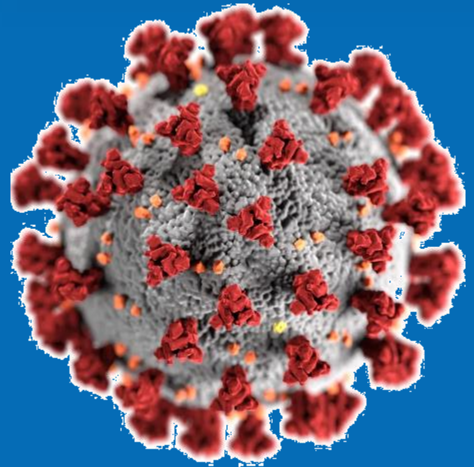
United States Environmental Protection Agency

EPA Tools & Resources Webinar:
Detection of SARS CoV-2 in Wastewater to Inform Public Health

Jay Garland, PhD
US EPA Office of Research and Development



December 16, 2020

SARS-CoV-2 in Sewage

- **Virus is shed in feces by individuals with symptomatic and asymptomatic infection**
- **Variable SARS-CoV-2 load in feces: 10^3-10^7 RNA copies/gram[1]**
- **Approximately 75-80% US is served by municipal sewage systems[2]**
- **SARS-CoV-2 has been detected in raw sewage**
  - US, Europe, Australia, Africa, etc.
  - Up to 10^7 RNA copies/L[3]
- **Low risk of wastewater as vehicle for transmission**
  - Limited reports of infectious virus in feces[4,5]; none from sewage
  - No additional risk to wastewater workers[6]
  - Treatment and disinfection are likely effective

Photo credit: https://www.usgs.gov

[1]Foladori et al. 2020. Science of the Total Environment 743:140444; [2] USEPA. 2016. EPA-830-R15005; [3]https://www.lacsd.org/civicax/filebank/blobdload.aspx?blobid=%2022002; [4]Xiao et al., Emerging Infectious Diseases, 26(8), 1920-1922; [5] Zhou et al. 2020. Nature Medicine 26:1077-1083; [6]https://www.osha.gov/SLTC/covid-19/solid-waste-wastewater-mgmt.html

Wastewater Surveillance

Illicit Drugs in Municipal Sewage

Proposed New Nonintrusive Tool to Heighten Public Awareness of Societal Use of Illicit-Abused Drugs and Their F

Christian G. Daughton

DOI: 10.1021/bk-2001-07
Publication Date: July 30,

Environmental Health Perspectives • VOLUME 116 | NUMBER 8 | August 2008

Estimating Community Drug Abuse b

Ettore Zuccato, Chiara Chiabrando, Sara Castiglioni, R

Department of Environmental Health Sciences, Istituto di Ric

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NEWS & ANALYSIS | INFECTIOUS DISEASE

Israel's Silent Polio Epidemic Breaks All the Rules

Leslie Roberts

\+ See all authors and affiliations

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doi:10.1017/S095026881000316X

REVIEW ARTICLE

Role of environmental poliovirus s
polio eradication and beyond

AEM
Journals.ASM.org

gs

Research

Environmental surveillance of poliovirus
Dakar, Senegal (2007-2013)

Abdou Kader Ndiaye[1,&], Pape Amadou Mbathio Diop[1], Ousma

AMERICAN SOCIETY FOR MICROBIOLOGY | mSphere®

RESEARCH ARTICLE
Applied and Environmental Science

Check for updates

Retrospective Surveillance of Wastewater To Examine Seasonal Dynamics of Enterovirus Infections

Nichole E. Brinkman,[a,b] G. Shay Fout,[a] Scott P. Keely[a,b]

Wastewater-based SARS-CoV-2 Surveillance

- Complements existing COVID-19 surveillance systems
- Advantages
  - Non-invasive
  - Pool of individuals
  - Asymptomatic and symptomatic individuals
  - Inexpensive
  - Data for communities where individual testing data are underutilized or unavailable
  - Scalable
  - Unbiased
  - Can be a leading indicator of changes in community-level infection

Outline for Presentation

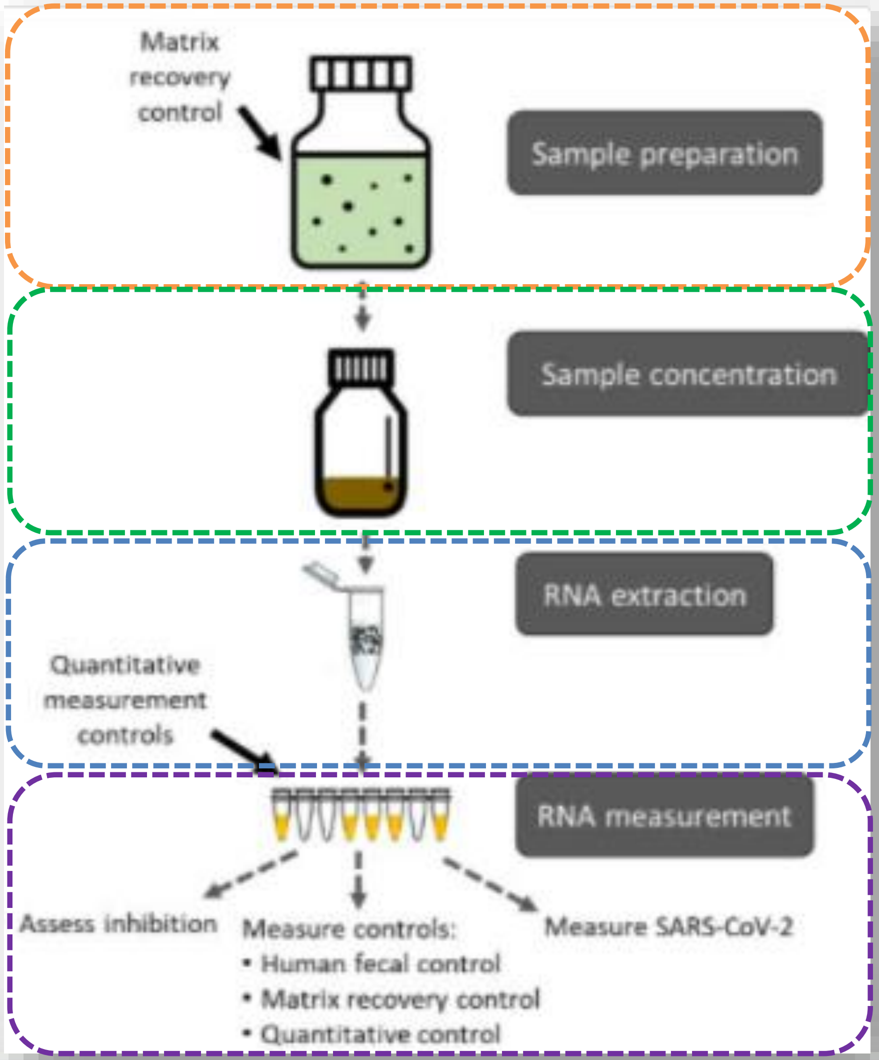
- Analytical method development
- Understanding dilution and degradation in the sewer
- Relating the sewer signal to community case rates
- Building a statewide network of sampling
- Translating the information into public health decisions

Method Considerations

Sample Type
Untreated wastewater
Primary sludge
Volume

Sample Preparation
Storage temperature
Homogenization
Additives
Matrix Spike
Clarification

Sample Concentration
Ultrafiltration
Electronegative membrane filtration
Polyethylene glycol (PEG) precipitation



https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance/testing-methods.html

Nucleic Acid Extraction
Silica columns
Magnetic beads
Precipitation

RNA/DNA Measurement
RT-qPCR
RT-ddPCR
Genetic targets

Other Considerations
Biosafety
Supply Chain issues
Practicality (time, equipment)
QA/QC

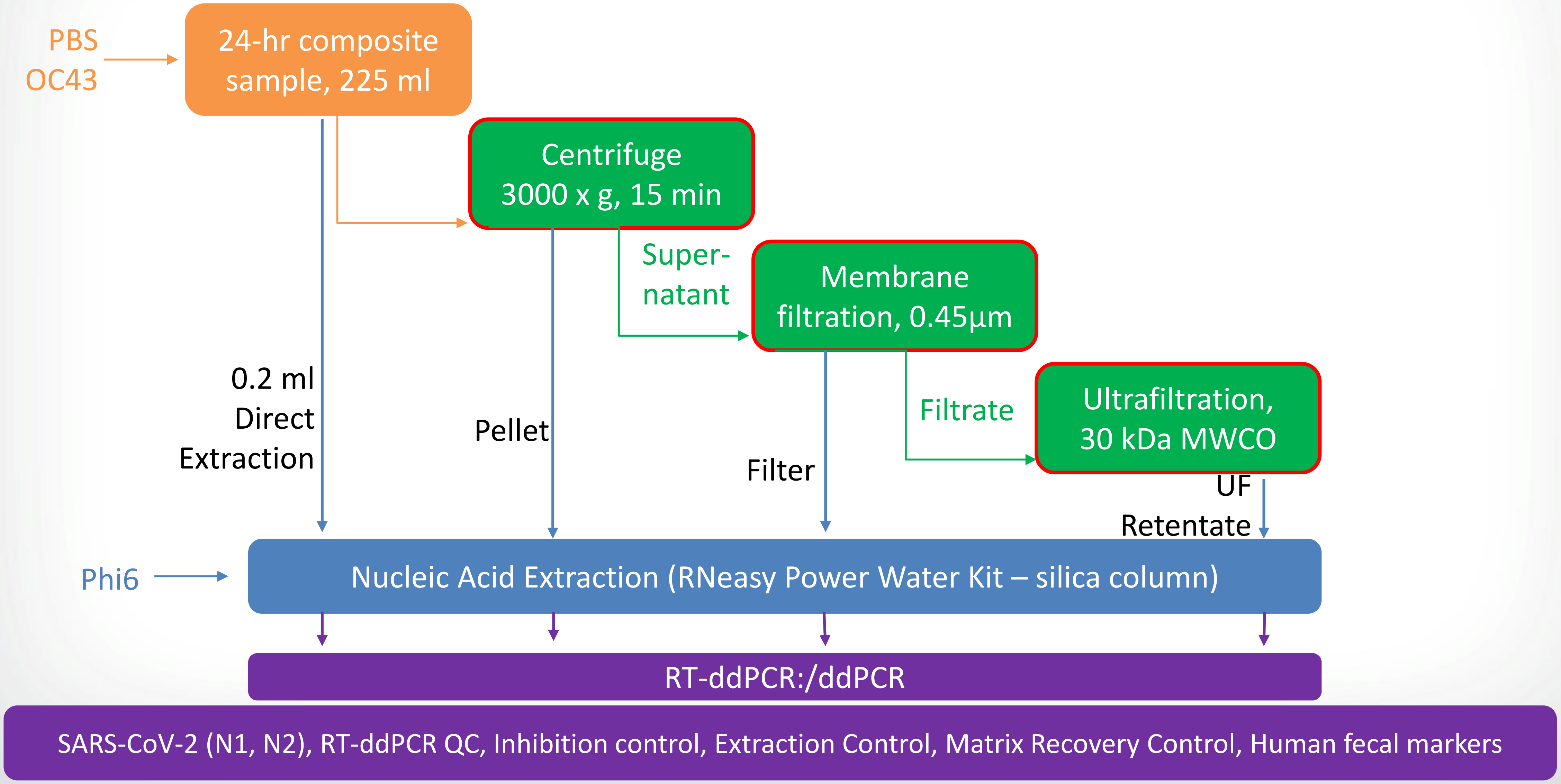
Biosafety

- Wastewater risk is the same
- Increased risk with processes that could generate aerosols
  - Centrifugation
  - Membrane filtration
- CDC recommendations[1]
  - Biosafety Level 2 laboratory
  - Biosafety Level 3 precautions
    - Respiratory protection
    - Designated donning/doffing area
- Borrowing lab space in AWBERC Biocontainment Suite
- Safety, Health and Environmental Management (SHEM)
- ORD's BioRisk Management Advisory Committee

Brian Morris

[1]https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html#environmental

EPA Sample Processing and Analysis

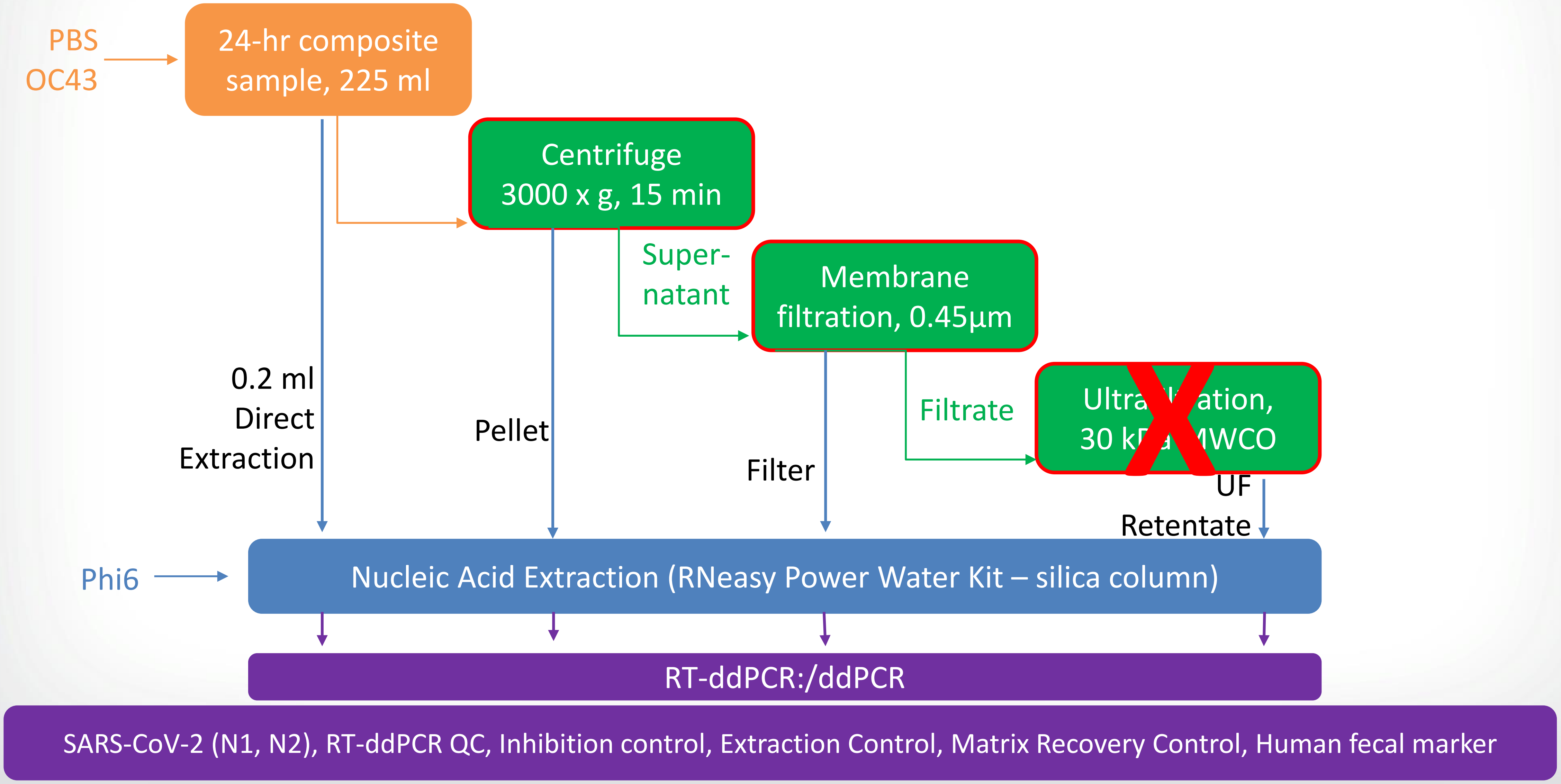


Method Performance Metrics

- **Limit of Detection**
  - 655 RNA Molecules/L
- **Recovery Efficiency**
  - Endogenous virus
    - crAssphage 84%
    - PMMoV 27%
  - Matrix spike
    - Betacoronavirus OC43 (up to 50%)
- **RT-ddPCR Inhibition**
  - Minimal (< 20%)

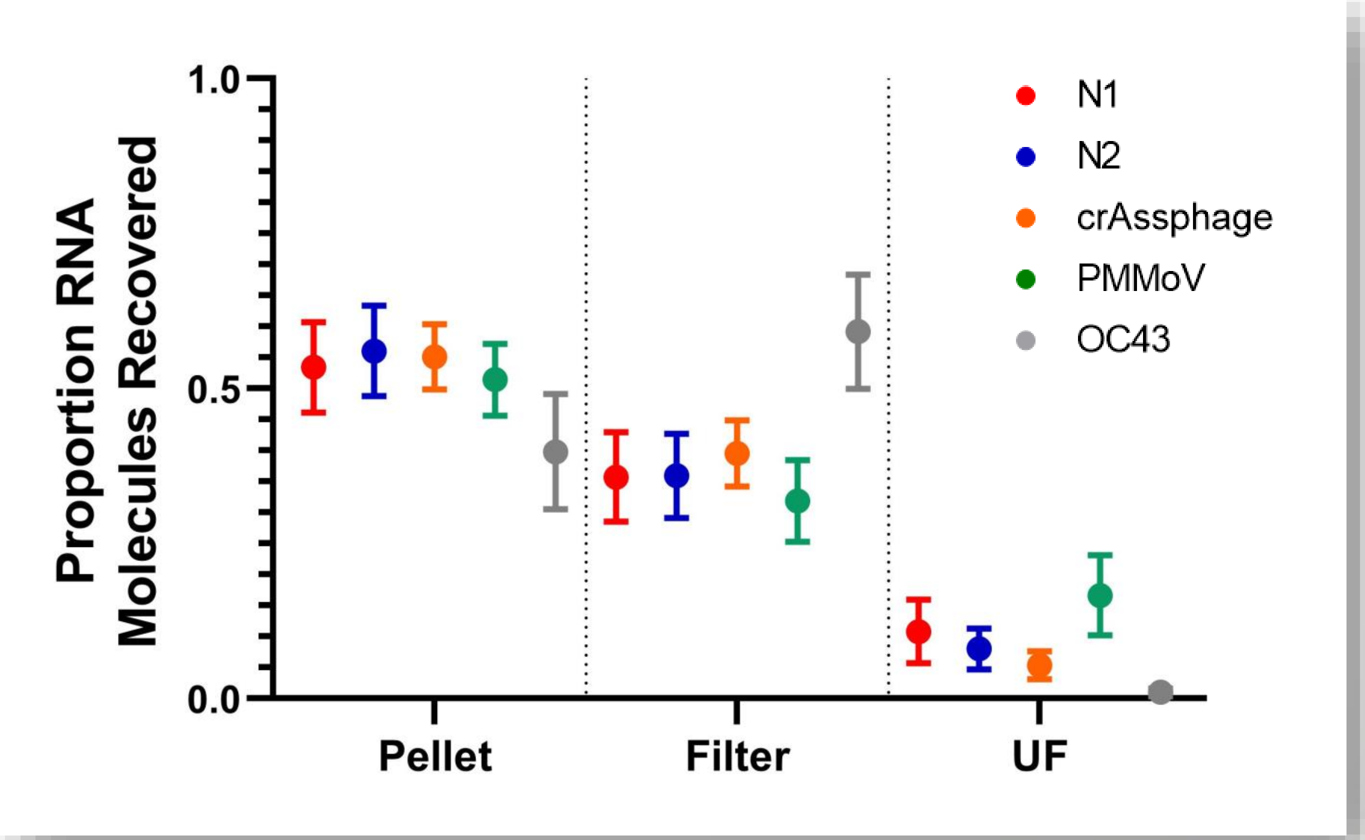
Chloe Hart

Supply Chain Disruption #1



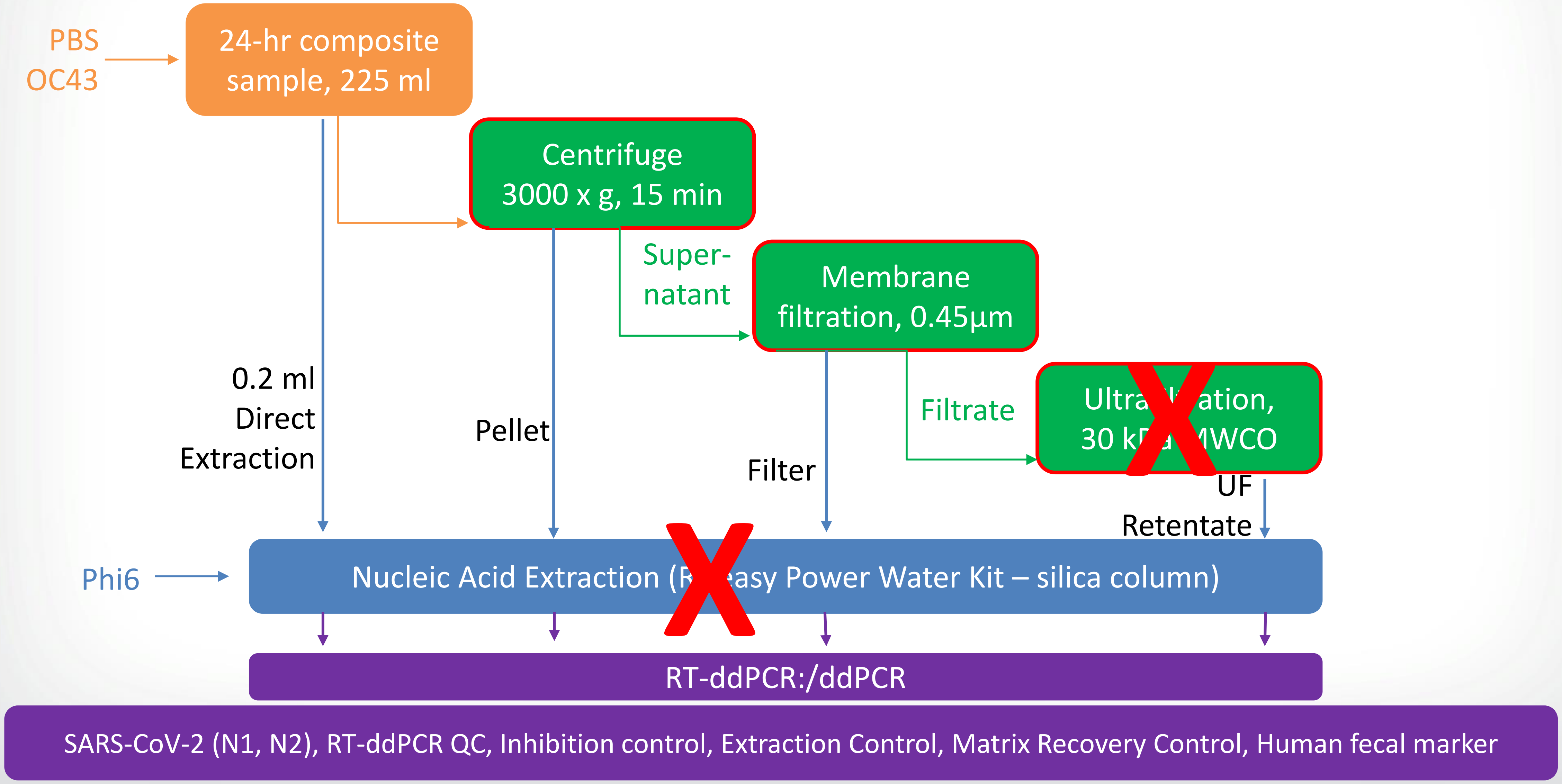
Supply Chain Disruption #1

Ultrafiltration – Millipore Centricon Plus-70 centrifugal Units

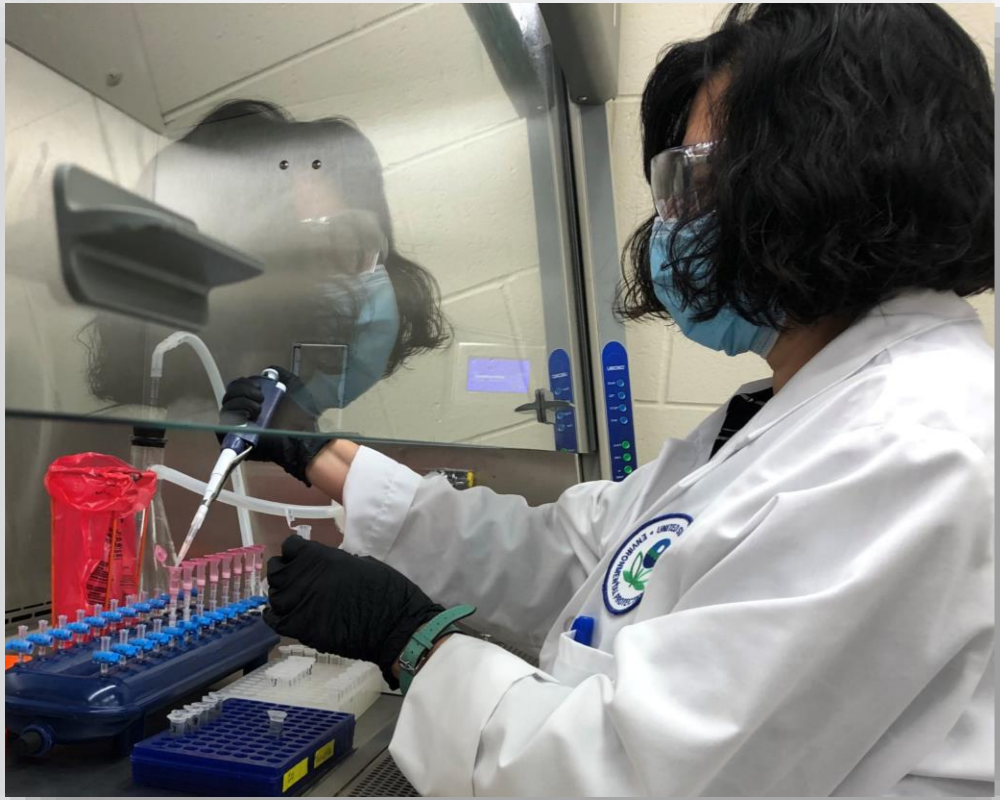


~ 90% measurable virus in pellet and filter fractions

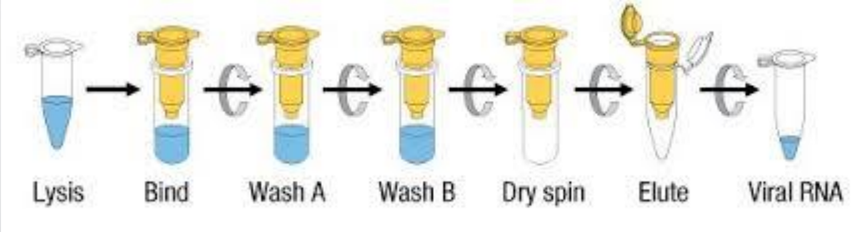
Supply Chain Disruption #2



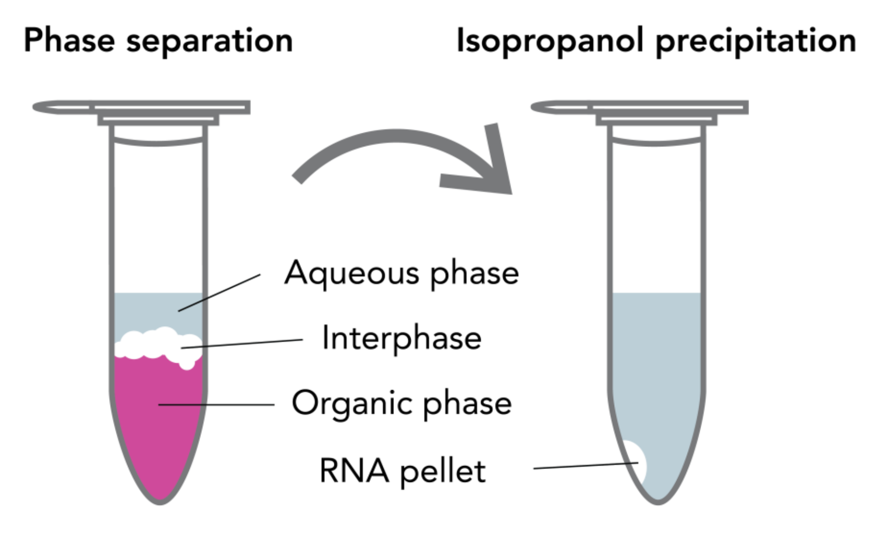
Supply Chain Disruption #2



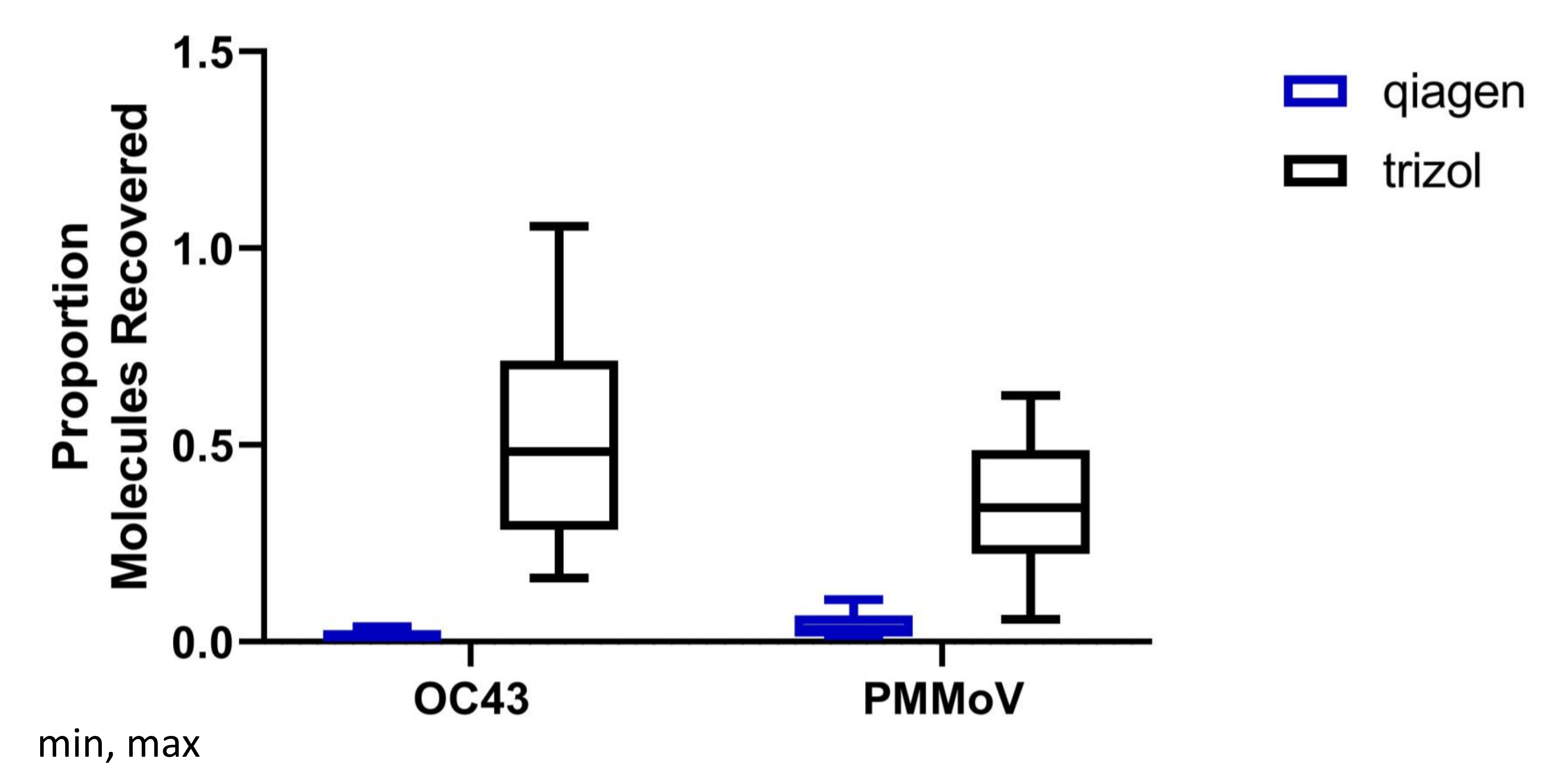
Ana Braam



Trizol-Chloroform Extraction
RNA precipitation

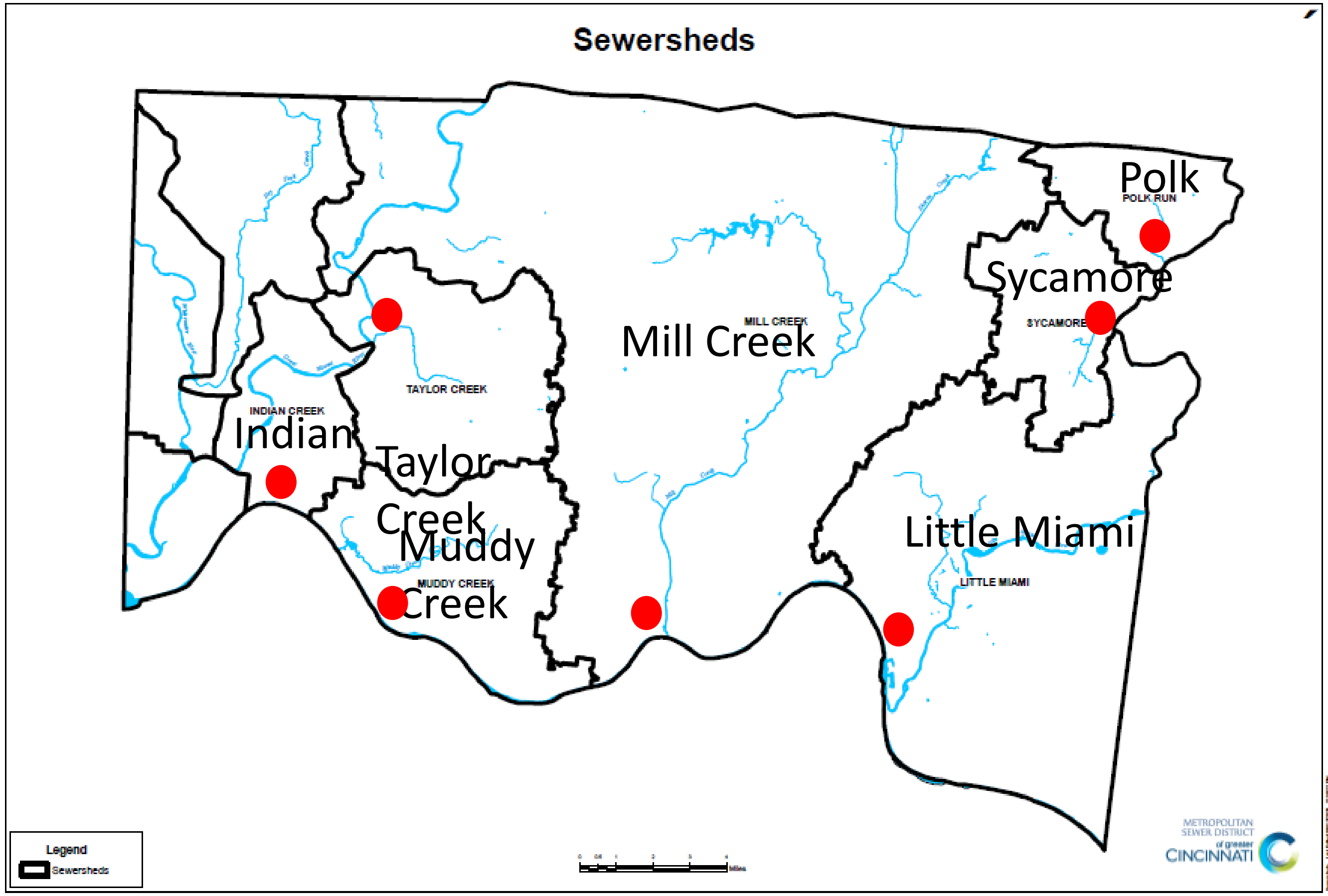


Recovery Efficiency



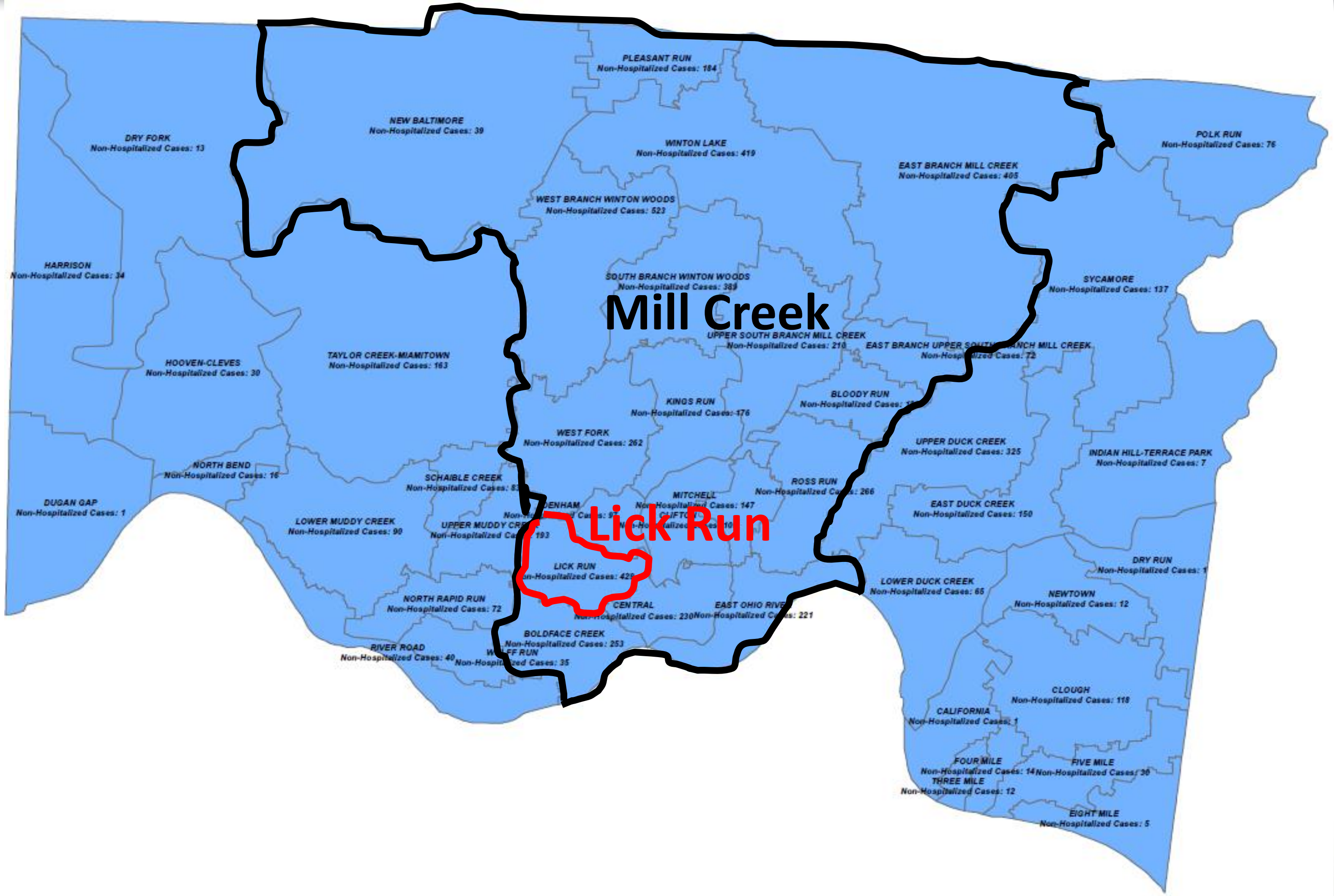
New extraction approach increased recovery efficiency 10-fold

Metropolitan Sewer District of Cincinnati



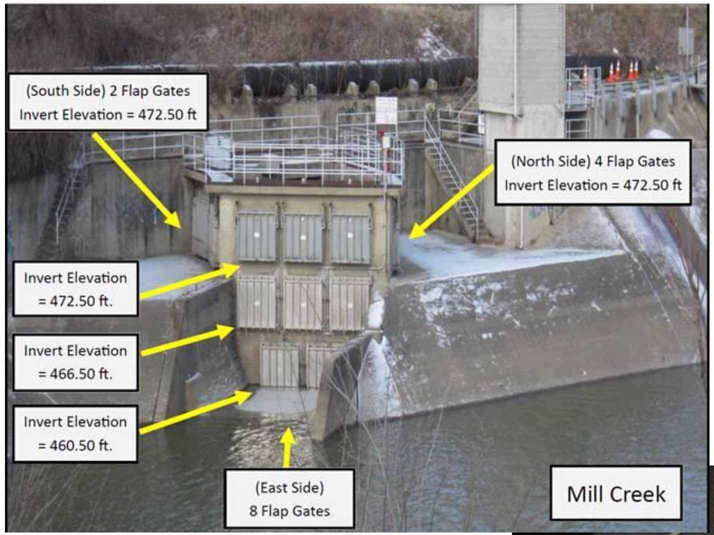
Sewershed	MGD	% Industrial	% Combined	Dilution
Mill Creek	118	5.0	40	0.5:1
Taylor Creek	3	0	0	1.8:1

Sub-Sewershed Sampling: Cincinnati



Sub-Sewershed Sampling – Lick Run

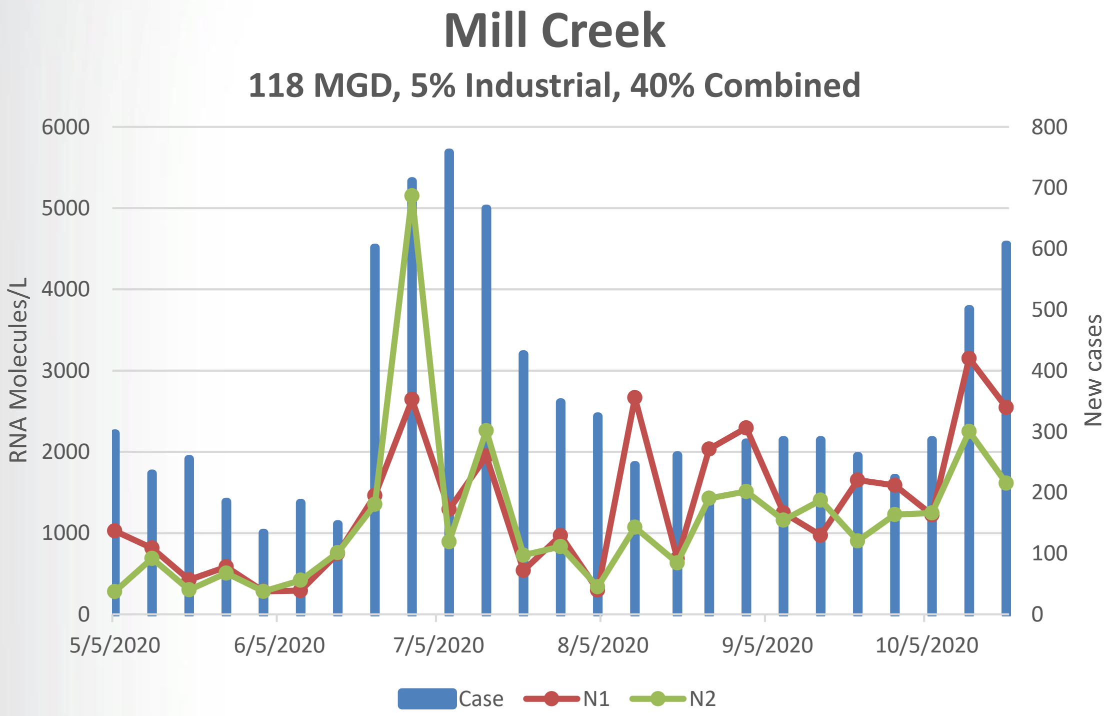
Combined Sewer Overflow



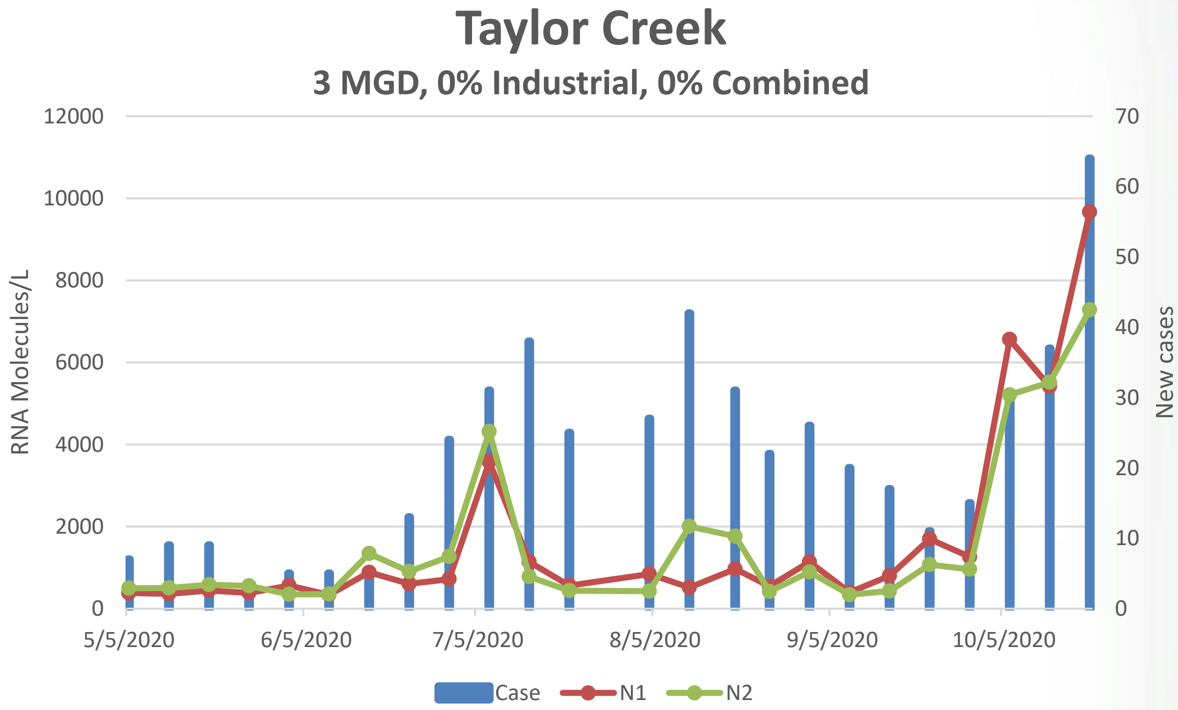
Remote Composite Sampler
~10L between 8-11 am
~500 ml every 15 min

Access to Sewer

Accounting for Dilution Impacts

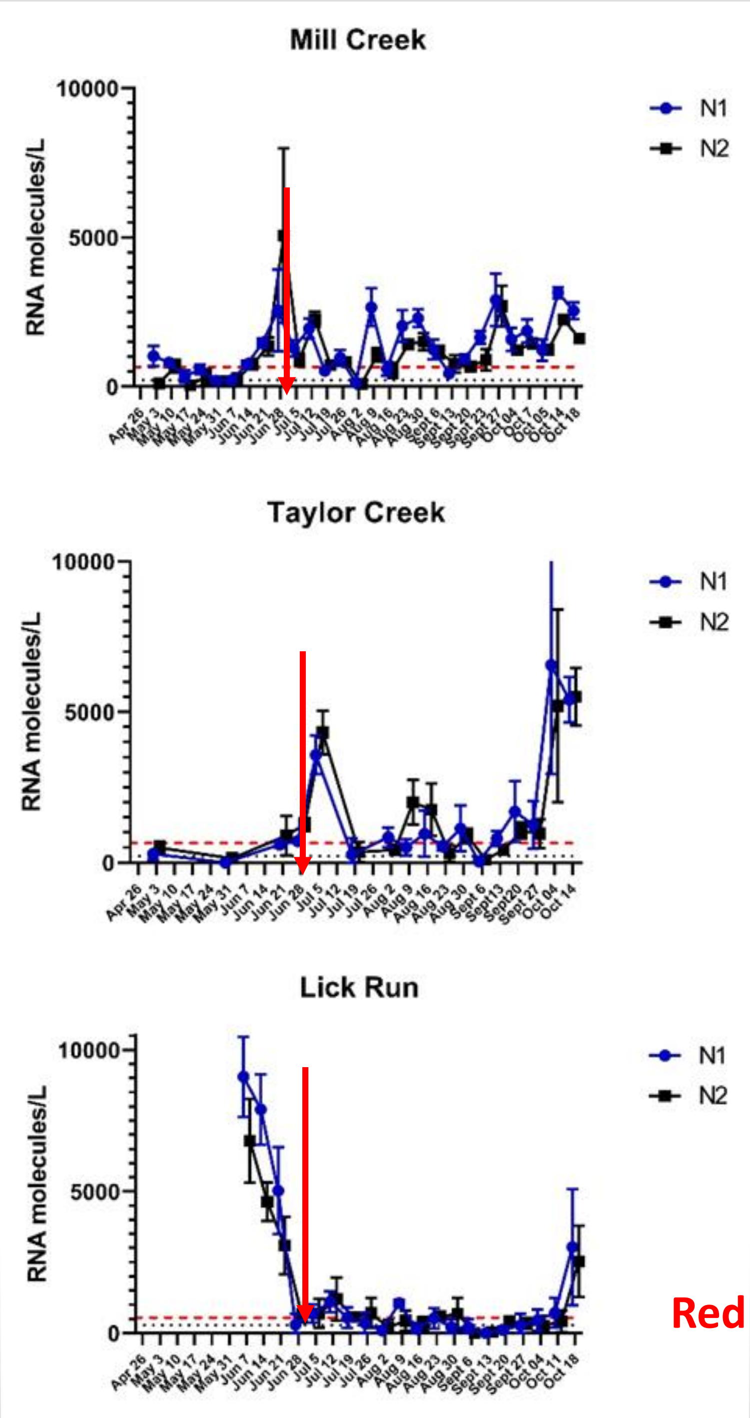


	Correlation Coefficient	P-value
N1	0.43	0.0322
N1/crAv	0.59	0.00195
N2	0.483	0.0148
N2/crAv	0.563	0.000385



	Correlation Coefficient	P-value
N1	0.6	0.00201
N1/crAv	0.505	0.012
N2	0.521	0.00925
N2/crAv	0.529	0.00809

Flow volume
TSS
crAssphage
PMMov
HF183

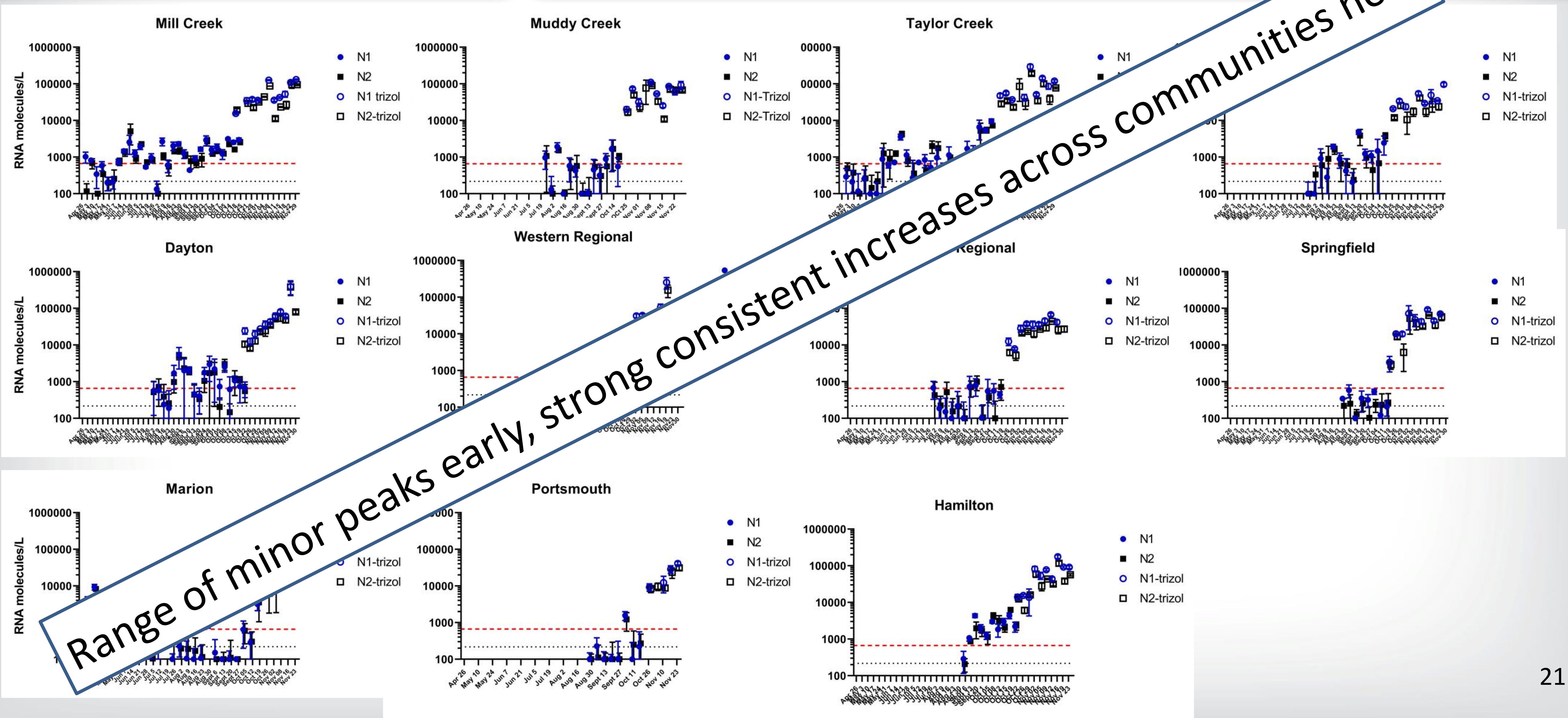


Different Views of Community Infection

Potential role of sentinel sites?

Red Line – County Infection Peak in early July

Temporal Trends of SARS-CoV-2 in Sewersheds



Range of minor peaks early, strong consistent increases across communities now

What do these data mean?

- If you want to relate SARS-CoV-2 wastewater data to the number of infected individuals, you need to know:
  - Concentration of SARS-CoV-2 in wastewater
    - Measured concentration
    - Recovery Efficiency
    - Dilution
    - Decay
  - And how much SARS-CoV-2 shed in feces (uncertain)

- Or focus on relative changes at a given site

Developing the Ohio Wastewater Monitoring Network

May
2020

Governor DeWine initiates wastewater SARS-CoV-2 monitoring project

June
2020

Ohio EPA - $2,000,000 for wastewater monitoring project via CARES funds

ODH is project lead

Ohio WRC project coordinator

Monitoring and Analyzing
July 2020

- 7 large cities

- 15 locations sampled

- 3 laboratories – OSU, UT, US EPA

Workgroups created

Part of CDC national monitoring network

Working on analytical methods

Working on data analysis

Adding Sites
August – October 2020

- Medium and smaller cities

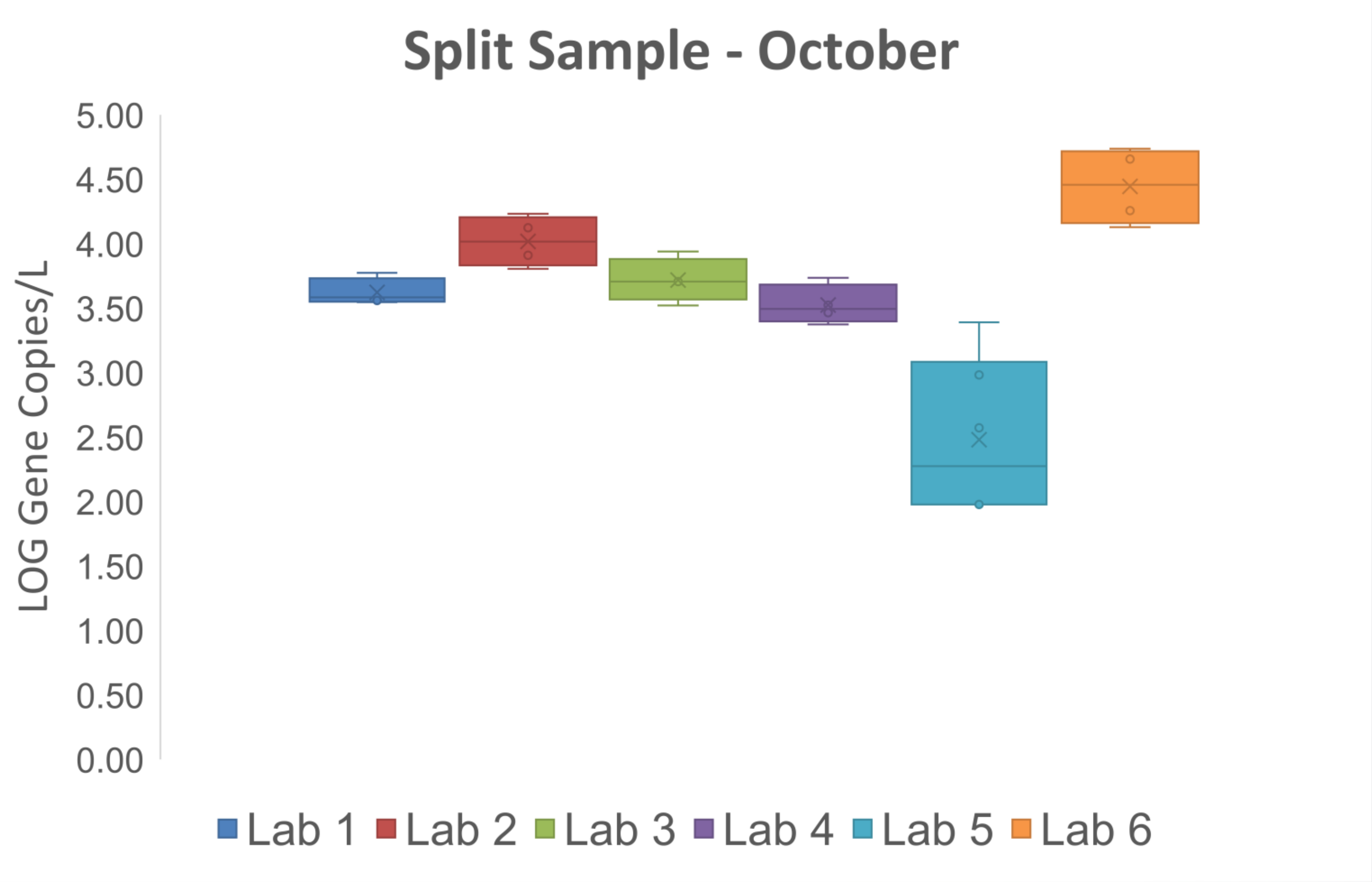
- 4 added laboratories – UA, KSU, Commercial lab, BGSU

- Sampling frequency twice a week

- Curently 52 sites

Interlaboratory Comparisons

- Once a month
- SARS-CoV-2 positive sample send to all the labs
- Normal protocols performed
- Results analyzed

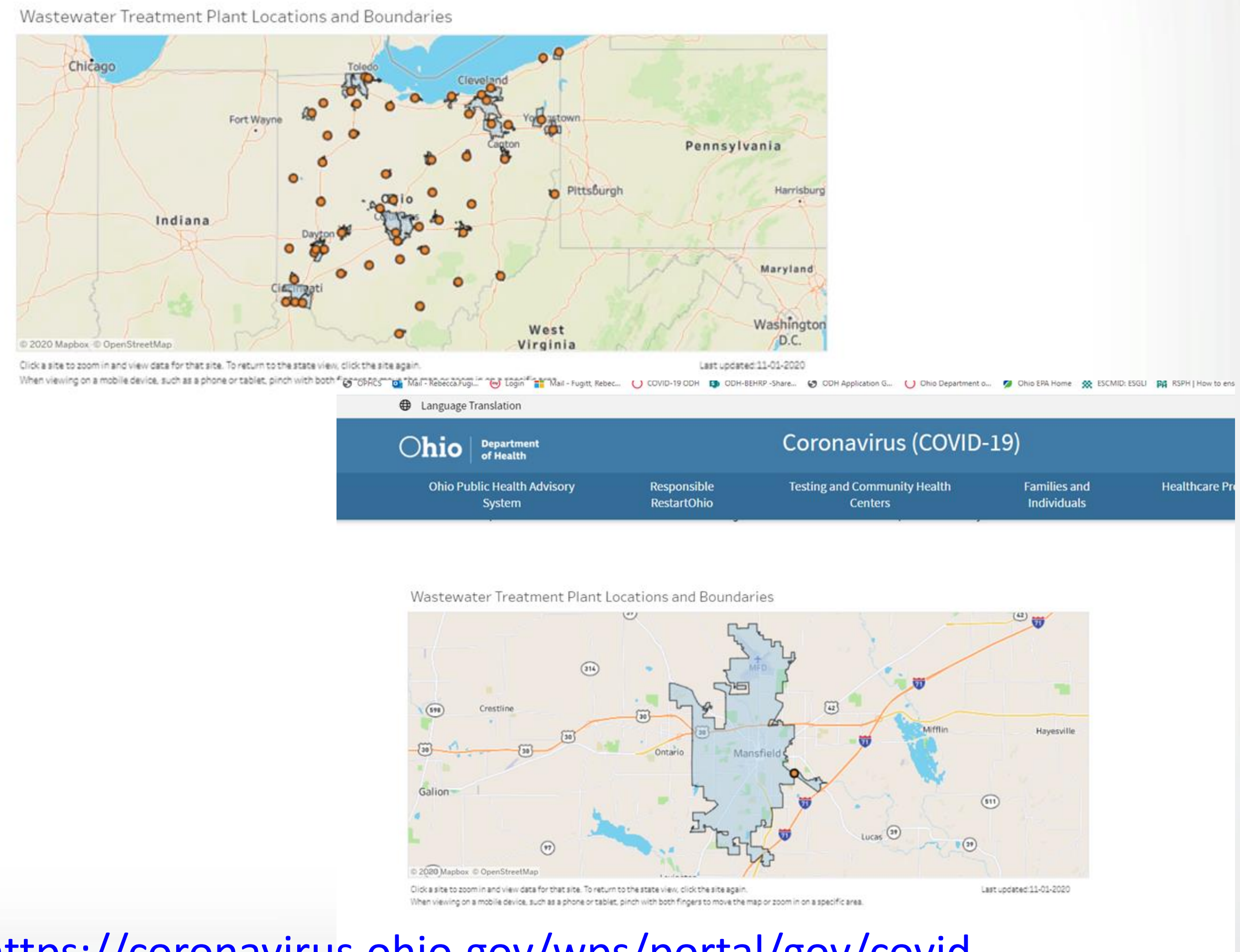


Ohio Wastewater Monitoring Network

The focus is on **<u>trends or significant changes</u>** in the number of viral gene copies detected.

Currently action is taken when at least 3 samples show a sustained increase of at least 10-fold (1 log)

- Notify the local health district and utility
- Provide information on how to interpret the data and link to message toolkit
- Notify the state pandemic testing team for linkages to establish pop-up testing sites and the state contact tracing team to offer assistance



https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/wastewater

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Ohio Public Health Applications

Development of toolkit for local health districts and utilities

- Additional messaging to public on best practices – social media, twitter

https://coronavirus.ohio.gov/wps/portal/gov/covid-19/healthcare-providers-and-local-health-districts/for-local-health-districts-and-governments

New focus on monitoring multiple sites on campus to support colleges/universities across state

Ohio is coordinating on data reporting approaches and with CDC on their National Wastewater Surveillance System

https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html

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Collaborations

Monitoring SARS-CoV-2 virus in wastewater as an indicator of changes in community-level infection is a topic of interest to many different organizations, and EPA is committed to leveraging partnerships and collaborations to achieve results. Some examples:

- **CDC** – Weekly exchange with staff scientists to both provide status of EPA work and info on the National Wastewater Surveillance System
- **Ohio Wastewater Monitoring Network** – Committed to conducting samples as part of lab network, provided initial guidance on sample handling, coordinated interlaboratory comparisons, and developed standard data collection formats for entire network
- **Public Utilities** – Research collaboration with Cincinnati MSD, reached out to Ohio utilities organizations (i.e., AOMWA, OWEA) early in the pandemic, participated in initial meetings with potential participating utilities in Ohio, presented on status/progress to California WEA
- **States** – Provided technical assistance to Arkansas, Maryland, New Jersey and New Mexico as they developed their wastewater surveillance efforts
- **Research Community** – Participated in the Water Research Foundation International Virtual Summit on the topic in April and subsequent interlaboratory comparison of methods organized by WRF, shared results with Global Water Research Coalition's Workgroup on SARS-CoV2 sewage surveillance

Final Summary

- **Analytical Method Development**
  - No standard method, but many options available (useful to address supply chain)
  - Quality Control for assessing method performance (recovery efficiency, inhibition control)
- **Dilution/Degradation in Sewer System**
  - Ongoing comparison of different approaches to normalize for dilution
  - Use existing temperature dependent rates, targeted studies on industrial wastes
- **Relation of Sewer Signal to Infection rates**
  - Accounting for recovery efficiency, dilution, degradation
  - Need better data on shedding rates

Final Summary

- **Developing a network**
  - – Linking wastewater utilities, environmental analytical labs, public health agencies
  - – Network of labs to increase capacity if needed; build in QA/QC
- **Translating data to public health decisions**
  - – Focus on trends or significant changes in the concentration ato reinforce public messaging
    - As models to predict infection are refined
  - – Early warning?
    - Relative turnaround time of individual and wastewater data key
    - Sentinel sites might be very useful, but attributes of these sites may vary across pandemic cycle
  - – Targeted sampling to direct individual testing/actions
    - e.g., university dormitory monitoring

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