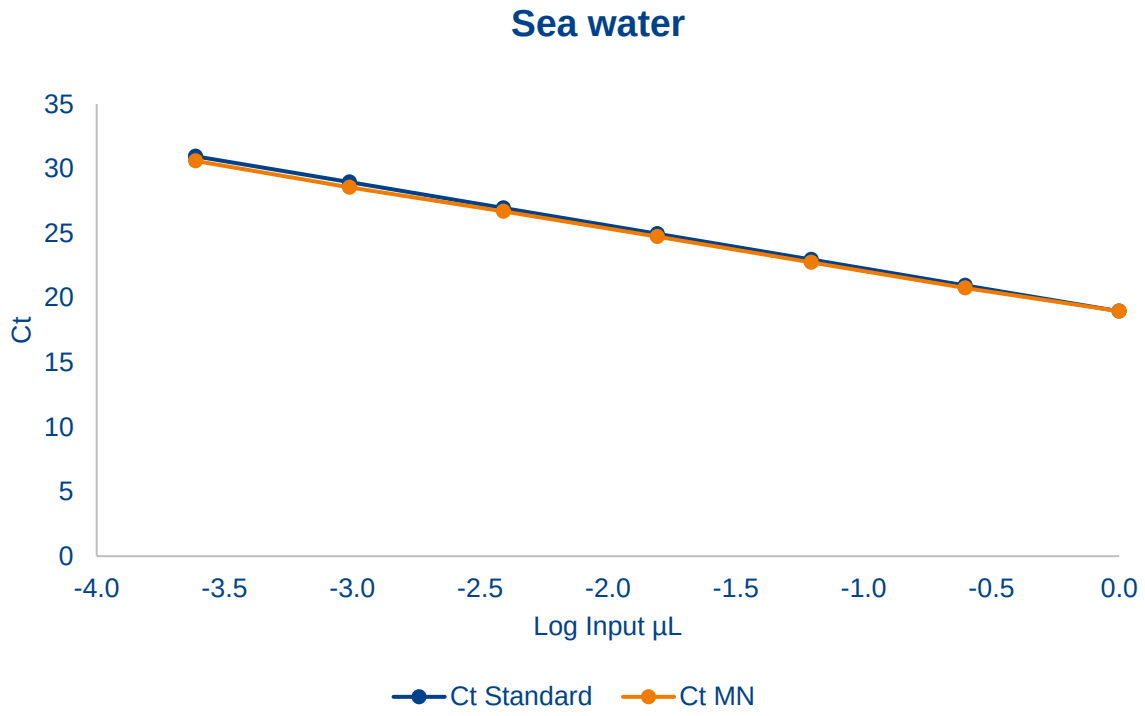




NucleoMag® DNA/RNA Water

Minimized downstream inhibition

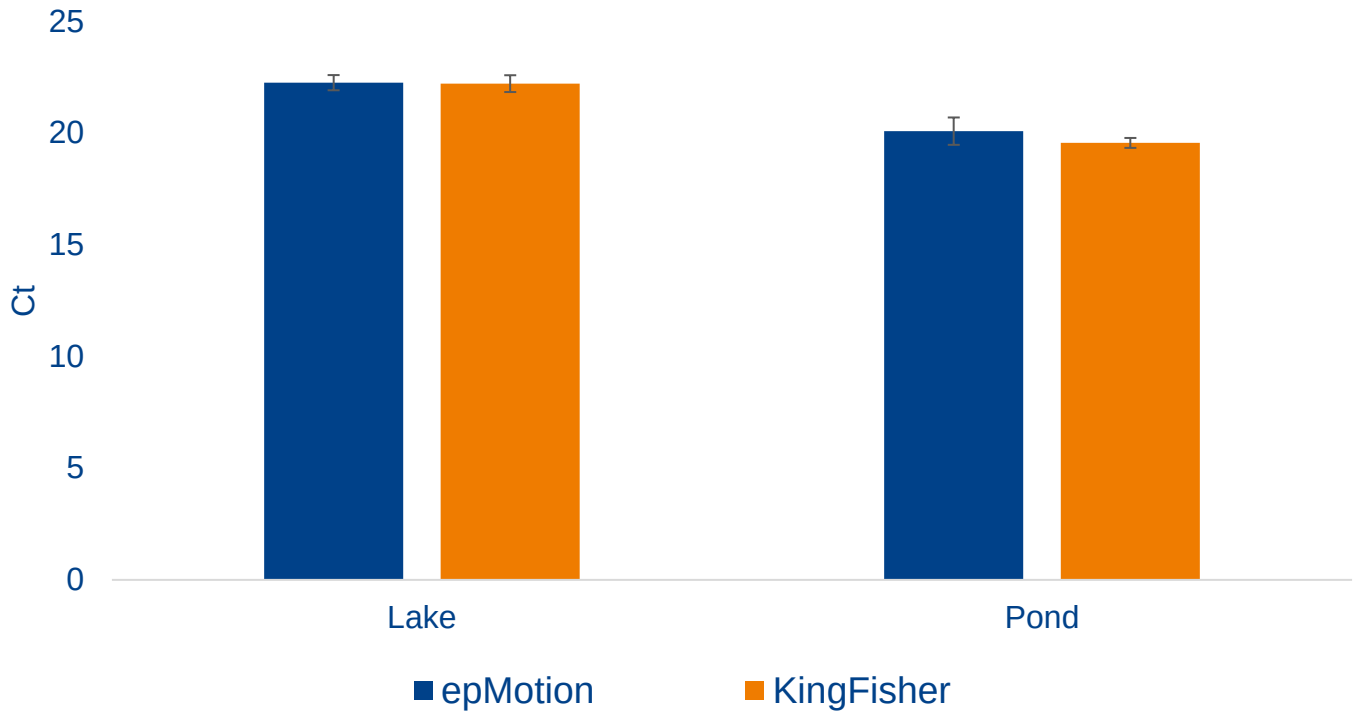




No PCR inhibition detectable with multiple sample types

NucleoMag® DNA/RNA Water

HTP-friendly

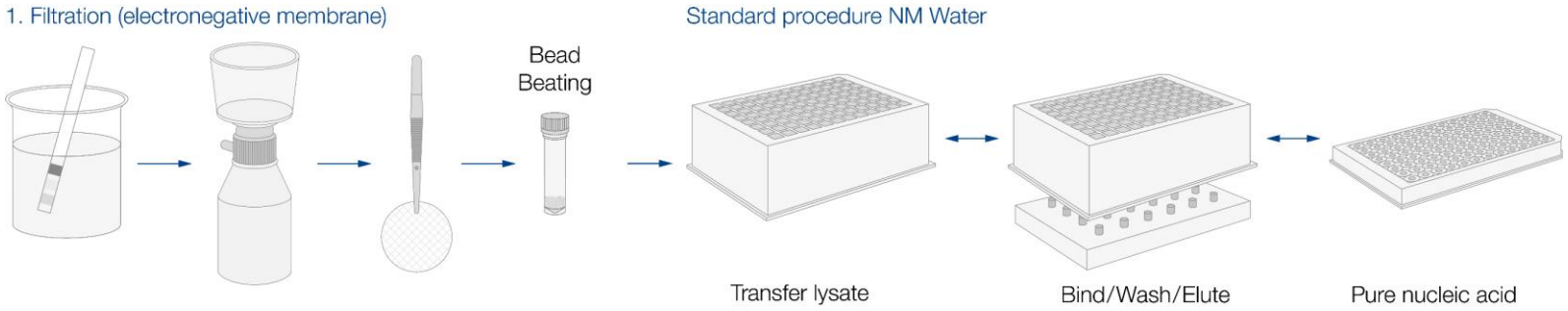


Compatible with multiple automation platforms

Sample preparation and concentration techniques

Filtration with electronegative membrane (Ahmed et. al 2015)

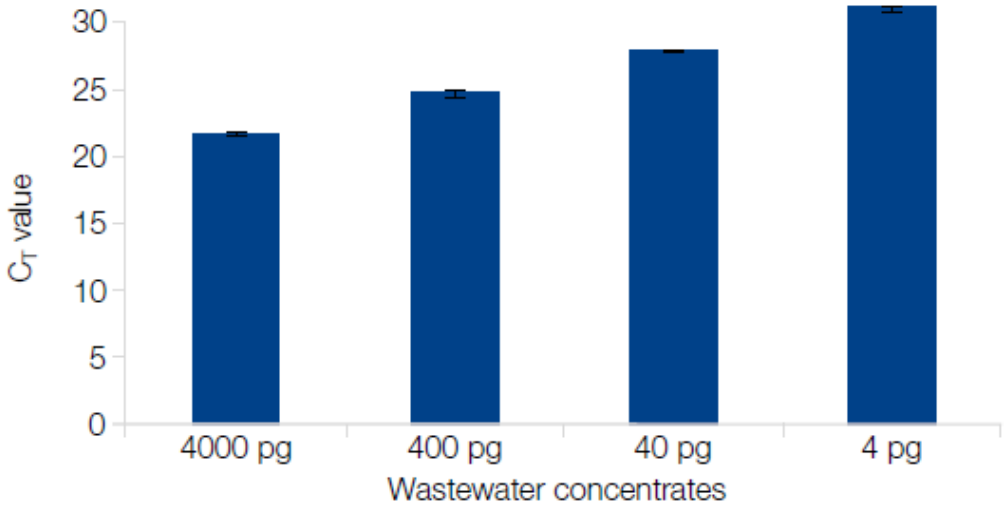
- Collect water and remove debris by sedimentation
- Adjust pH of water to 3.5 with HCL (positive net charge of viral particles)
- Filter samples: negatively charged 0.45 µM mixed cellulose ester membrane
- Extract SARS-CoV-2 RNA from filter with the MACHEREY-NAGEL NucleoMag® RNA/DNA Water extraction kit



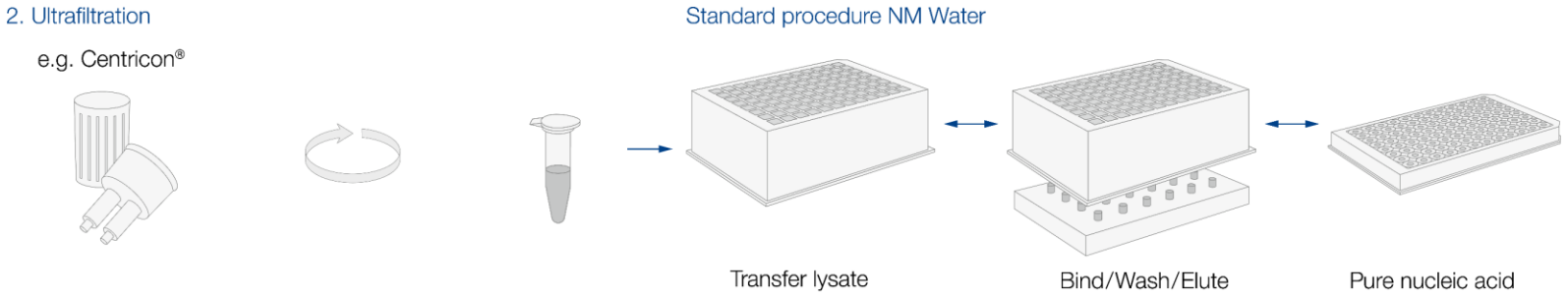
Sample preparation and concentration techniques

Ultrafiltration

- Remove larger debris from 40 ml wastewater by centrifugation (4,600-4,700 x g for 30 min)
- Subject supernatant to ultrafiltration, i.e. using Centricon® Plus-70 (Merck)
- Extract SARS-CoV-2 RNA from recovered liquid concentrate (100-200 µl) with the MACHEREY-NAGEL NucleoMag® RNA/DNA Water extraction kit



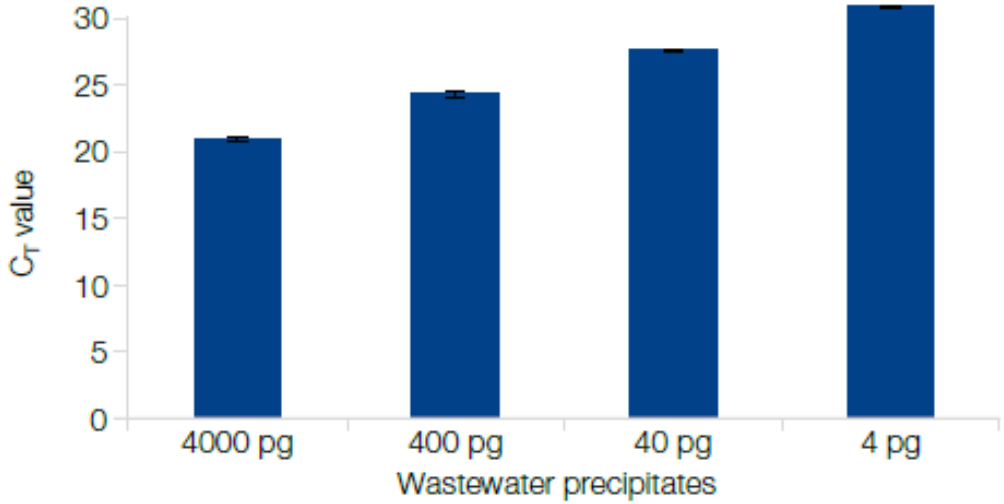
Detection of MS2 bacteriophage RNA in wastewater concentrates



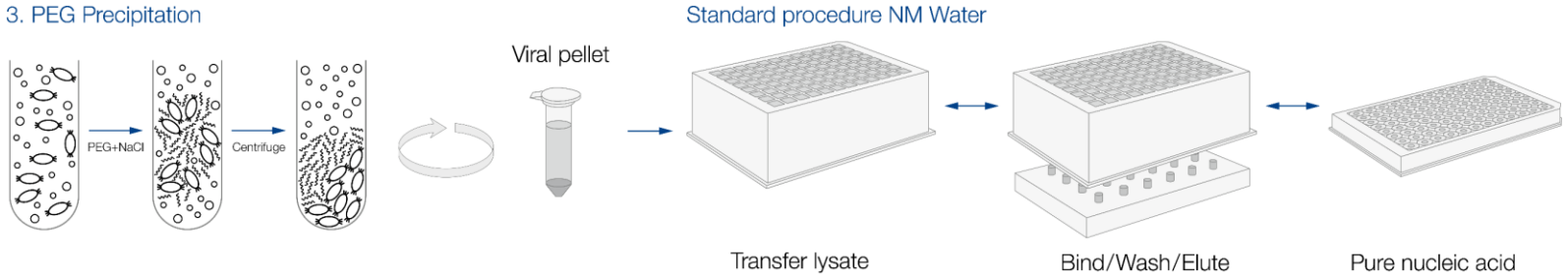
Sample preparation and concentration techniques

PEG Precipitation

- Filter raw sewage through 0.2 µM membrane to remove debris
- Add PEG and NaCl to 40 mL filtrate
- Centrifuge at 12,000 x g for 2 h
- Resuspend the viral pellet and use as input for the MACHEREY-NAGEL NucleoMag® RNA/DNA Water extraction kit



Detection of MS2 bacteriophage RNA in wastewater precipitates



Science of The Total Environment

Available online 29 July 2021, 149298

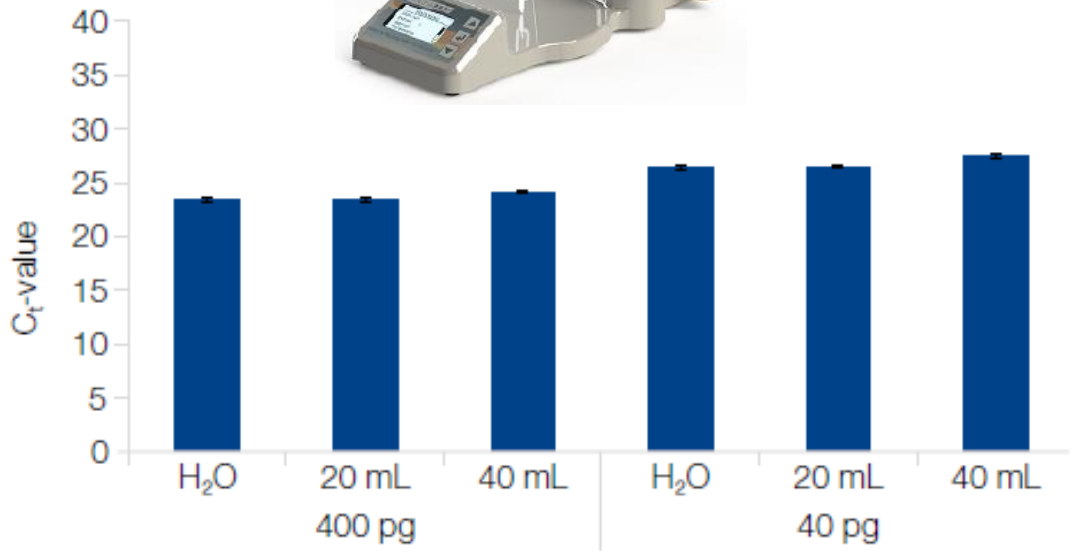
Systematic assessment of SARS-CoV-2 virus in wastewater, rivers and drinking water – A catchment-wide appraisal

Vhahangwele Masindi [a, b] ✉, Spyros Foteinis [c], Kefilwe Nduli [a], Vhahangwele Akinwekomi [a]

Sample preparation and concentration techniques

INNOVAPREP CP-Select™ concentrator pipet

- Filter raw sewage through 0.2 µM membrane to remove debris
- Concentrate 20 - 40 mL filtrate
- Extract viral RNA from 200 ul concentrate with the MACHEREY-NAGEL NucleoMag® RNA/DNA Water extraction kit
- Extraction is automatable on various compatible platforms



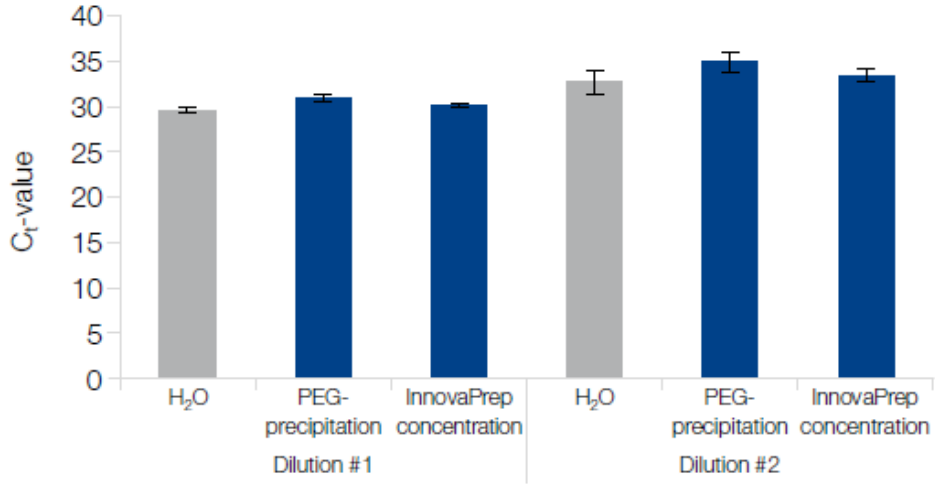
NucleoMag® DNA/RNA Water allows reliable, inhibition-free qPCR detection of MS2 phage RNA from wastewater concentrates.

Sample preparation and concentration techniques

INNOVAPREP CP-Select™ concentrator pipet with Nimbus Presto

- New application note for NucleoMag DNA/RNA Water on the Hamilton Nimbus-Presto workstation

NIMBUS Presto workstation	
Technology	Automated liquid handling platform (Hamilton NIMBUS) with integrated magnetic rod processing unit (KingFisher™ Presto)
Capacity	1–96 samples (≤ 200 µL sample volume
Processable volume	50–5000 µL

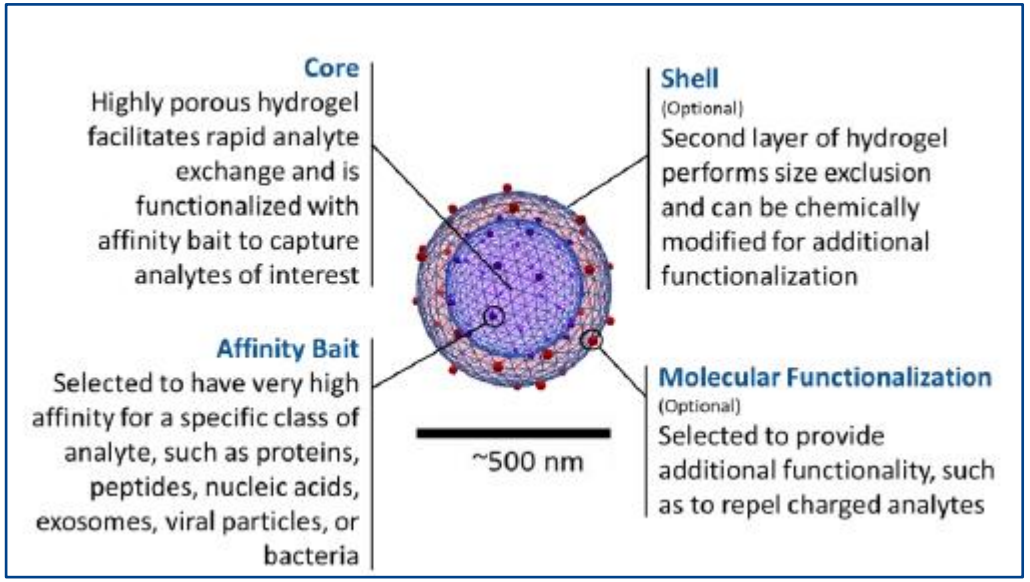


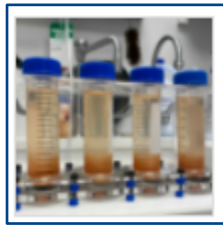
Armored Enterovirus RNA recovery from synthetic wastewater

Sample preparation and concentration techniques

Nanotrap® Magnetic Virus Particles

- Affinity dye captures intact viral particles
- Hydrogel structure enables rapid viral binding
- Magnetically functionalized for easy isolation
- Apply lysis buffer from the MACHEREY-NAGEL NucleoMag® RNA/DNA Water kit and follow standard extraction protocol





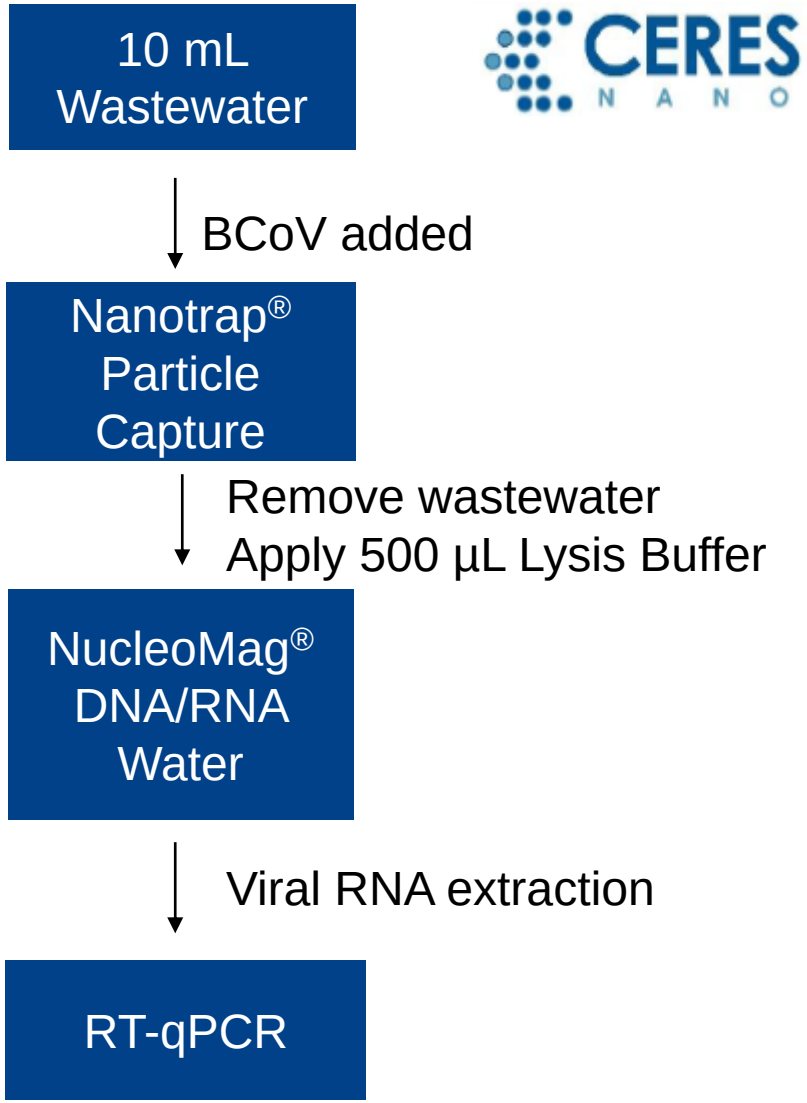
SARS-CoV-2 Wastewater RNA Concentration and Extraction (Nanotrap® and NucleoMag® RNA Water)

Brett Rasile[1], Kendra Maas[1]
[1]University of Connecticut

Sample preparation and concentration techniques

Nanotrap® Magnetic Virus Particles

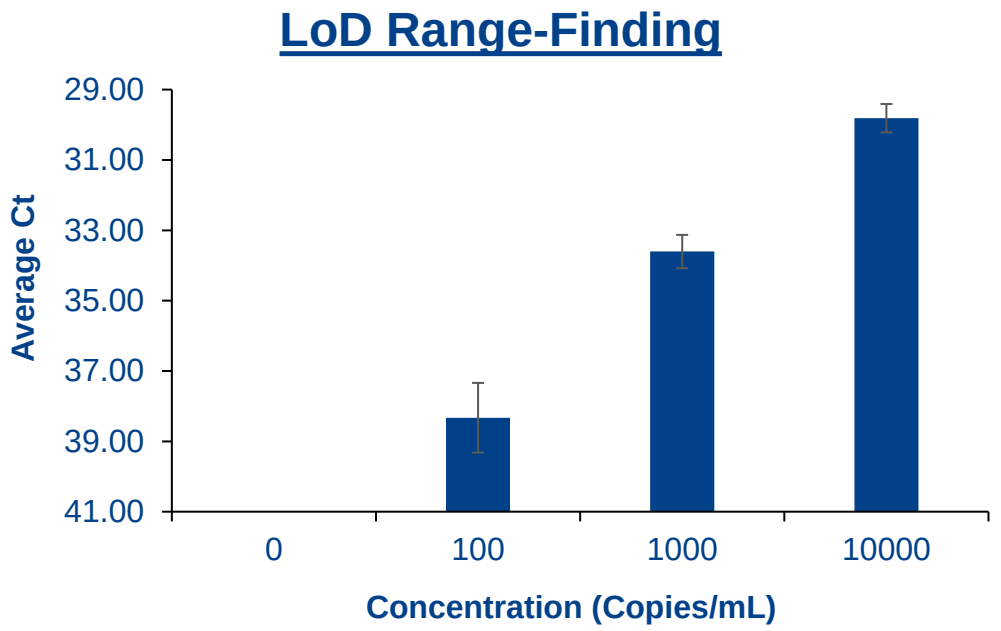
- Simple wastewater concentration and viral RNA extraction techniques combined in one workflow
- Excellent sensitivity from 10 ml wastewater
- Enhanced inhibitor removal
- Manual or automatable
  - Process up to 96 samples from raw sewage to PCR analysis in 4.5 hours on a KingFisher Apex



Sample preparation and concentration techniques

Nanotrap® Magnetic Virus Particles

- Heat inactivated SARS-CoV-2 spiked into 10 ml wastewater
- 150 µL Nanotrap® beads used to concentrate intact viral particles
- NucleoMag® DNA/RNA Water lysis buffer applied to viral particles, then follow standard extraction protocol
- RT-qPCR performed using CDC nCoV-2019 N1 EUA assay

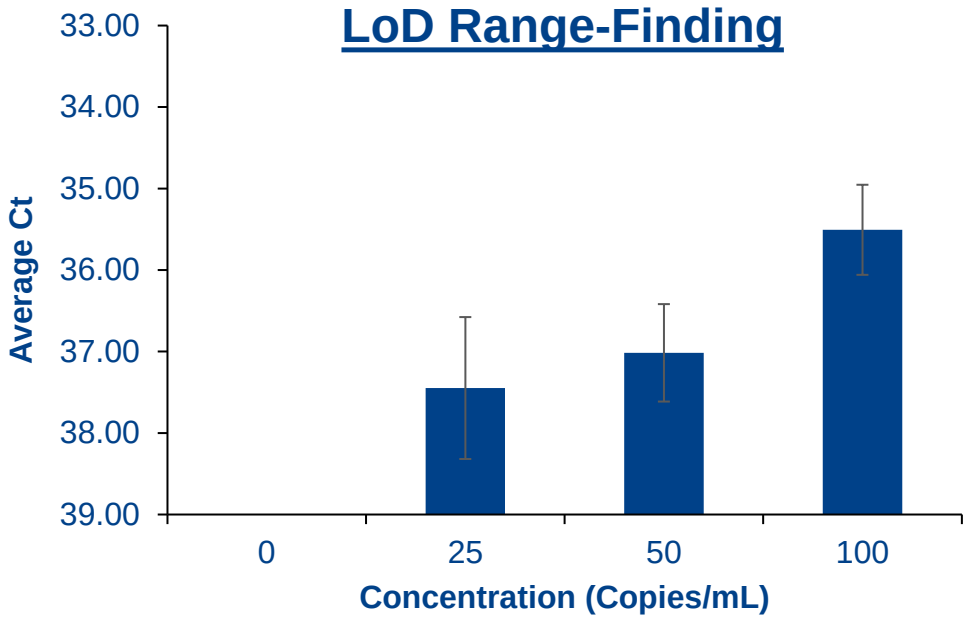


Range-finding study of Nanotrap®/NucleoMag® DNA/RNA Water SARS-CoV-2 Wastewater Concentration and Extraction Method

Sample preparation and concentration techniques

Nanotrap® Magnetic Virus Particles

- Heat inactivated SARS-CoV-2 spiked into 10 ml wastewater
- 150 µL Nanotrap® beads used to concentrate intact viral particles
- NucleoMag® DNA/RNA Water lysis buffer applied to viral particles, then follow standard extraction protocol
- RT-qPCR performed using Promega SARS-CoV-2 Wastewater RT-qPCR Kit for N1
- Limit of Detection ~50 copies/mL
  - SARS-CoV-2 was detected at 25 copies/mL in only 85% of the biological replicates



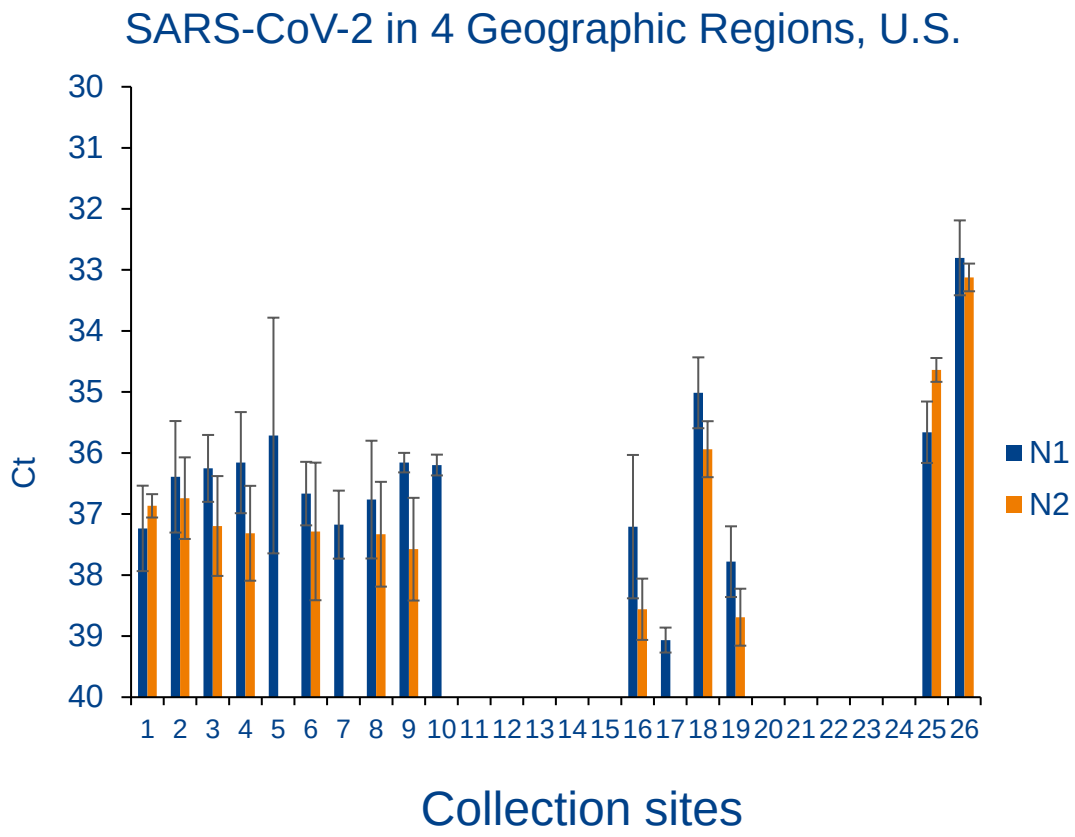
Range-finding study of Nanotrap®/NucleoMag® DNA/RNA Water SARS-CoV-2 Wastewater Concentration and Extraction Method

Sample preparation and concentration techniques

Nanotrap® Magnetic Virus Particles

- Wastewater samples donated from multiple locations analyzed for the presence of SARS-CoV-2
  - Seattle, WA (samples 1-9)
  - San Bernadino, CA (samples 10-15)
  - Storrs, CT (samples 16-19)
  - Los Angeles, CA (samples 20-26)

- Results corresponded to known positives and also revealed positives previously undetected in some collection sites



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Thanks for listening!

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