

# COVID-19 Wastewater Surveillance:

## Scalable Solutions for Detection in Low to High Throughput Workflows



MACHEREY-NAGEL Seminar: Case Studies in Wastewater Surveillance

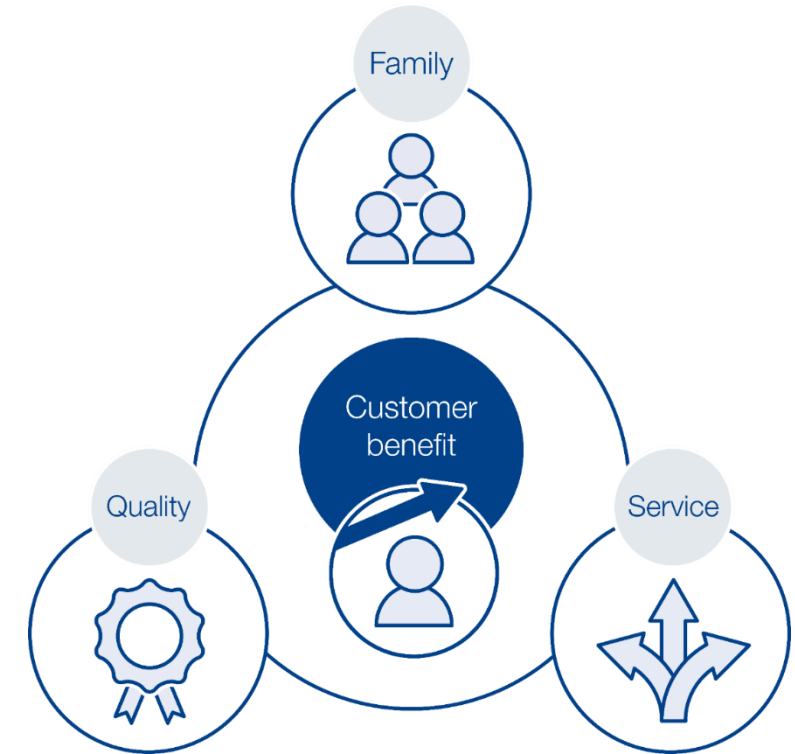
Cynthia Ripoll, PhD

August 2, 2022



# MACHEREY-NAGEL - Bioanalysis

## MACHEREY-NAGEL (MN) Mission



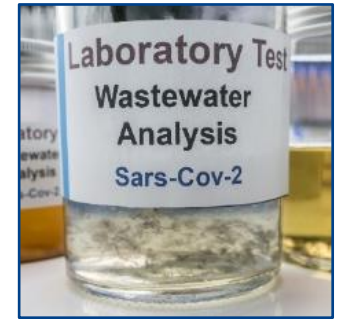
- Nucleic acid purification made easy, fast, affordable and reliable

# 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

## Challenges



# Featured extraction kit: NucleoMag<sup>®</sup> DNA/RNA Water

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Magnetic bead based viral RNA extraction

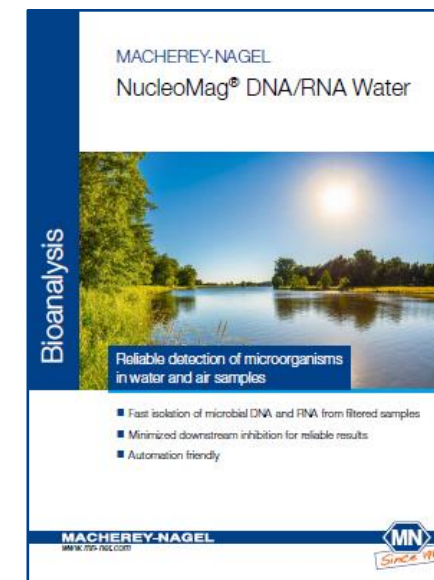




# Wastewater testing methods

## Magnetic Bead Technology

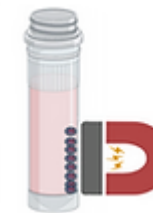
- NucleoMag<sup>®</sup> DNA/RNA Water
  - Manual or automated extraction
  - Scalable and flexible
  - Inhibitor removal technology



## Compatible with multiple wastewater concentration techniques

- Technical notes posted on product webpage
- Today's focus: compatibility with technology from Ceres Nanosciences
  - Nanotrap<sup>®</sup> Magnetic Virus Particles

# Ceres Nanosciences Technology



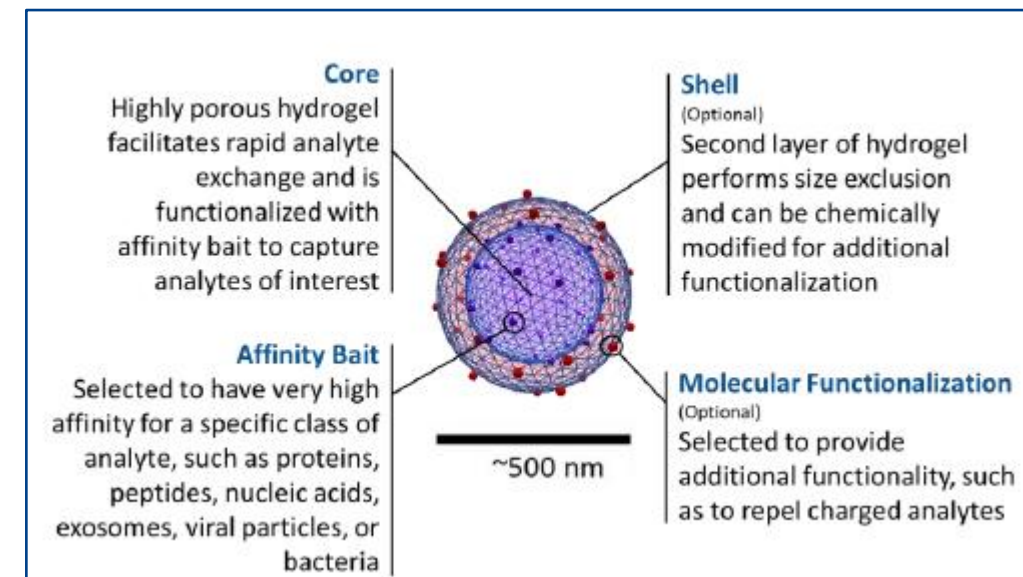
## Nanotrap<sup>®</sup> Magnetic Virus Particles

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

### MACHEREY-NAGEL

SARS-CoV-2 extraction from wastewater concentrated with Nanotrap<sup>®</sup> Magnetic Virus Particles

Automatable concentration and purification of viral RNA from wastewater



Application Note: Nanotrap<sup>®</sup> + ddPCR Assay in Wastewater



Nanotrap<sup>®</sup> Particles are Compatible with RT-ddPCR Assay for SARS-CoV-2 Variant Detection in Wastewater

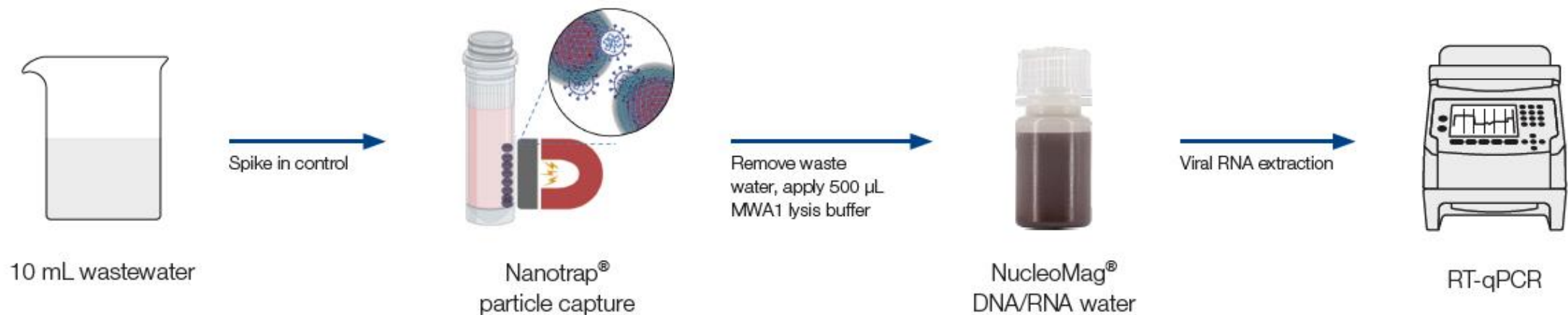
# Workflow overview



## Nanotrap<sup>®</sup> Magnetic Virus Particles



- Simple pathogen concentration and viral RNA extraction combined in one workflow
- Manual or automatable
  - Process up to 96 samples from raw sewage to PCR analysis in 4.5 hours on a KingFisher Apex





# Method comparison – University of Washington

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Data courtesy of:

Sarah Philo, PhD Candidate

Scott Meschke, PhD

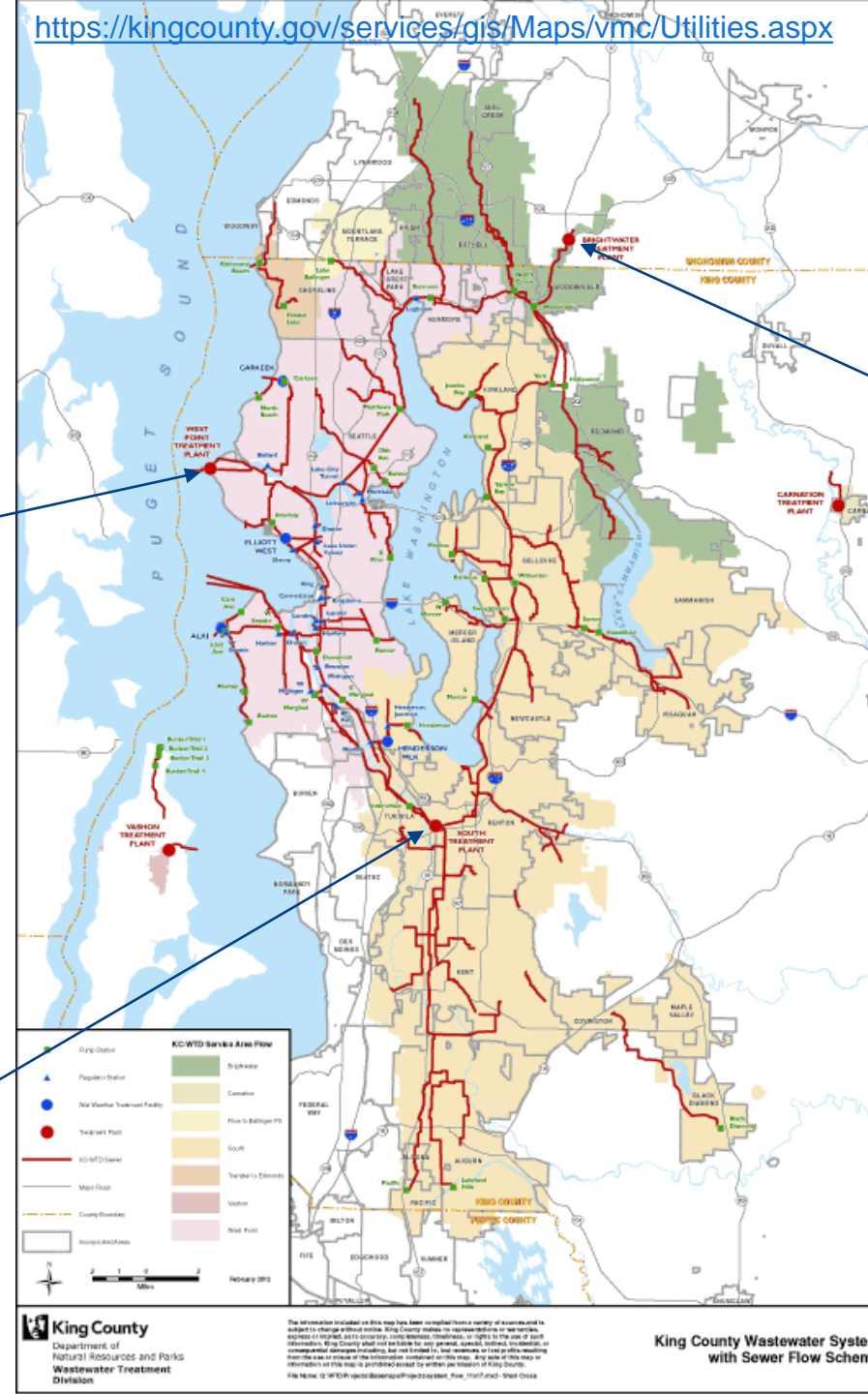


# Wastewater Sampling Locations

- 1° influent wastewater collected weekly
- 3 King County WWTP locations

# West Point

# South Plant



# Brightwater



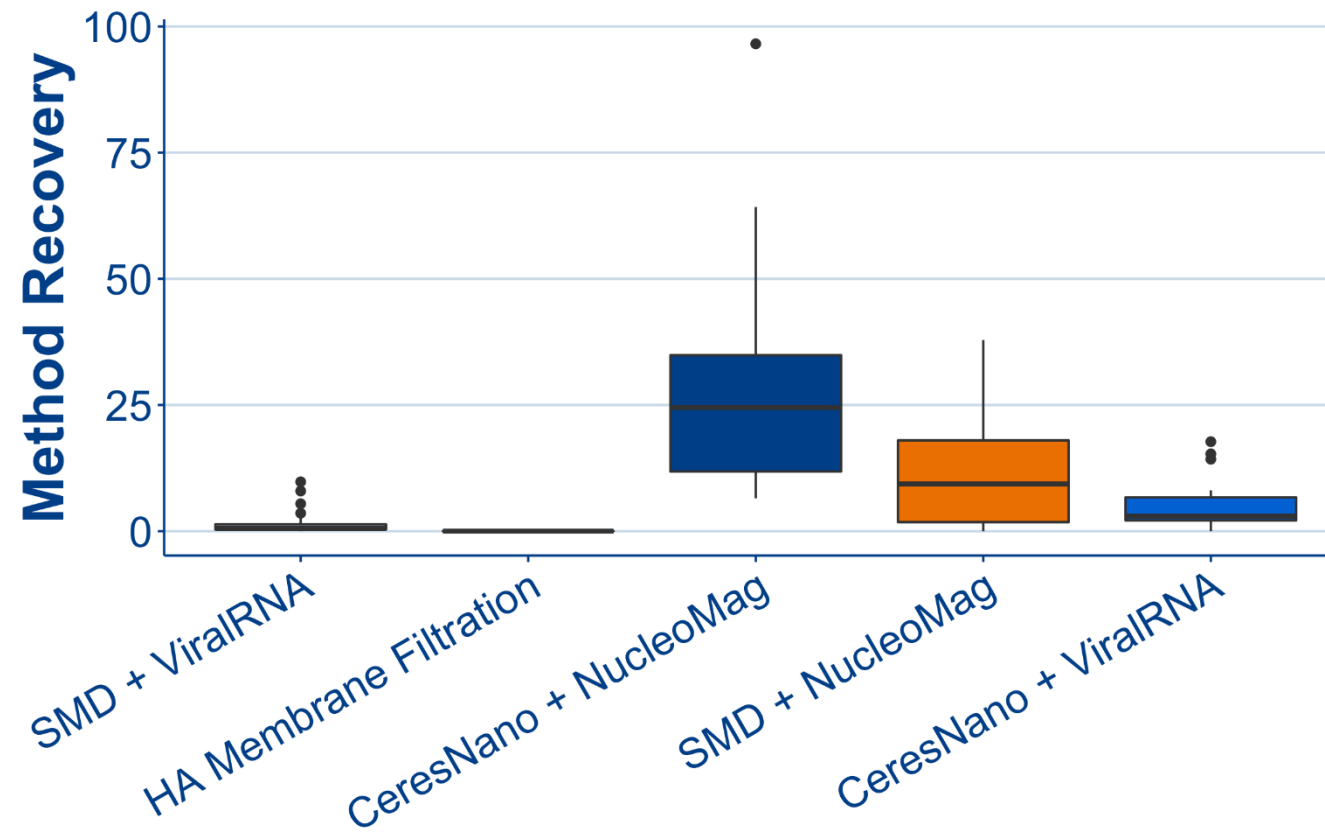
# Comparison of concentration and extraction techniques

- Lab work carried out at the Environmental and Occupational Health Microbiology Lab at the University of Washington, Seattle

Concentration Method ( <i>n</i> )	Extraction Kit	Volume Concentrated
Skimmed Milk Pellet Extraction (36)	QIAamp® Viral RNA Mini Kit	100 mL
Skimmed Milk Pellet Extraction (15)	NucleoMag® DNA/RNA Water	100 mL
Ceres Nanotrap® Particles (15)	QIAamp® Viral RNA Mini Kit	40 mL
Ceres Nanotrap® Particles (18)	NucleoMag® DNA/RNA Water	40 mL
HA Membrane Filtration (15)	NucleoMag® DNA/RNA Water	100 mL

# OC43 Method Recovery Efficiency

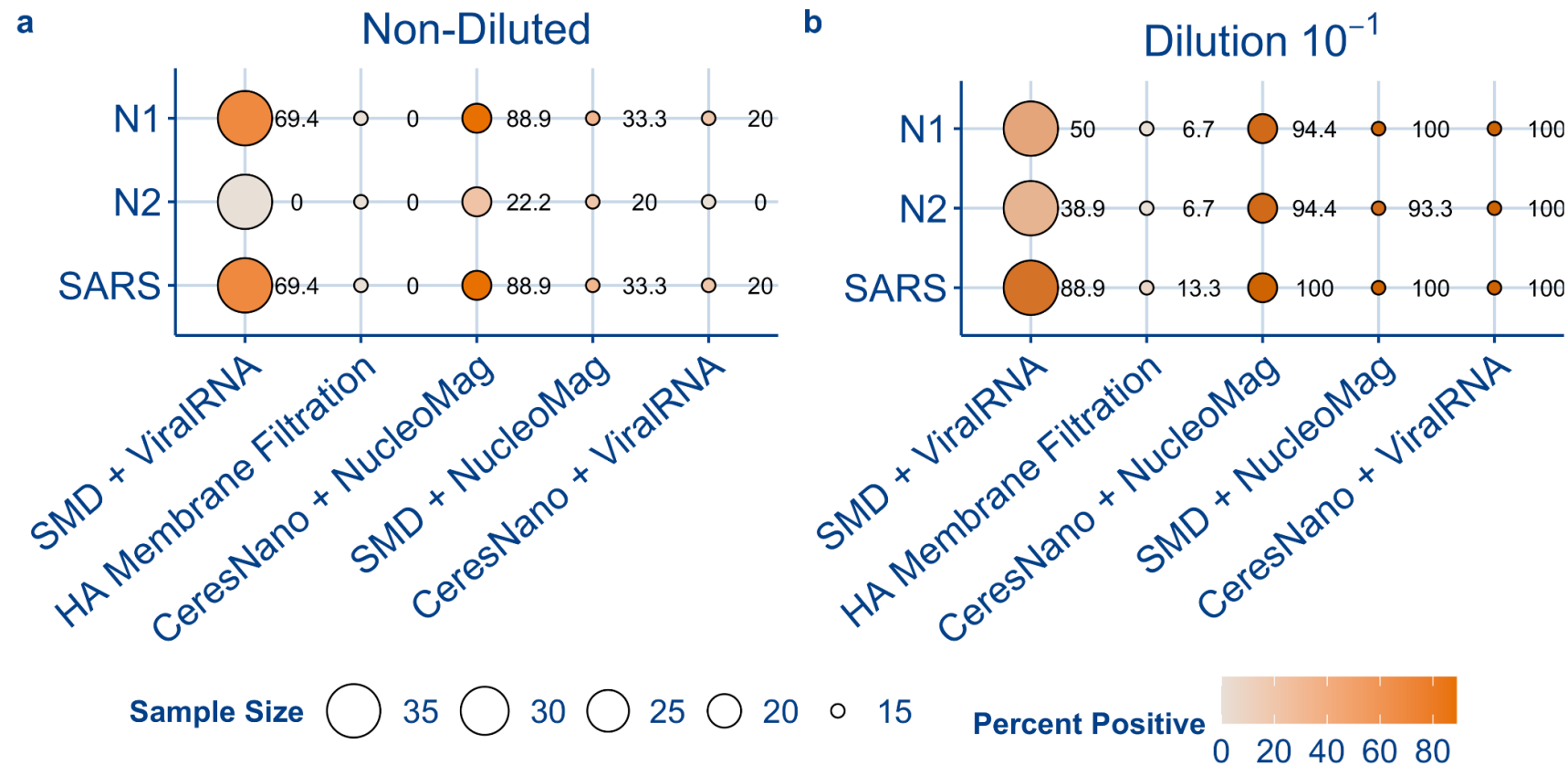
- Seeded Human Coronavirus OC43 at  $3.3 \times 10^4$  TCID<sub>50</sub>/L to serve as recovery control
- NucleoMag<sup>®</sup> DNA/RNA Water kit results in higher OC43 recovery compared to other methods





# Percent Positivity for SARS-CoV-2 Assays

- Higher SARS-CoV-2 detection in  $10^{-1}$  diluted reactions
- Ceres Nanotrap<sup>®</sup> particles + NucleoMag<sup>®</sup> DNA/RNA Water showed highest SARS-CoV-2 detection in undiluted reactions



## Conclusions

- Pathogen concentration and RNA extraction protocols chosen highly affects OC43 recovery efficiency and SARS-CoV-2 detection
- Need to use dilutions to assess performance
  - Particularly if switching to a new concentration or RNA extraction protocol
- Combination of Nanotrap<sup>®</sup> Magnetic Virus Particles from Ceres Nanosciences and the NucleoMag<sup>®</sup> DNA/RNA Water kit from MACHEREY-NAGEL demonstrates the highest recovery and lowest SARS-CoV-2 inhibition in Seattle-area wastewater



# Implementation of techniques in a wastewater surveillance program – UCLA

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Data courtesy of:

Merel Bot, MSc.

Roel Ophoff, PhD



# UCLA Wastewater Surveillance Program

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## Overview

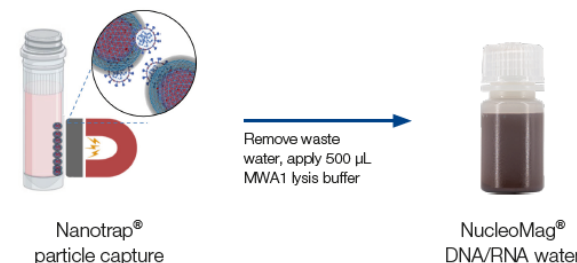
- Surveillance program monitoring UCLA student housing
- 24 hr composite samples pulled twice per week using autosamplers (~90/week)
- Samples from both on-campus and off-campus locations
  - 32 on-campus sites
  - 11 off-campus sites
- Testing ~ 12,000 students on campus and 3,100 off campus



# UCLA Wastewater Surveillance Program

## Method Development

- Previous – PEG concentration and column based extraction method
  - Slow turn around time
  - Manual, prone to error, more hands-on
  - Lower sensitivity
- Current - Nanotrap<sup>®</sup> Magnetic Virus Particles for concentration and NucleoMag<sup>®</sup> DNA/RNA Water for viral RNA extraction
  - Automated on a epMotion 5075 (liquid handling steps) and KingFisher Apex (concentration and extraction)
  - Same day turn around time
  - Quadrupled output
  - Can operate with one technician (with a lunch break!)

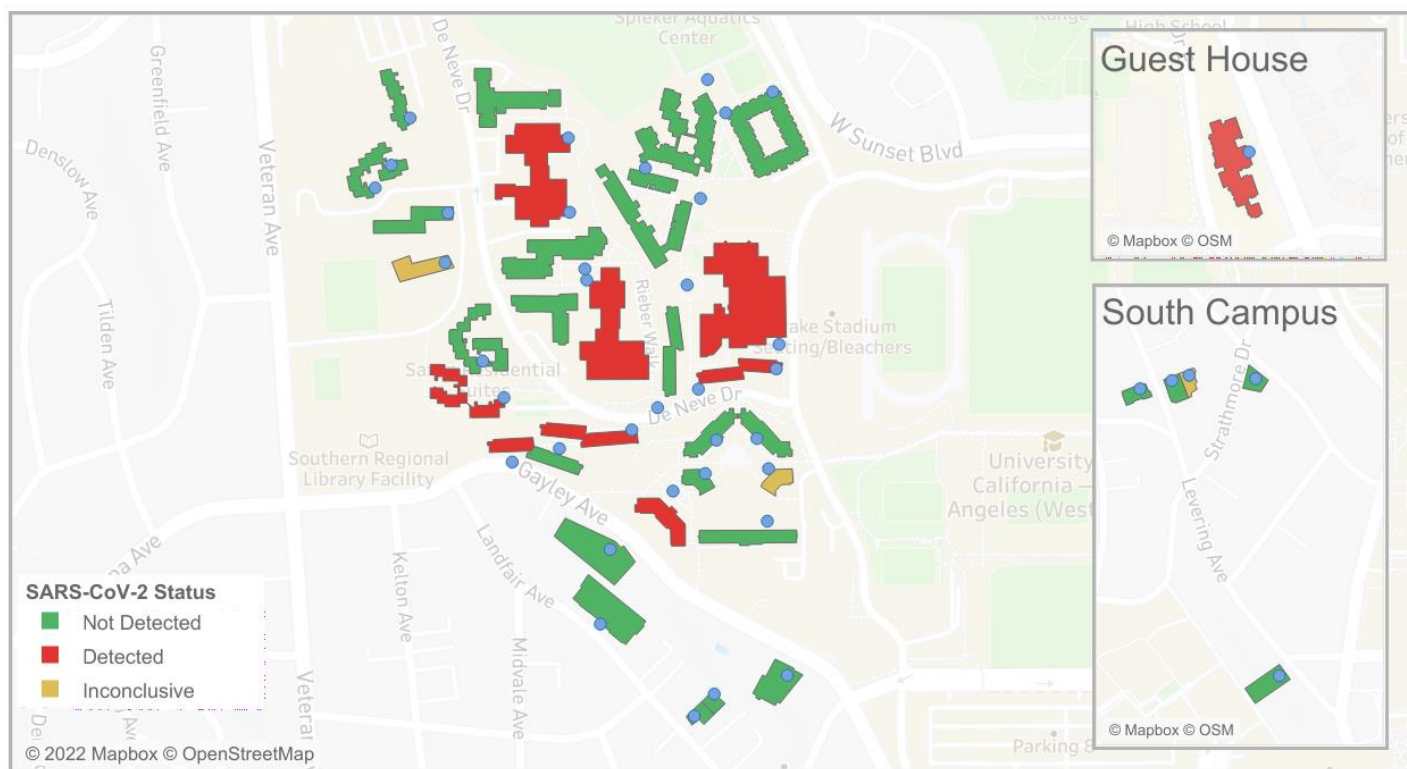


# UCLA Wastewater Surveillance Program

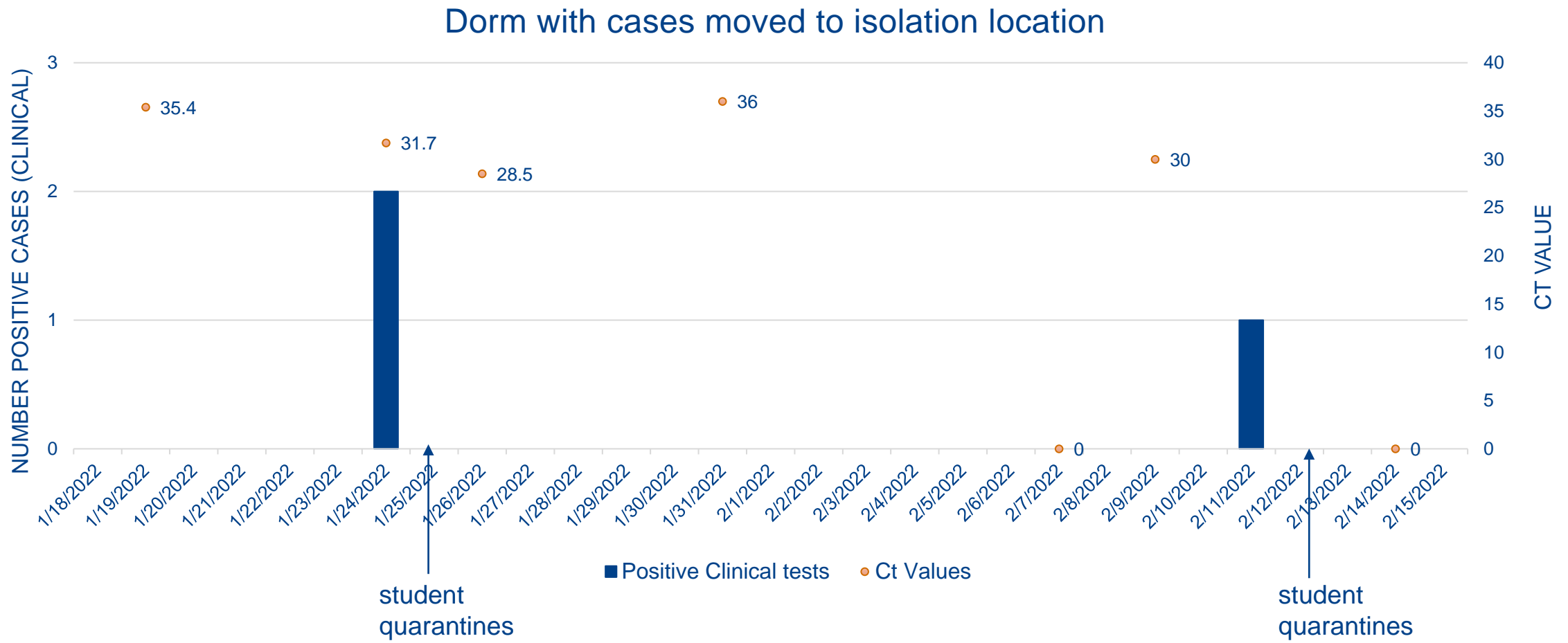
- Data provided same day to UCLA contact tracer team
- $>10^5$  copies/L triggers student notification in affected building
- $10^4 - 10^5$  copies/L trend analysis
- Individual testing recommended

## COVID-19 Wastewater Collection Sample Collection Data by Date

[Click to view historical trends](#)

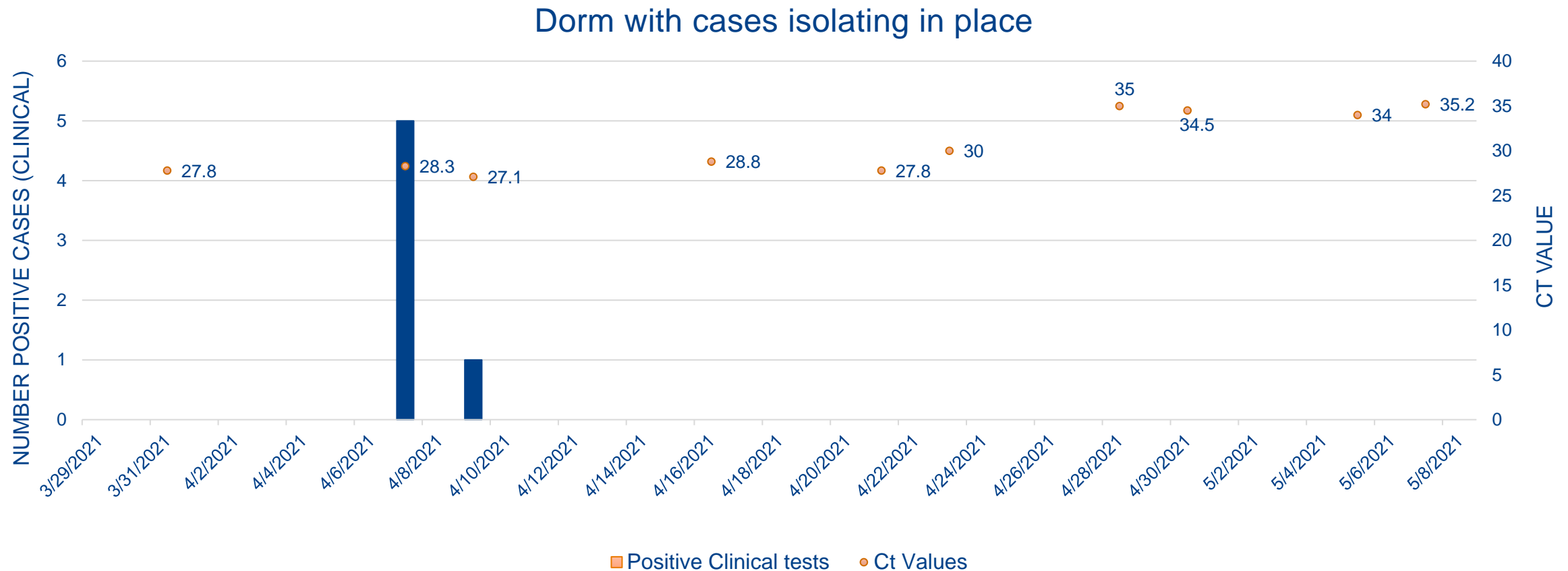


# UCLA Wastewater Surveillance Program



- Sensitivity - can detect a single positive case

# UCLA Wastewater Surveillance Program



- Wastewater surveillance indicates positive cases prior to exhibition of symptoms or respiratory testing
- Data crucial for project funding



# UCLA Wastewater Surveillance Program

## Benefits to the tested population

- Positive cases detected prior to symptom manifestation or in asymptomatic individuals
- Reduces further spread of COVID-19 in student population
- Important SARS-CoV-2 detection mechanism on campus
- Overall cost significantly less compared to widespread individual respiratory testing
- Less invasive than individual testing

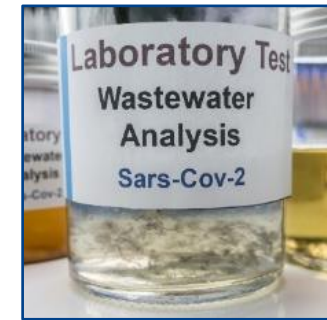


# Summary

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- Wastewater surveillance for SARS-CoV-2 is cost effective and efficient with the right tools
- MACHERY-NAGEL's NucleoMag® DNA/RNA Water kit is an excellent option for viral RNA extraction from complex samples
  - Scalable
  - Manual or automatable
  - Inhibitor removal technology
  - Compatible with multiple wastewater concentration techniques
- Combination of Ceres Nanotrap® particles and MN's Water kit provides excellent sensitivity for wastewater surveillance programs
  - Automatable and manual options
  - Proven techniques with multiple users and technical/application notes
  - Methodology useful for other applications beyond COVID-19 surveillance





# Acknowledgements

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Thank you!

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