Long-term Monitoring and Sequencing of SARS-CoV-2 in Wastewater

Eunice Varughese, PhD

US Environmental Protection Agency

Office of Research and Development

University of Cincinnati - Environmental Health Department Seminar

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Research Team and Partners

EPA/ORD

Nichole Brinkman

Maitreyi Nagarkar

Chloe Hart

Emily Wheaton

Scott Keely

Michael Jahne

Jay Garland

Bruce Smith

Brian Morris

Ana Braam

Barry Wiechman

Sarah Okum

Utilities

Metropolitan Sewer District of Greater Cincinnati

Robin Schaefer

City of Dayton

Chris Clark, Walter Schroder

City of Marion

Steve Morris

City of Portsmouth

Tommy Stewart

Montgomery County

Jim Davis

City of Hamilton

Mark Smith

City of Springfield

Jeff Yinger

Hamilton County Public Health Department

Chris Griffith

Ohio Water Resources Center

Zuzana Bohrerova

Ohio Department of Health

Rebecca Fugitt

Ohio EPA

Brian Hall

Tiffani Kavalec

University Labs

Ohio State University

University of Toledo

Kent State University

University of Akron

Bowling Green State University

Commercial Lab

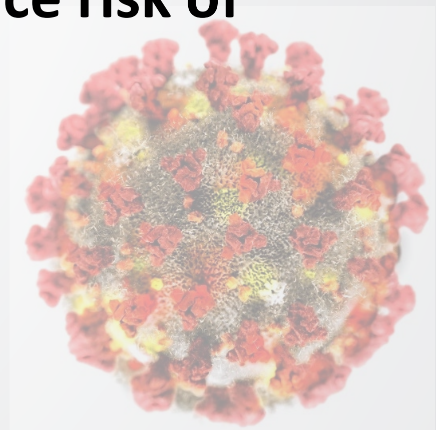
LuminUltra

Private Lab

Battelle

EPA SARS-CoV-2 Capabilities and Research

- **EPA has capabilities to respond to environmental emergencies and address biological contamination**
- **The world continues to learn much about COVID-19 – EPA has the expertise to add to that knowledge, especially in the areas of exposure, wastewater, and cleaning and disinfection**
- **EPA researchers are building on an expansive body of world-class research and applying that knowledge to reduce the risk of exposure to SARS-CoV-2**
- **Aimed to help states & territories, tribes and local governments (e.g., public health agencies) guide the public, businesses and institutions to reduce risk of SARS-CoV-2**
- **Partnering with CDC, state and local agencies and others**



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

OPEN

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Ox

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

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REVIEW ARTICLE

Role of environmental poliovirus s polio eradication and beyond

Research

Environmental surveillance of poliovirus

Dakar, Senegal (2007-2013)

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

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

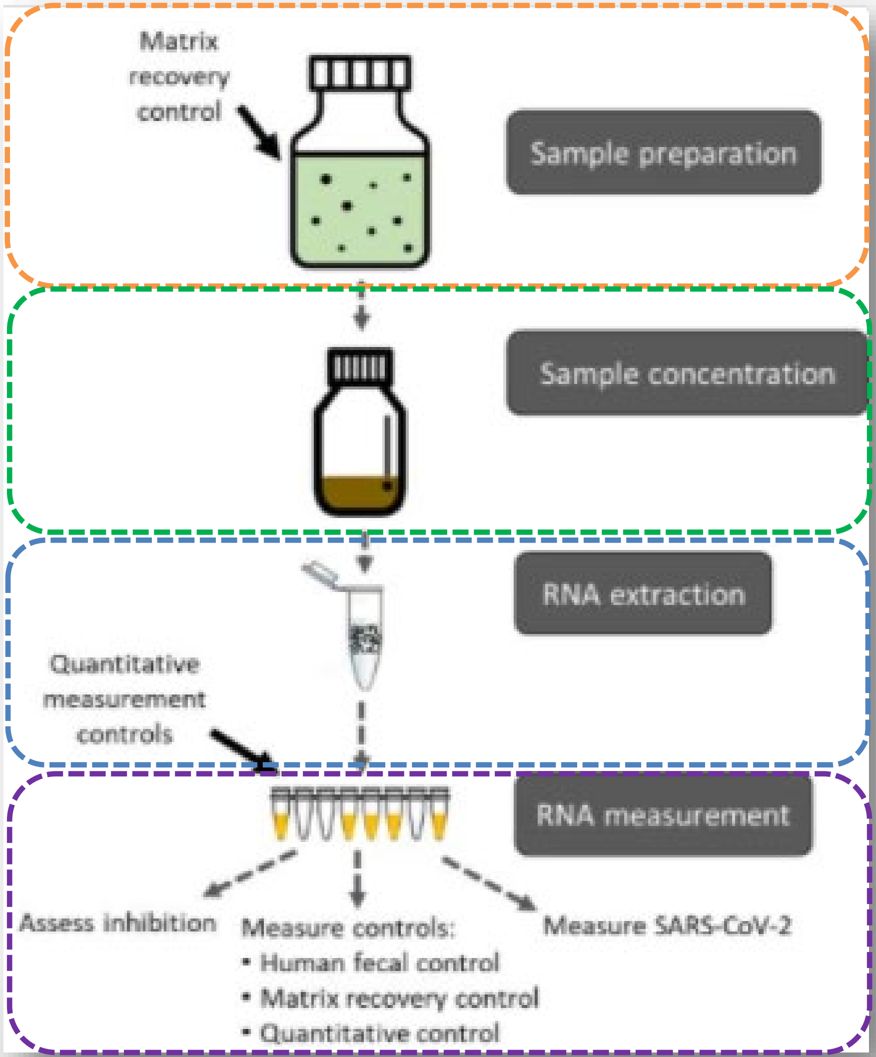
- Understanding the virus in wastewater
  - Analytical method development
  - Understanding dilution and degradation in the sewer
  - Relating the sewer signal to community case rates
- Building a statewide network of sampling & linking to public health decisions
- Next Steps
  - Monitoring this pandemic
  - Preparing for the next potential pandemic

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



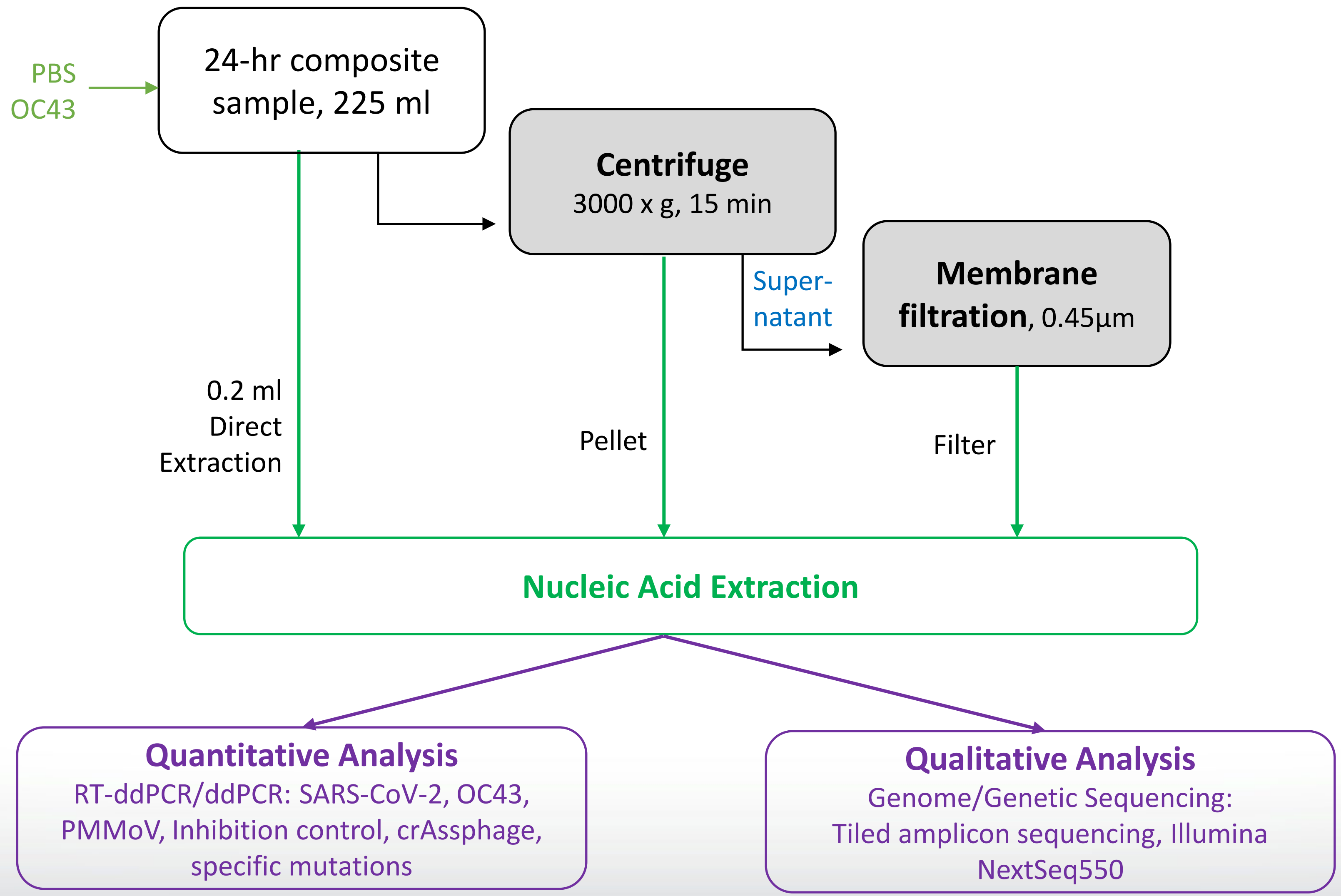
https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance/testing-methods.html

Nucleic Acid Extraction
Silica columns
Magnetic beads
Precipitation

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

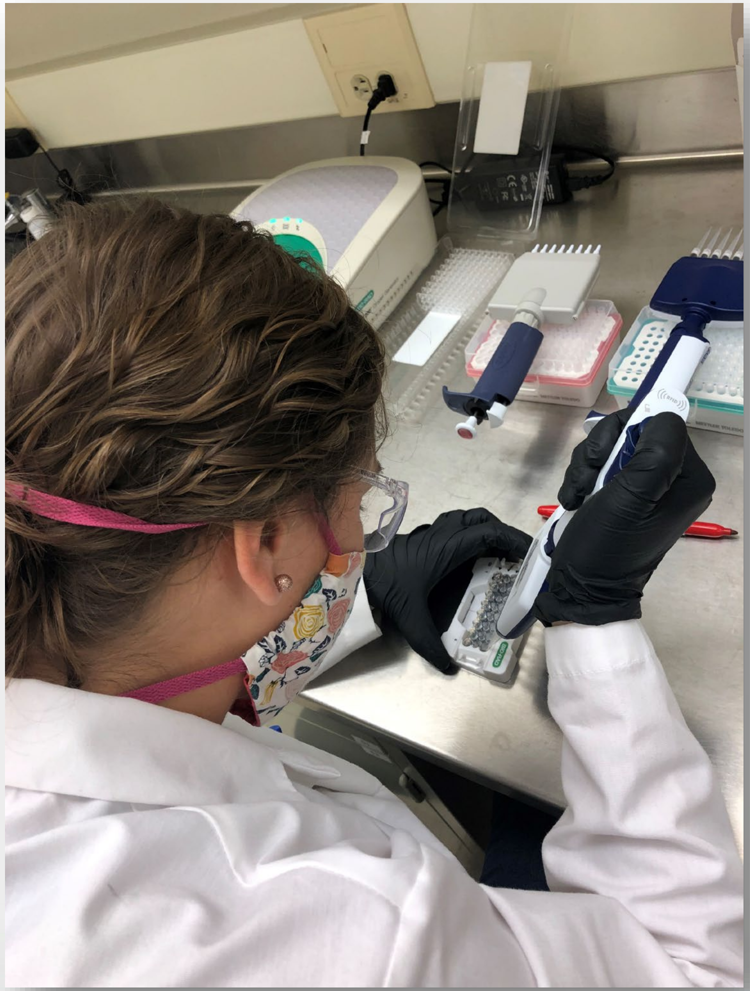
Other Considerations
Biosafety
Supply Chain issues
Practicality (time, equipment)
QA/QC

USEPA Sample Processing and Analysis



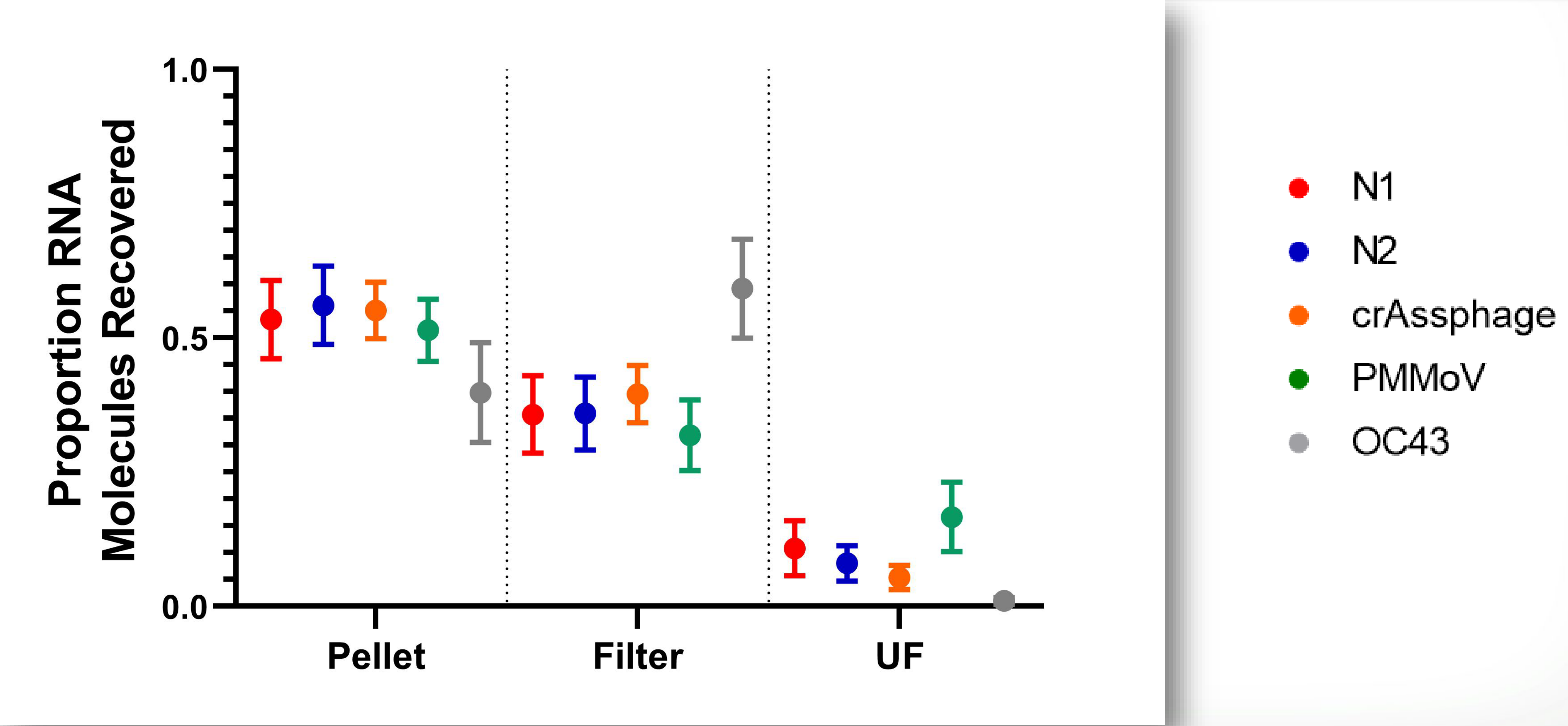
Method Performance Metrics

- **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%)



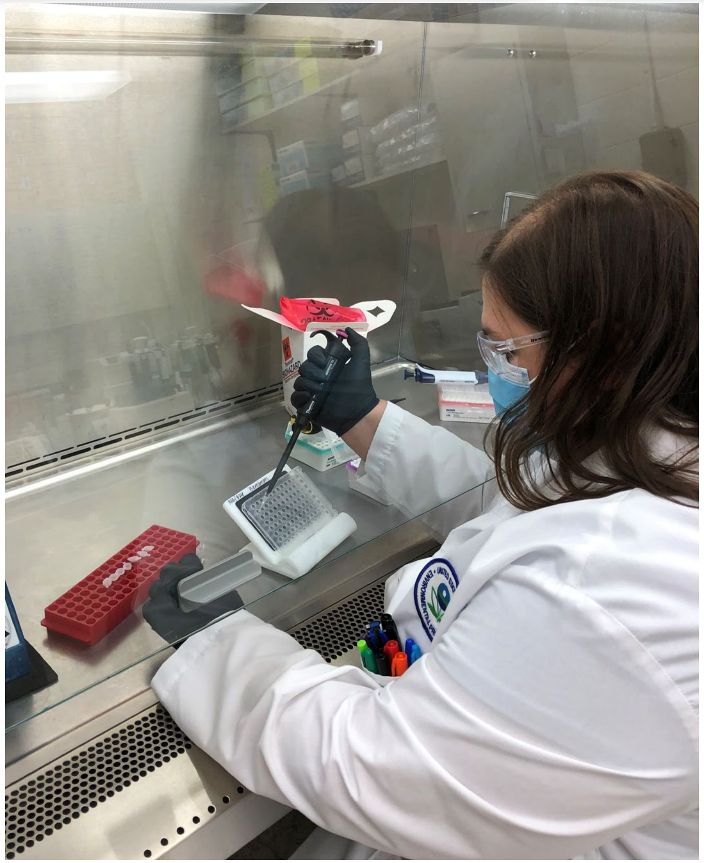
Chloe Hart

Recovery in Different Fractions



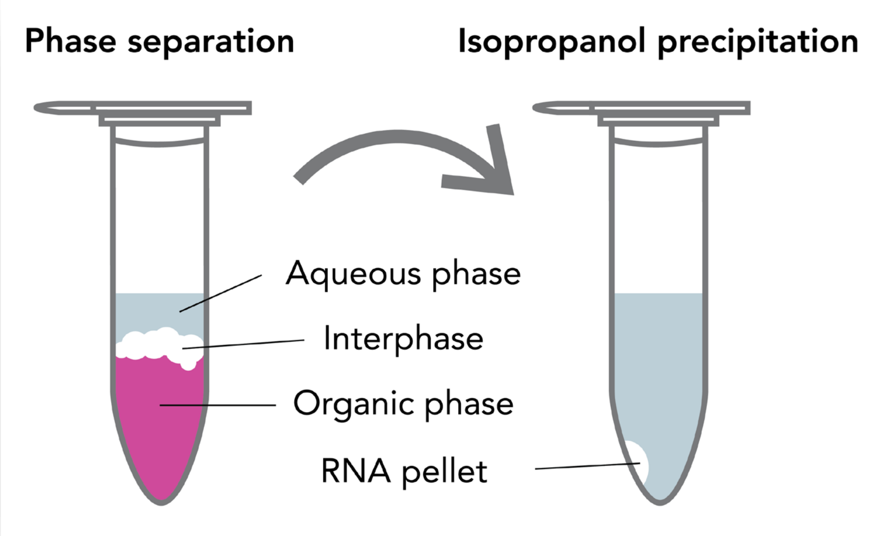
~ 90% measurable virus in pellet and filter fractions

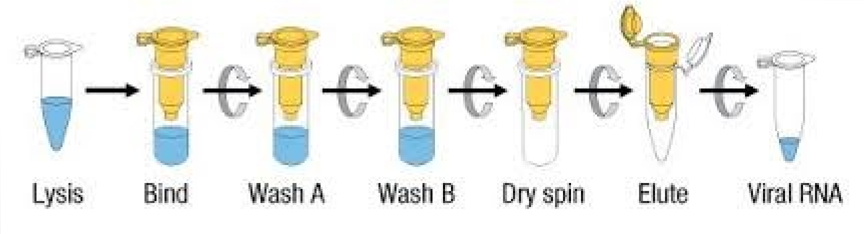
Alternative RNA Extraction



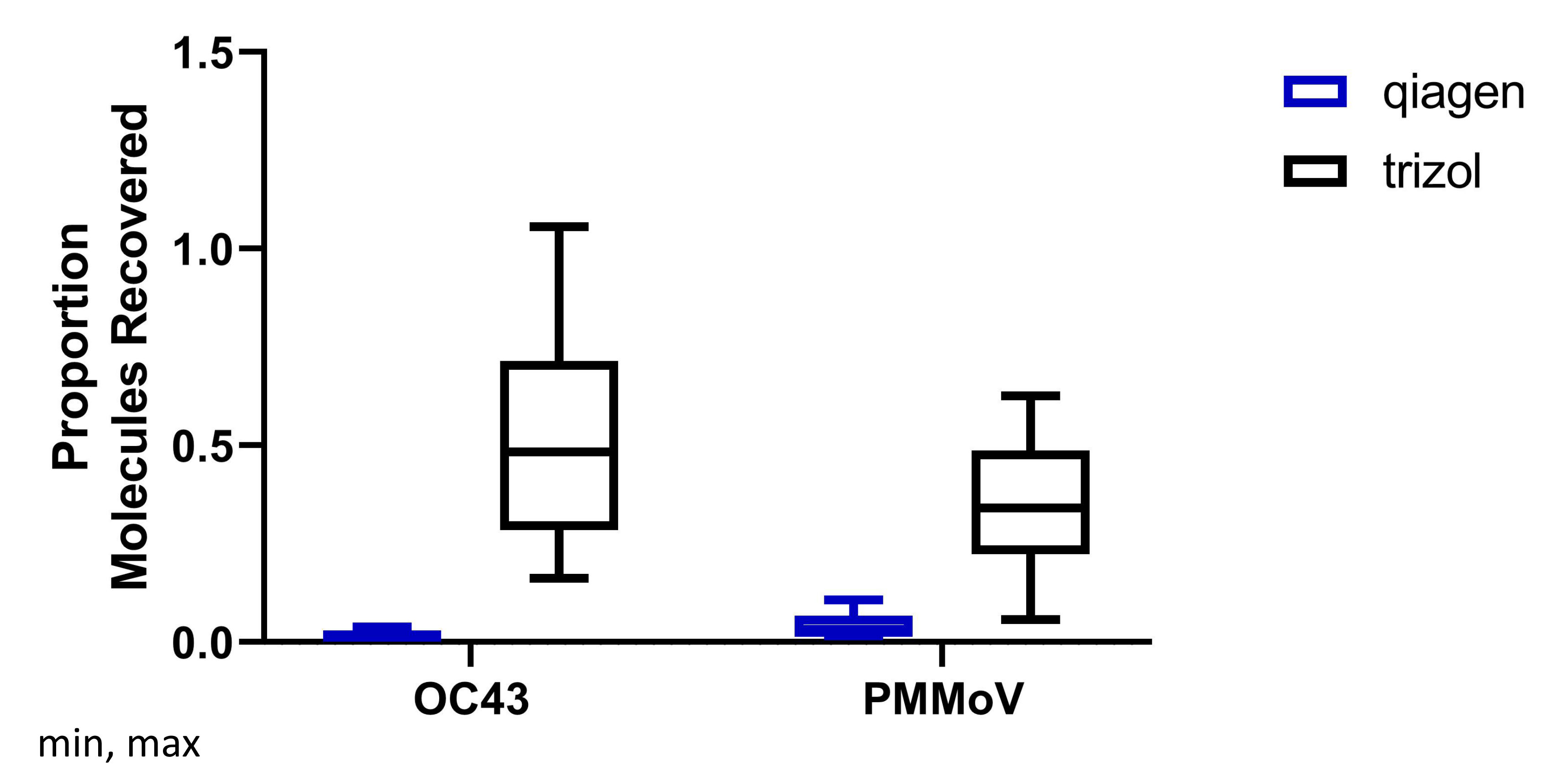
Emily Wheaton

Trizol-Chloroform Extraction
RNA precipitation





Recovery Efficiency



New extraction approach increased recovery efficiency 10 fold

Ohio Wastewater Monitoring Network (OWMN)

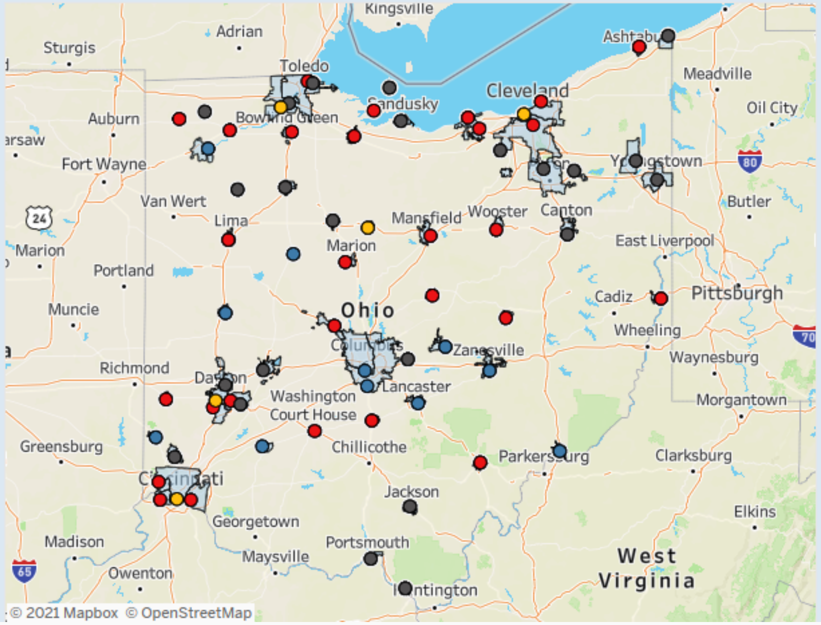
- **Statewide Network**
  - Started July 2020
  - Coordinated by Ohio Water Resources Center at OSU
  - Leveraged expertise and resources
  - 67 utilities, twice a week
  - 9 labs (university, commercial, private, government)
  - Dashboard with results updated daily
- **Goal**
  - Monitor trend of SARS-CoV-2 RNA in sewersheds
  - Screen for presence of SARS-CoV-2 variants of concern/interest (VOC/VOI) by sequencing and RT-ddPCR

Ohio Department of Health | Coronavirus (COVID-19)

COVID-19 Dashboard

Ohio Coronavirus Wastewater Monitoring Network

Wastewater Treatment Plant Locations and Boundaries

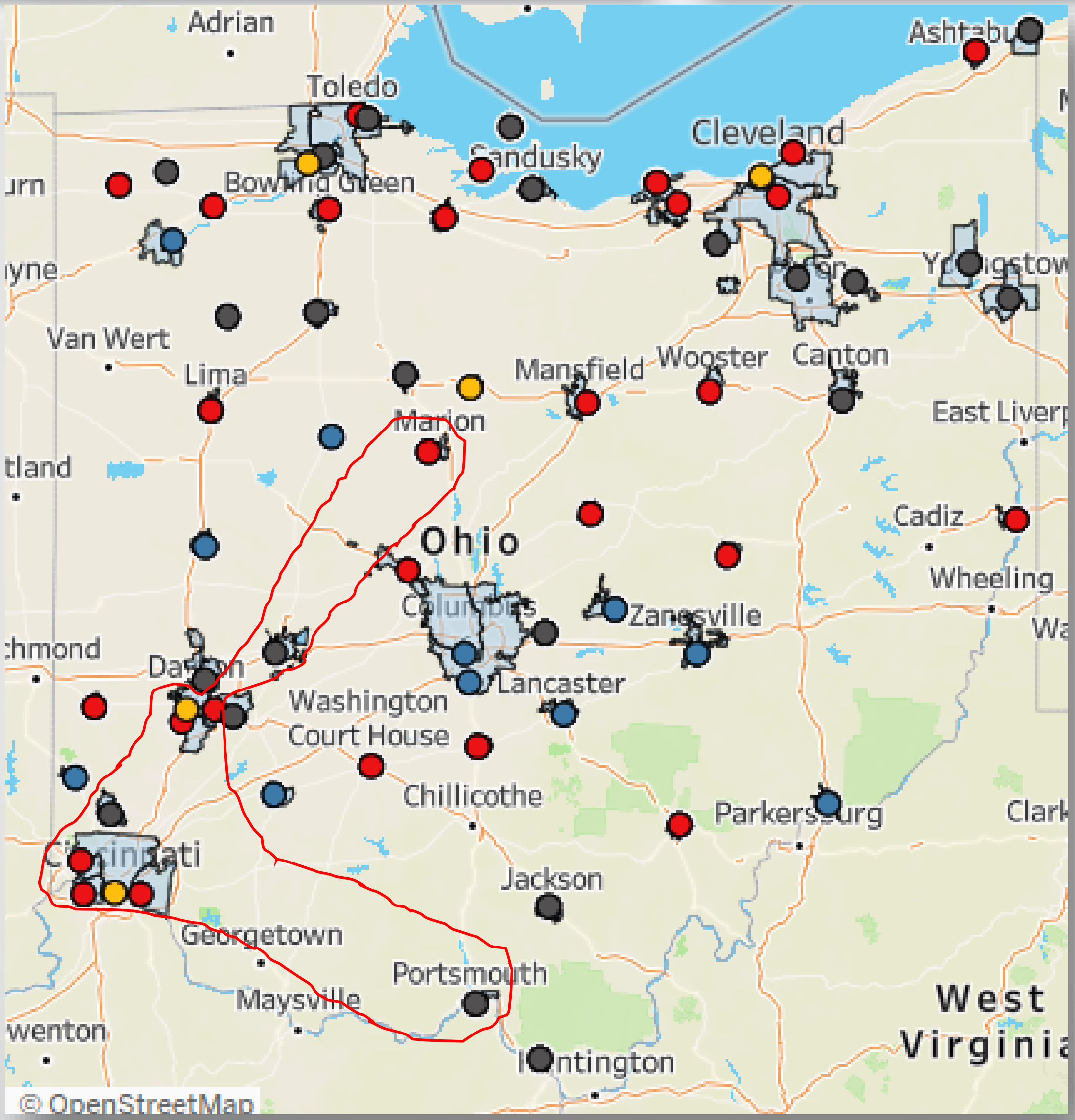


Athens WTP	↑
Circleville WWTP	↑
City of Marion WPC	↑
Eastern Regional WRF	↑
Little Miami WWTP	↑
Marysville WRF	↑
Muddy Creek WWTP	↑
Taylor Creek WWTP	↑
Western Regional WRF	↑
Port Clinton WWTP	↑
City of Eaton WWTP	↑
Washington Court House WWTP	↑
Mount Vernon WWTP	↑
City of Wooster WRRF	↑
Coshocton WWTP	↑
Elyria WWTP	↑

Legend: Red arrow ↑ = substantial increase (>100%), Yellow arrow ↗ = increase (50% to 100%), Gray arrow ↔ = steady (-49% to 49%), Blue arrow ↘ = decrease (<=-50%) [as of Aug 09, 2021]

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

US EPA Support to OWMN

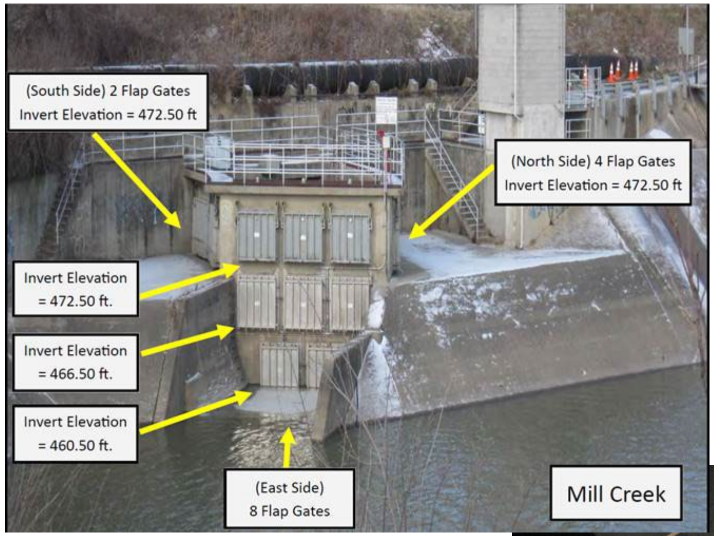


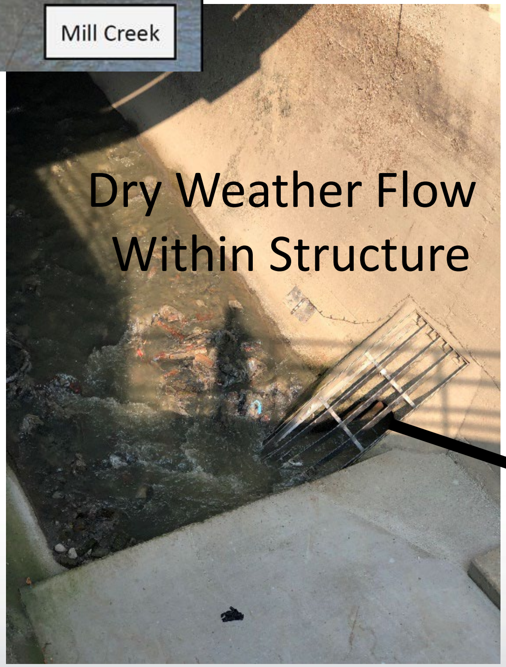
https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/other-resources/wastewater

- **Monitoring 12 sites**
  - 11 sewersheds
  - 1 subsewershed
- **1-2x weekly**
- **12-14 months of monitoring**
- **Quantitative data**
  - RT-ddPCR to assess community viral load and temporal trends
- **Qualitative data**
  - SARS-CoV-2 sequencing (full genome and spike gene) to screen for variants of concern (VOC) in communities
- **Interlab data comparisons**

Sub-Sewershed Sampling – Lick Run

Combined
Sewer
Overflow

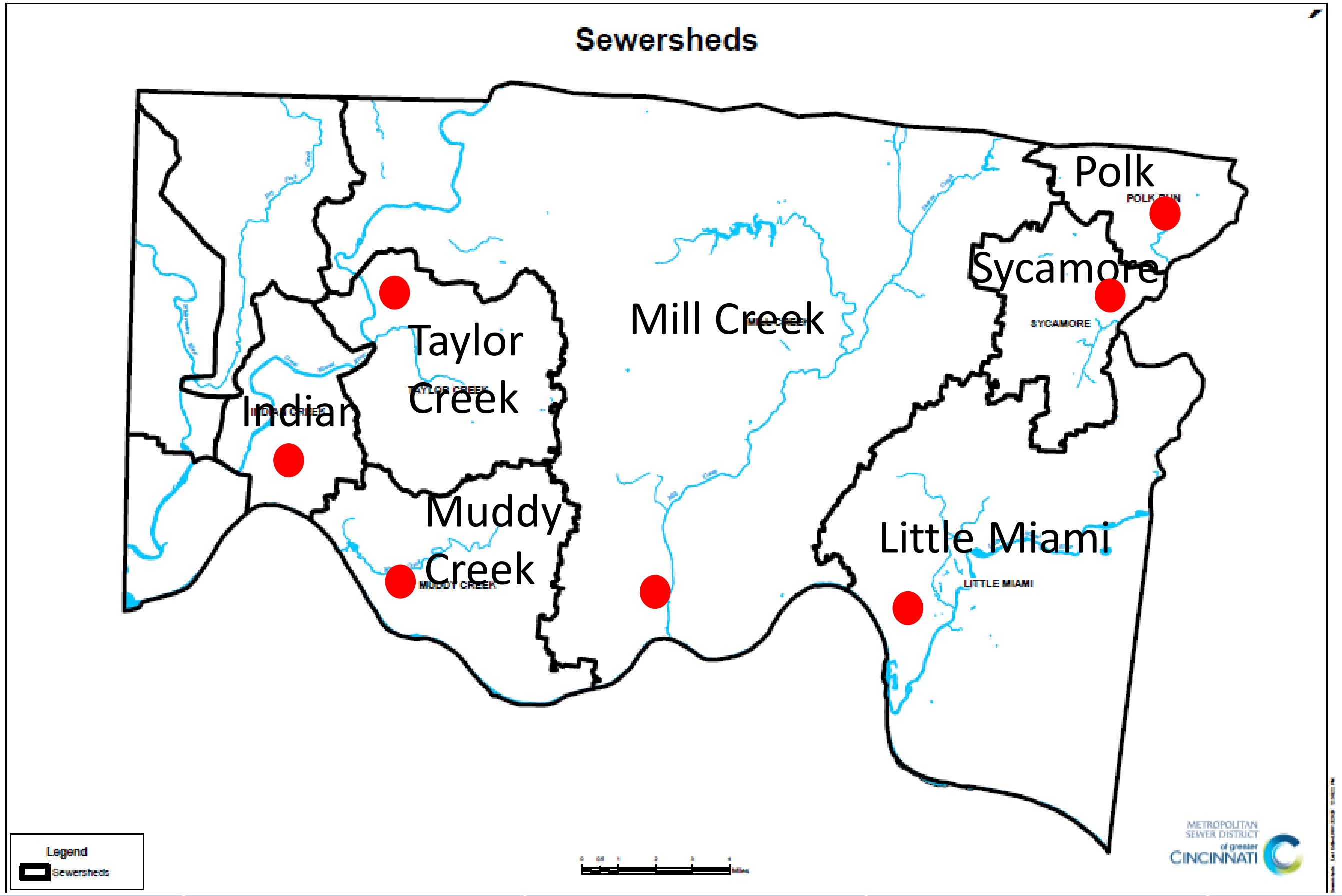




Remote Composite Sampler
~10L between 8-11 am
~500 ml every 15 min

Access to
Sewer

Metropolitan Sewer District of Cincinnati



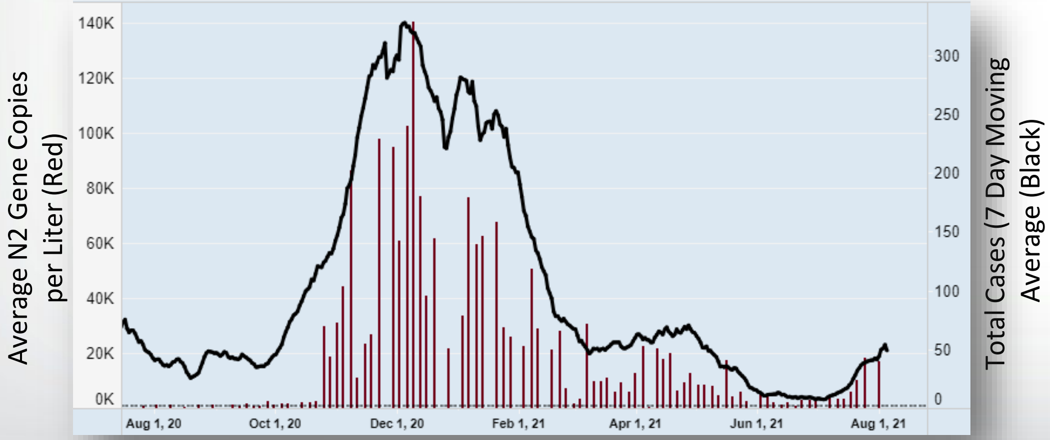
Sewershed	MGD	% Industrial	% Combined	Dilution
Mill Creek	118	5.0	40	0.5:1
Taylor Creek	3	0	0	1.8:1

OWMN Dashboard

Mill Creek WWTP	Levels Increasing	Calculated based on: 7/18/2021 - 8/1/2021

Viral Load

Wastewater and Clinical Data



- **Application**
  - The focus is on <u>trends or significant changes</u> in the number of viral gene copies detected at each site
  - Action is taken when at least 3 samples show a sustained increase of at least 10-fold (1 log)
- **Accomplishments**
  - Statewide network represents wastewater flow from nearly 5 million residents
  - 1 year of weekly data collected
  - Provided nearly 500 warnings to local health communities

Factors that influence the relationship between wastewater and clinical data

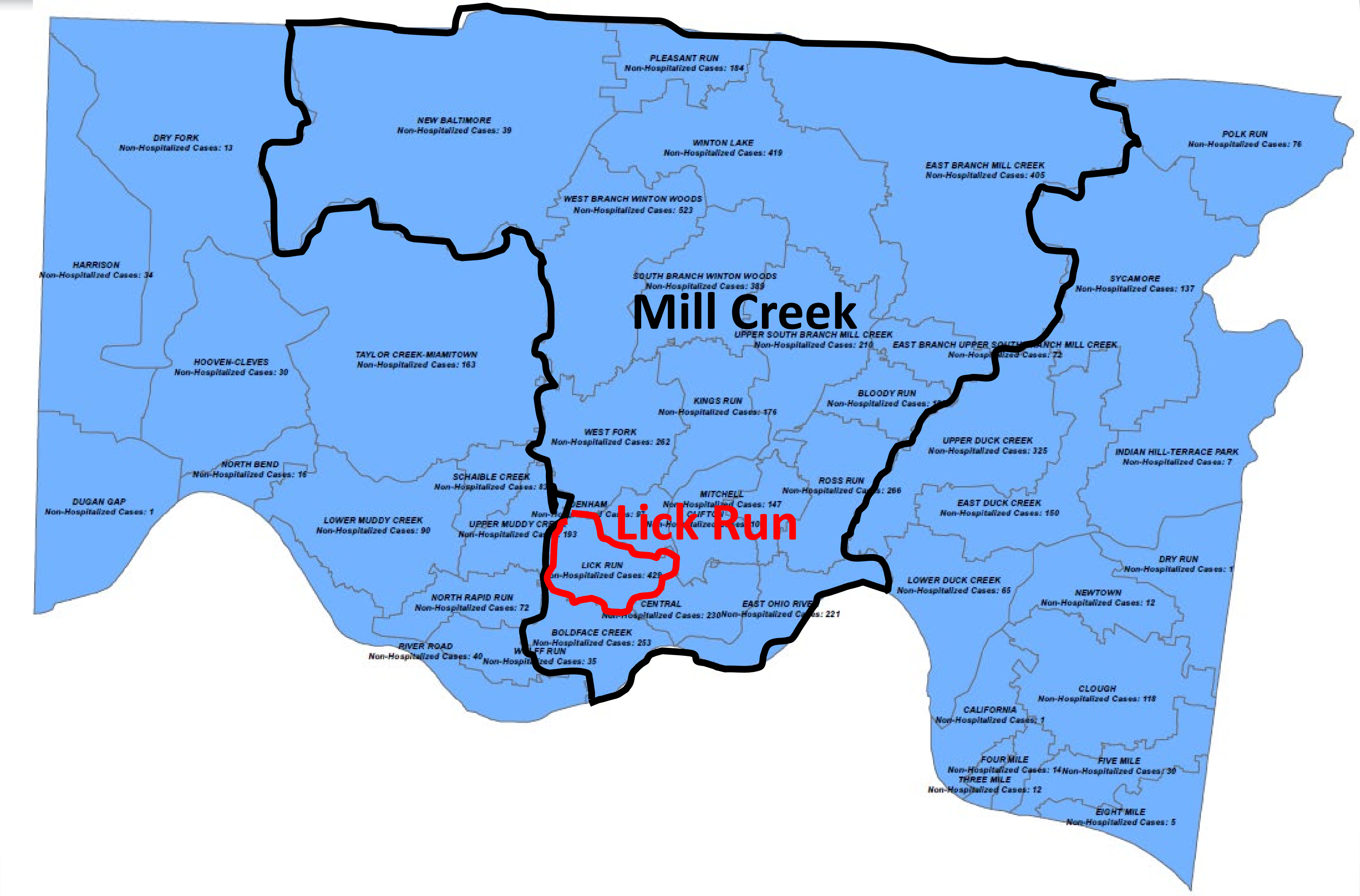
Factor	Factor Details	Data Parameters
Fecal shedding	Load, duration	Variable, up to 10^7 copies/g
Fate and transport of viral particle	Decay during conveyance	Decay rate
Sewershed Characteristics	Physical data	Flow, pH, temperature, TSS
Sampling Scheme	Frequency Type	Daily, weekly Grab, 24-hour flow-weighted composite
RNA Measurement	Concentration method Extraction method Processing recovery efficiency Analysis method Inhibition	PEG, HA filter, concentrating pipet Silica-column kits, magnetic bead kits, TRIzol OC43, BCoV, MHV RT-qPCR, RT-ddPCR Control RNA
Human Contribution	Fecal indicator organisms	Pepper mild mottle virus (PMMoV) crAssphage HF183
Health data	Point estimates from public health departments	Sewershed case counts

Case Study of Sewersheds

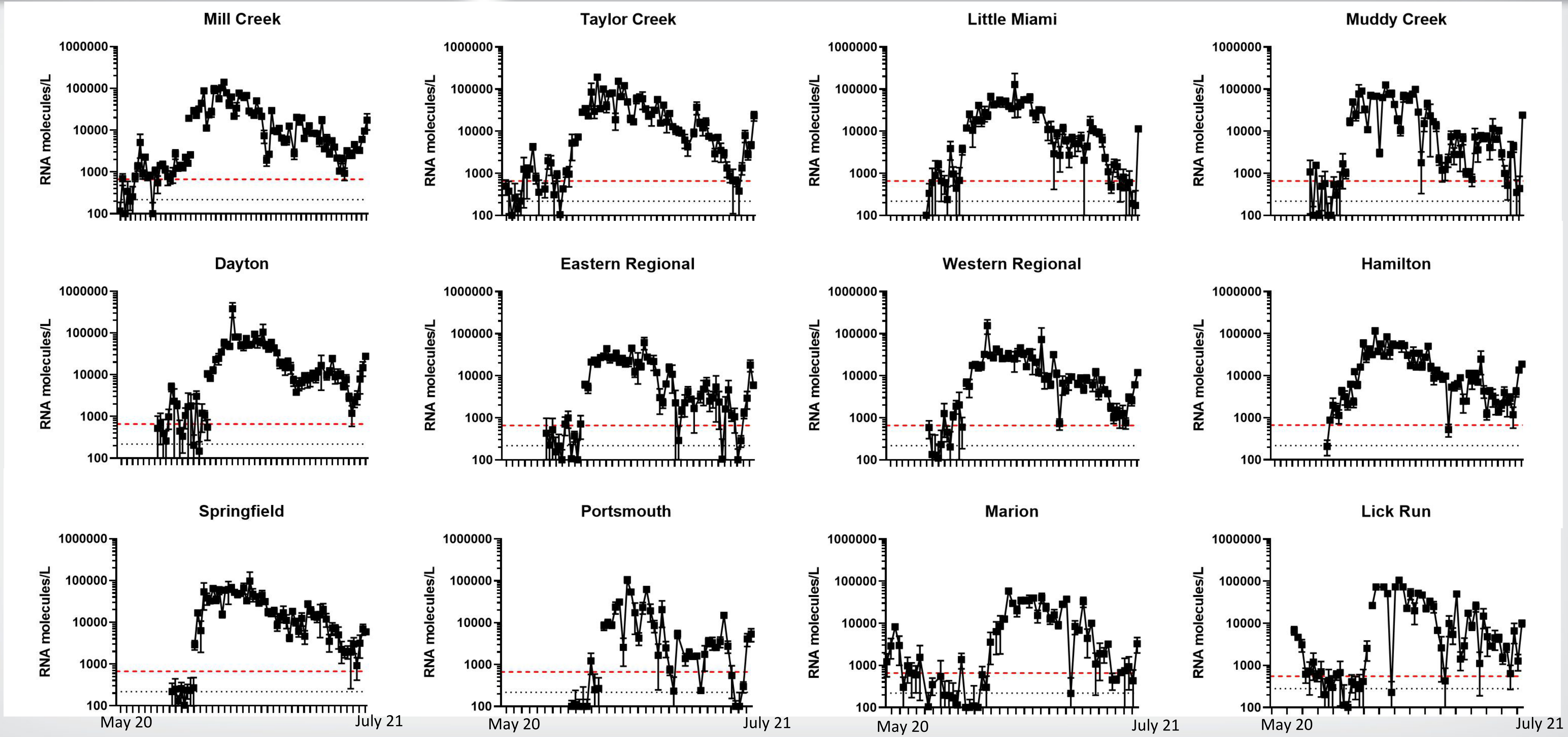
Parameter	Mill Creek	Taylor Creek
Population size	488,000	34,000
Mean MGD	93.17	2.79
MGD range	55.12-350.31	2.11-6.87
% Combined sewers	40	0
% Industrial flow	5	0
pH range	6.04-8.86	6.4-7.38
Mean TSS (mg/L)	247.87	340.96
TSS range	90-640	180-700

Do sewersheds with stormwater/industrial intrusion need to incorporate additional data parameters to understand relationship between wastewater and clinical case data?

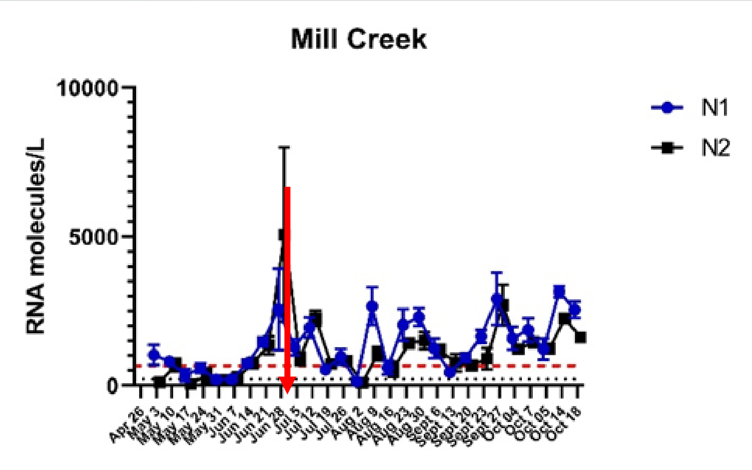
Sub-Sewershed Sampling: Cincinnati

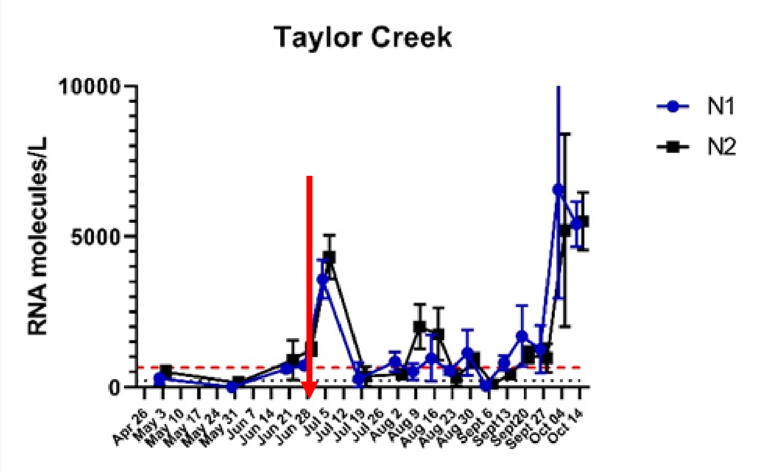


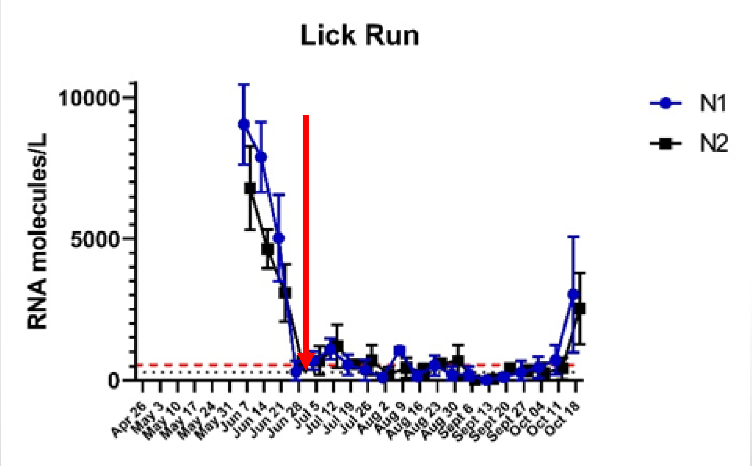
Wastewater SARS-CoV-2 RNA Trends: May 2020-July 2021



Different Views of Community Infection





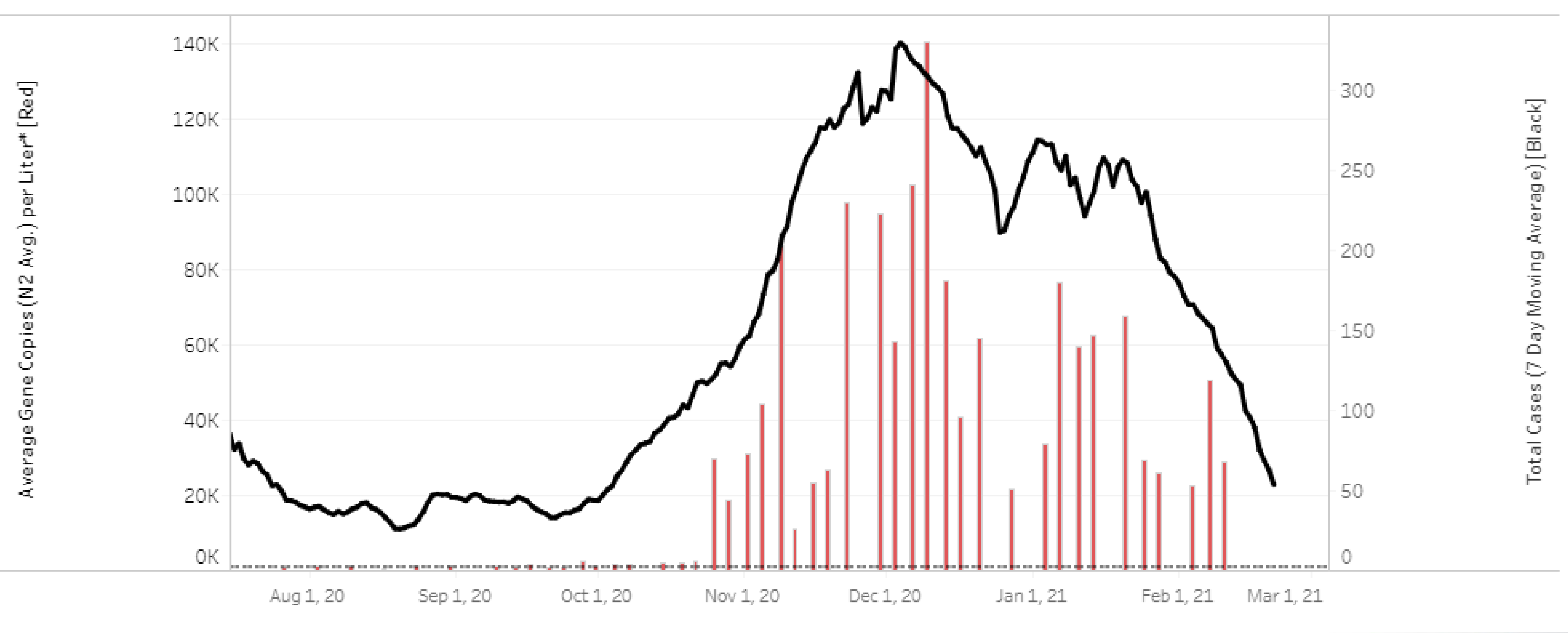


Potential role of sentinel sites?

Red Line – County Infection Peak in early July

Individual Site Example (Mill Creek)

From Dashboard



Wastewater Treatment Plant Locations and Boundaries

Wastewater RNA and Case Trend Data

May – October 2020

- N1 and N2 (raw and OC43-adjusted) concentrations were highly correlated (r = 0.87, BF_{10} >100)
- Raw and OC43-adjusted N1/N2 concentrations were strongly correlated (r = 0.64, BF_{10} > 100)
- Peak clinical cases: Mill Creek (7/27), Taylor Creek (8/3)
- Wastewater RNA peaked 1-2 weeks prior to peak in reported clinical data

Nagarkar et al., submitted

Flow Adjust Gene Copies vs. Sewershed Case Rates*

* 7 day rolling average

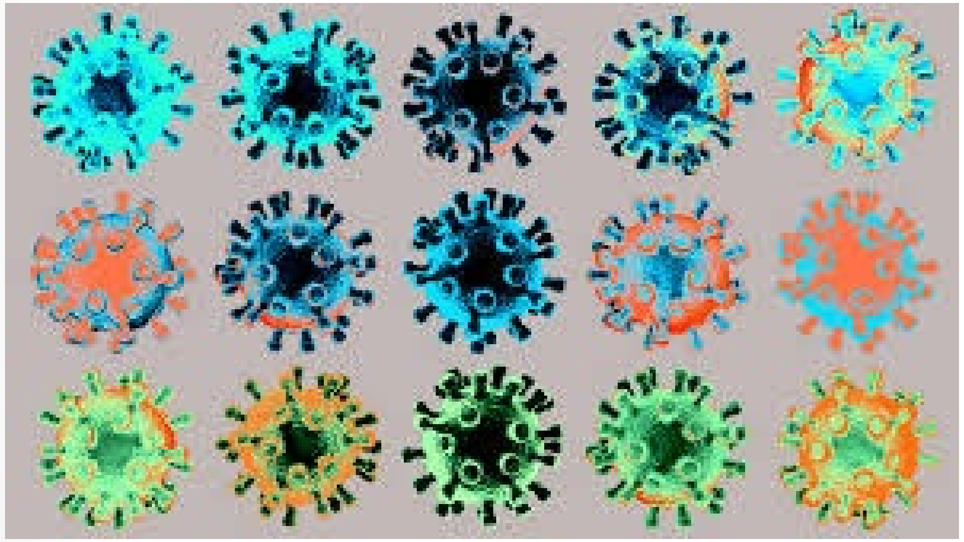
Site_name	R2 Load	p value Load	Lab	Population Served
Mill Creek WWTP	0.689	5.46E-13	USEPA	488,000
Eastern Regional WRF	0.674	2.85E-11	USEPA	36,150
Hamilton WRF	0.584	6.02E-09	USEPA	65,000
Little Miami WWTP	0.530	3.16E-08	USEPA	143,000
Muddy Creek WWTP	0.469	2.08E-07	USEPA	76,000
Springfield WWTP	0.495	5.83E-07	USEPA	60,000
Taylor Creek WWTP	0.442	1.14E-06	USEPA	34,000
City of Marion WPC	0.515	5.53E-06	USEPA	36,000
Dayton WWTP	0.318	1.97E-05	USEPA	269,850
Portsmouth Lawson Run WWTP	0.482	8.32E-05	USEPA	20,366
Western Regional WRF	0.222	1.42E-03	USEPA	79,000

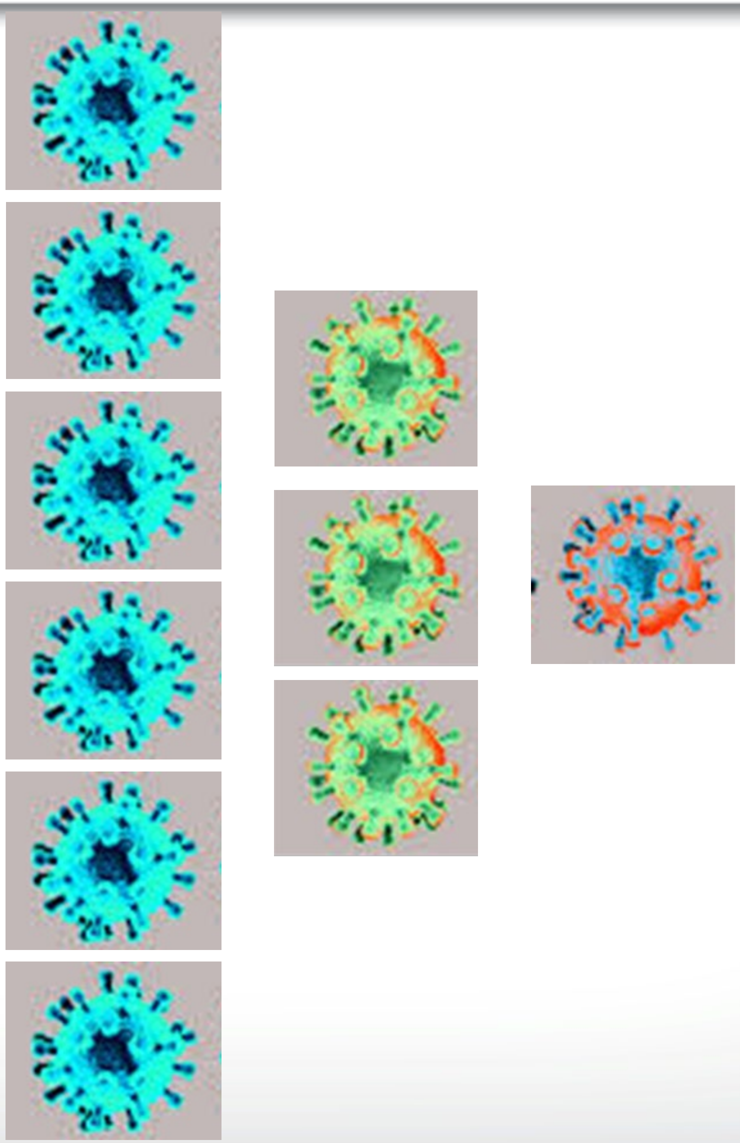
Optimization Areas

- **Analytical Method Development**
  - Predominantly particle associated
  - RNA extraction is critical step; standard kits show relatively low recovery
  - Quality Control for assessing method performance (recovery efficiency, inhibition control)
- **Dilution/Degradation in Sewer System**
  - Ongoing comparison of different approaches to normalize for dilution, flow adjusted can be used in standard composite sampling
  - Use existing temperature dependent rates, targeted studies on industrial wastes
- **Relation of Sewer Signal to Infection rates**
  - Statistical models show good correlation
  - Mechanistic models still under development to predict specific # of infected individuals (need better data on shedding rates)

Actual Distribution of Variants within a Community

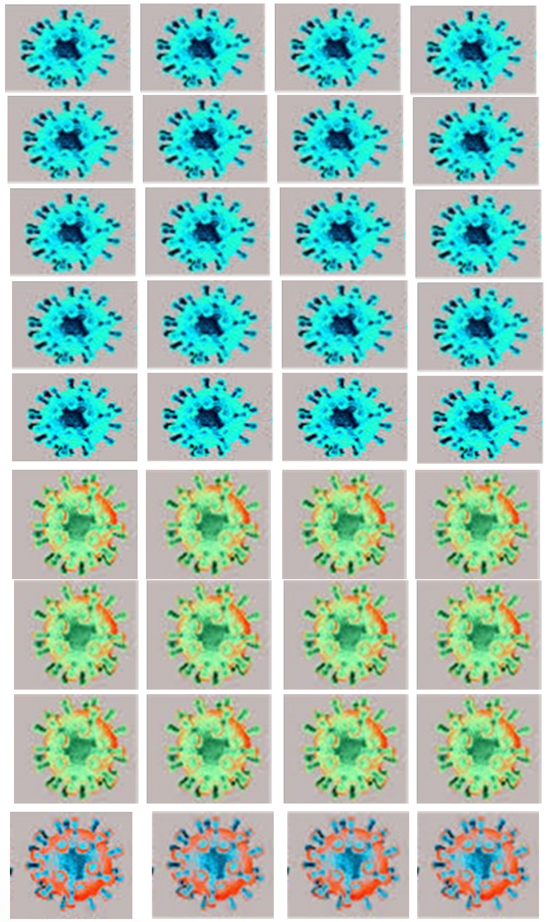
Range of Existing Variants of SARS-CoV-2





Estimating Variants in the Community by Analyzing COVID Patients

Patient 1
Patient 2
Patient3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9



Clinical samples with **enriched, clonal** type of SARS-CoV-2*

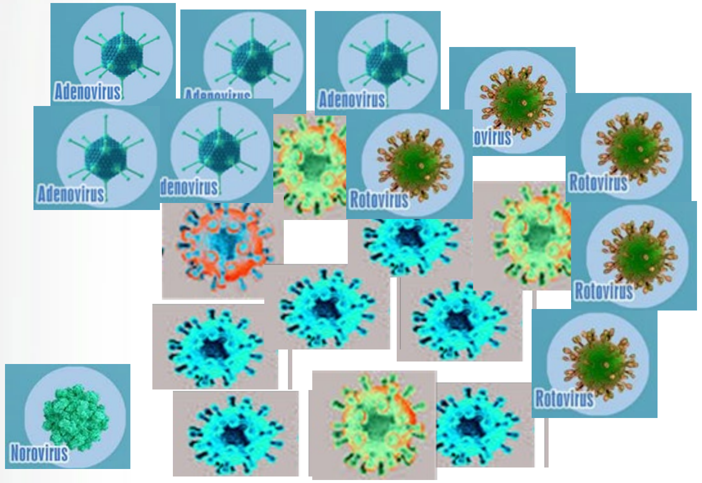
Extraction & Sequencing

* 10^4-10^7 copies per ml in throat/sputum samples

Patient 10-xxxx

(Pan et al. Lancet Infect. Dis. 20(4):411-412

Estimating Variants in the Community by Analyzing Wastewater



Single, diverse, unenriched
wastewater sample

Up to 10^5 per L N1 or N2 gene copies
~10^8 gene copies per L of crassphage
Many other human and non-human related viruses

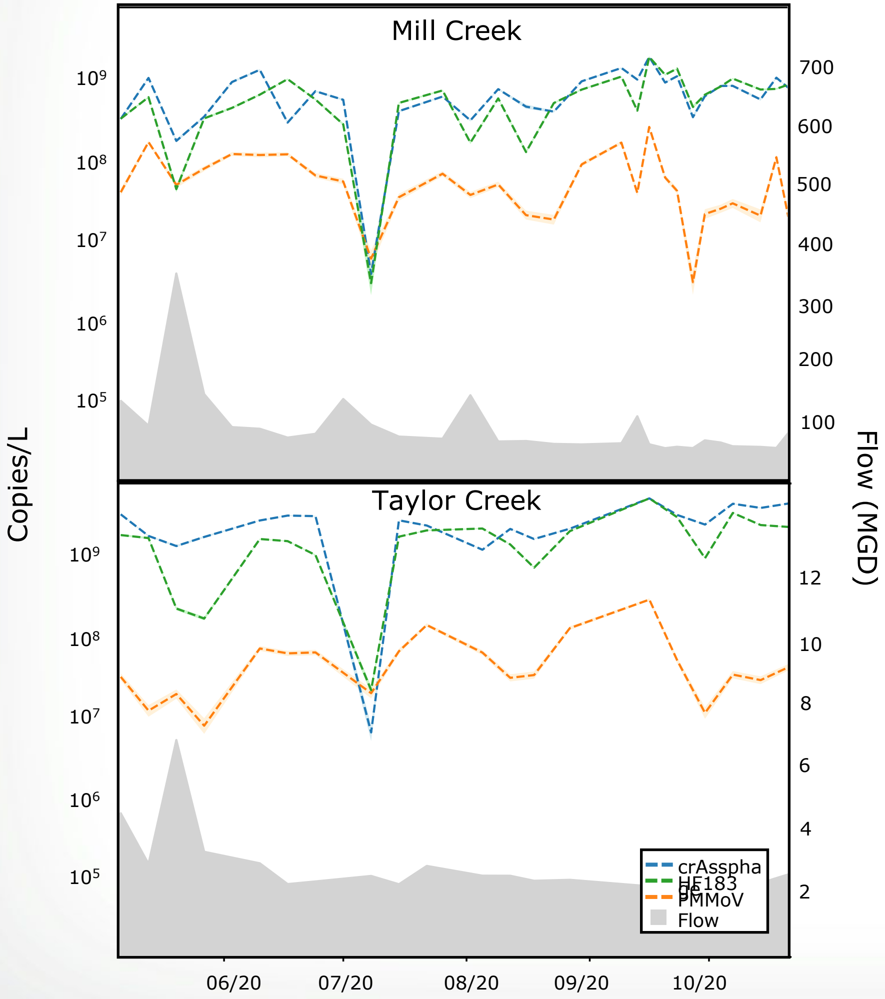
Extraction (if threshold levels)

Targeted PCR for mutations in variants of concern

Enrichment of SARS-CoV-2 Genome (multiplexed PCR)

Sequencing

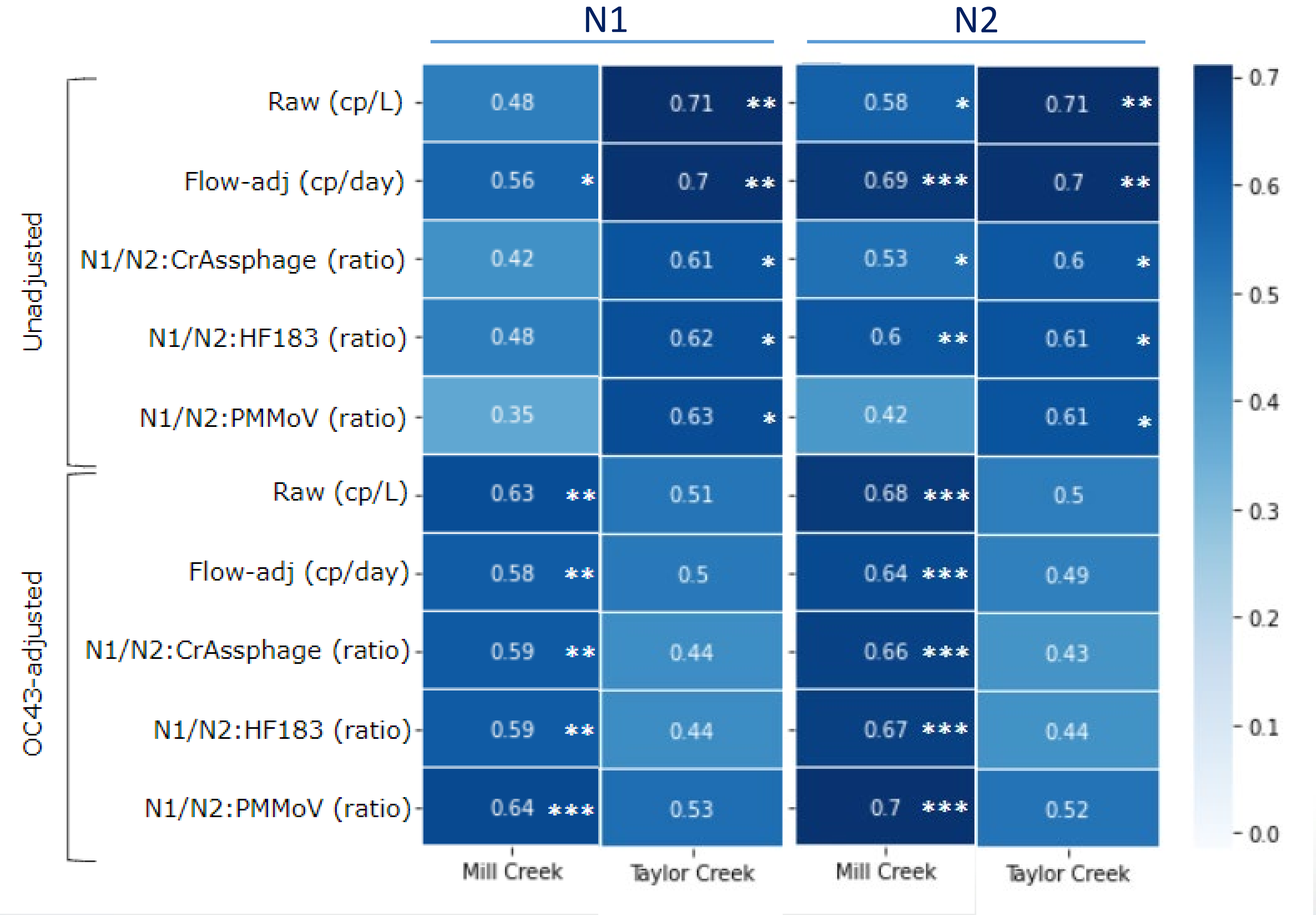
Fecal Indicators, Flow and TSS



		Mill Creek		Taylor Creek	
		Pearson's r	BF_{10}	Pearson's r	BF_{10}
crAssphage	HF183	0.9	***	0842	***
crAssphage	PMMoV	0.562	*	0.291	
PMMoV	HF183	0.455		0.559	
Flow (MGD)	crAssphage	-0.3		-0.062	
Flow (MGD)	PMMoV	0.054		-0.291	
Flow (MGD)	HF183	-0.502		-0.341	
TSS (mg/L)	crAssphage	0.318		-0.141	
TSS (mg/L)	PMMoV	0.085		-0.053	
TSS (mg/L)	HF183	0.359		-0.244	

Nagarkar et al., submitted

Correlations of Wastewater and New Case Data

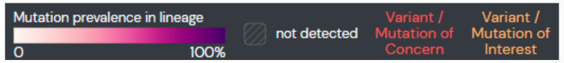


* = Bayes Factor $(BF)_{10} > 10$; ** = $BF_{10} > 30$; *** = $BF_{10} > 100$

Nagarkar et al., submitted

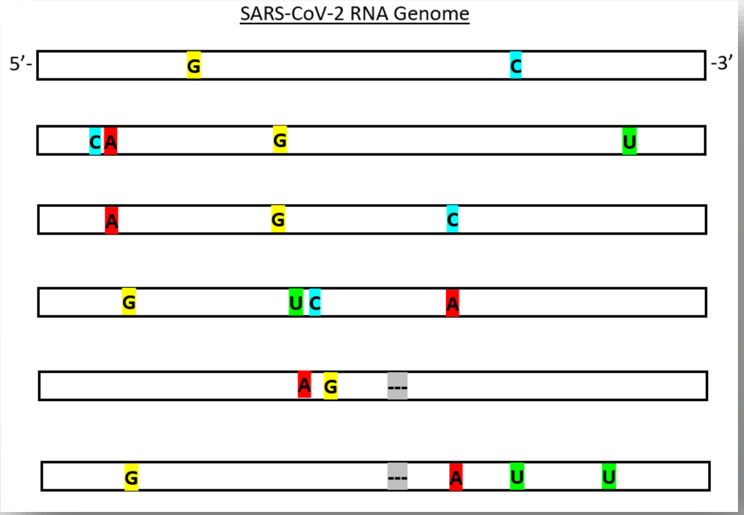
Variants of Concern (VOC) in Wastewater

S https://outbreak.info [as of Aug 09, 2021]



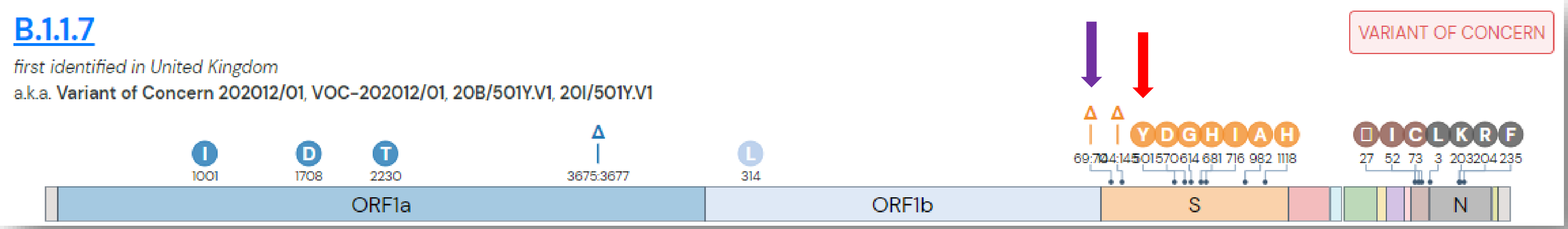
★ = CDC identified VOC

- Wastewater contains a mixture of variant genomes from human populations
- 2 approaches: RT-ddPCR mutation assays and tiled amplicon sequencing
- ODH is focused on key mutations in spike gene
- Results may be used by ODH to direct resources for clinical sequencing

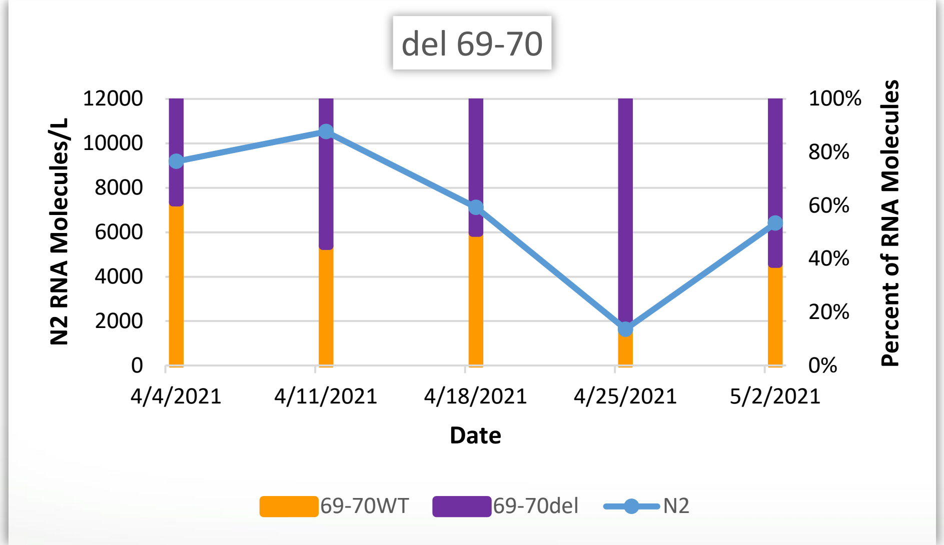


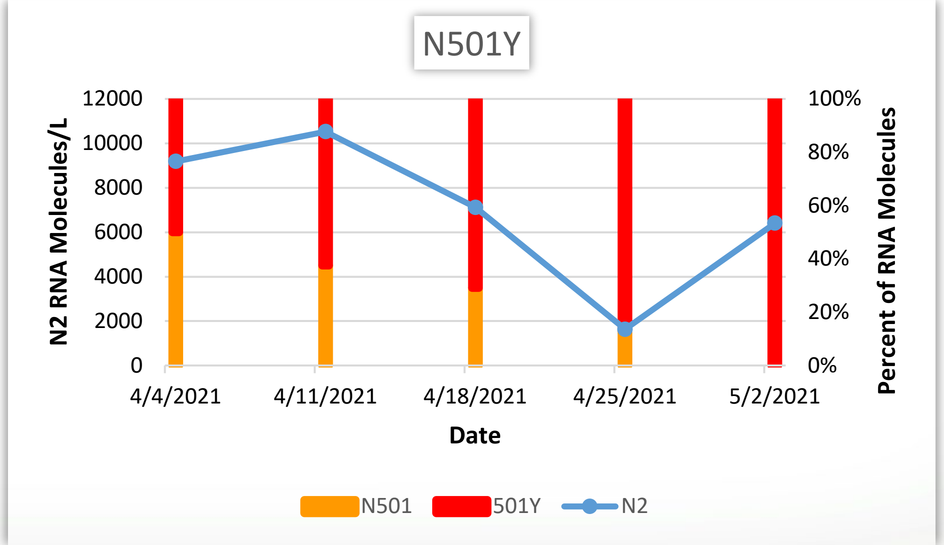
RT-ddPCR Mutation Assays

RT-ddPCR was used to monitor signatures of B.1.1.7 (Alpha) by detecting Spike: DEL69/70 and 501Y



https://outbreak.info

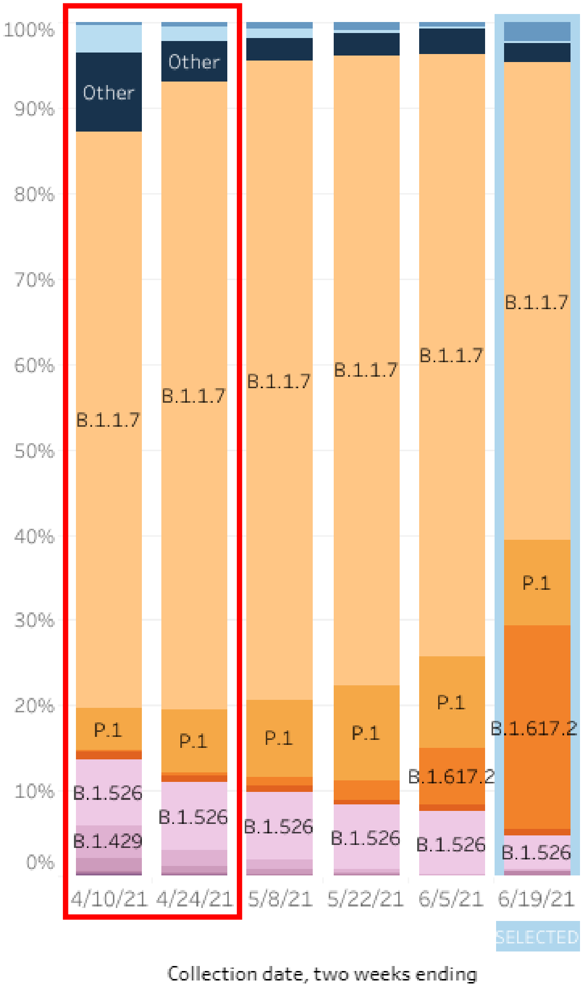




RNA sequences with deletions of nucleotides that result in absence of spike aa 69-70 increase over time

RNA sequences with nucleotides that change spike aa 501 increase over time

US Region 5 Distribution of B.1.1.7



Region 5 - Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin

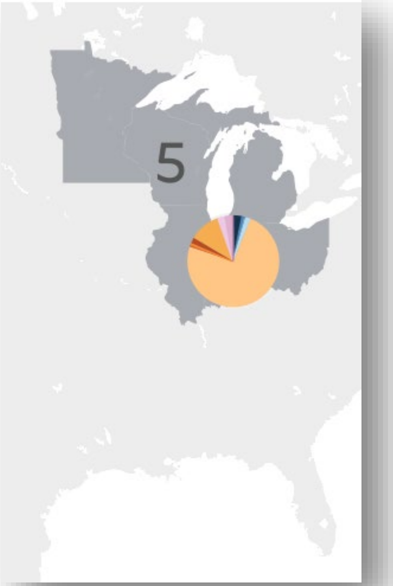
	Lineage	Type		%Total	95%CI
Most common lineages #	B.1.1.7	Alpha	VOC	56.0%	43.5-
	B.1.617.2	Delta	VOC	23.9%	15.0-
	P.1	Gamma	VOC	10.0%	5.4-1
	B.1.526	Iota	VOI	4.0%	1.7-9
	B.1			2.2%	1.1-4
	B.1.1.519	†		0.0%	NA
Additional VOI/VOC lineages #	B.1.351	Beta †	VOC	0.6%	0.3-1
	B.1.525	Eta †	VOI	0.6%	0.1-3
	B.1.427	Epsilon †	VOI	0.2%	0.0-2
	B.1.429	Epsilon †	VOI	0.0%	NA
	B.1.617.1	Kappa †	VOI	0.0%	NA
	B.1.617.3	†	VOI	0.0%	NA
	P.2	Zeta †	VOI	0.0%	NA
Other*	Other			2.4%	1.1-5

* Other represents >200 additional lineages, which are each circulating at <1% of viruses

† Fewer than 10 observations of this variant during the selected time/location context

Sublineages of P.1 and B.1.351 (P.1.1, P.1.2, B.1.351.2, B.1.351.3) are aggregated with the parent lineage and included in parent lineage's proportion. AY.1 and AY.2 are aggregated with B.1.617.2.

https://covid.cdc.gov/covid-data-tracker/#variant-proportions



Date	Human B.1.1.7% (95% CI)	Sewershed % del69/70¶	Sewershed % 501Y¶
4/10/21	66.7 (53.1-78.0)	58.2(38.9-77.5)	69.4(58.3-80.5)
4/24/21	73.2 (60.3-83.1)	72.2(59.5-84.9)	76.9(68.6-85.2)

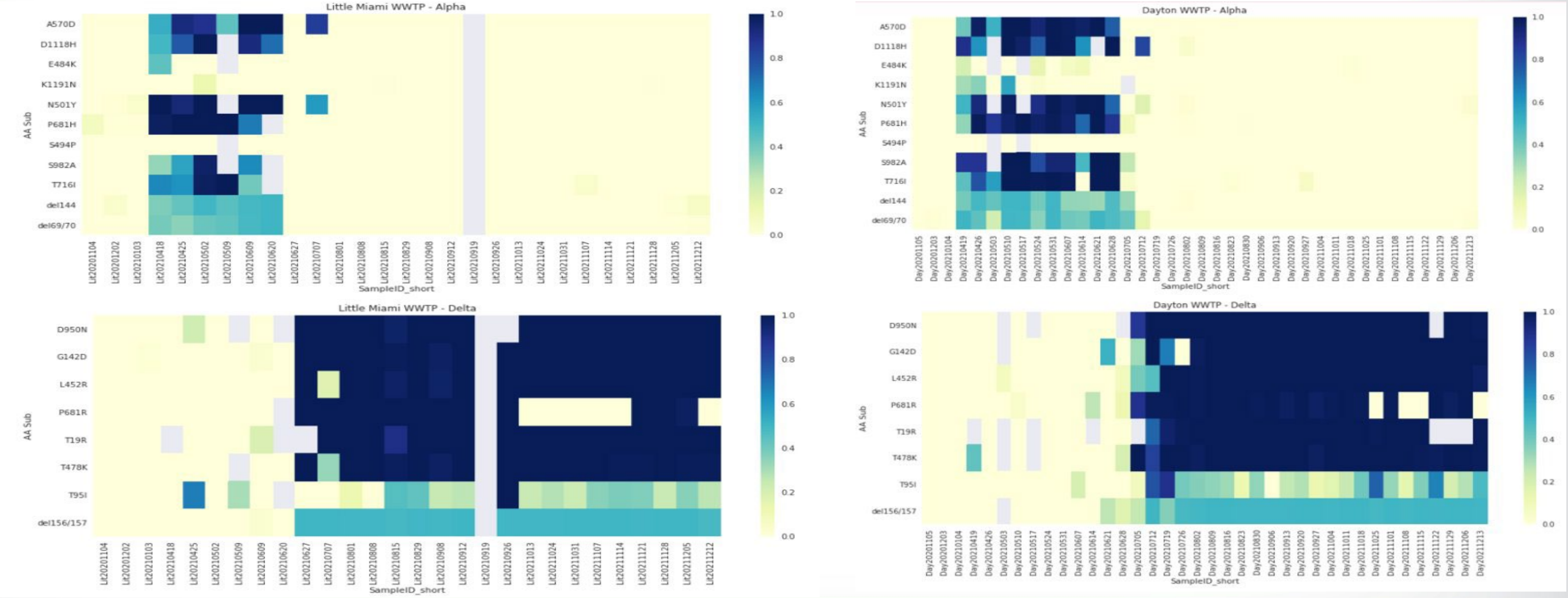
¶average of 10 Ohio sewersheds during sample dates April 11,12 and April 25,26
April 4 and 5: 69/70del 30.3(7.8-52.8); 501Y 33.5(13.5-53.5)

Alpha (B.1.1.7) and Delta (B.1.617.2) Signatures in Wastewater

Tiled amplicon sequencing from November 2020 through December 2021

Alpha (B.1.1.7)

Delta (B.1.617.2)



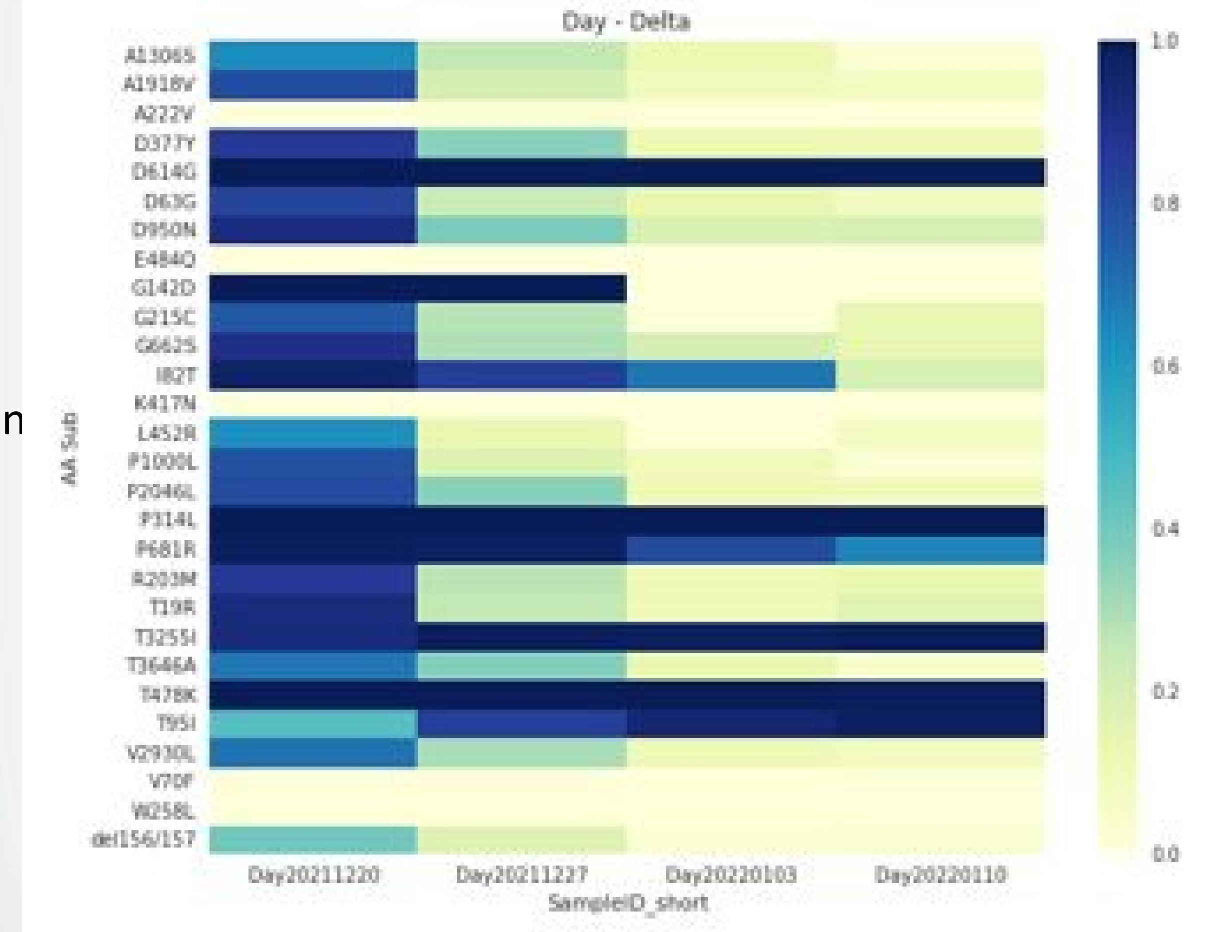
Little Miami

Dayton

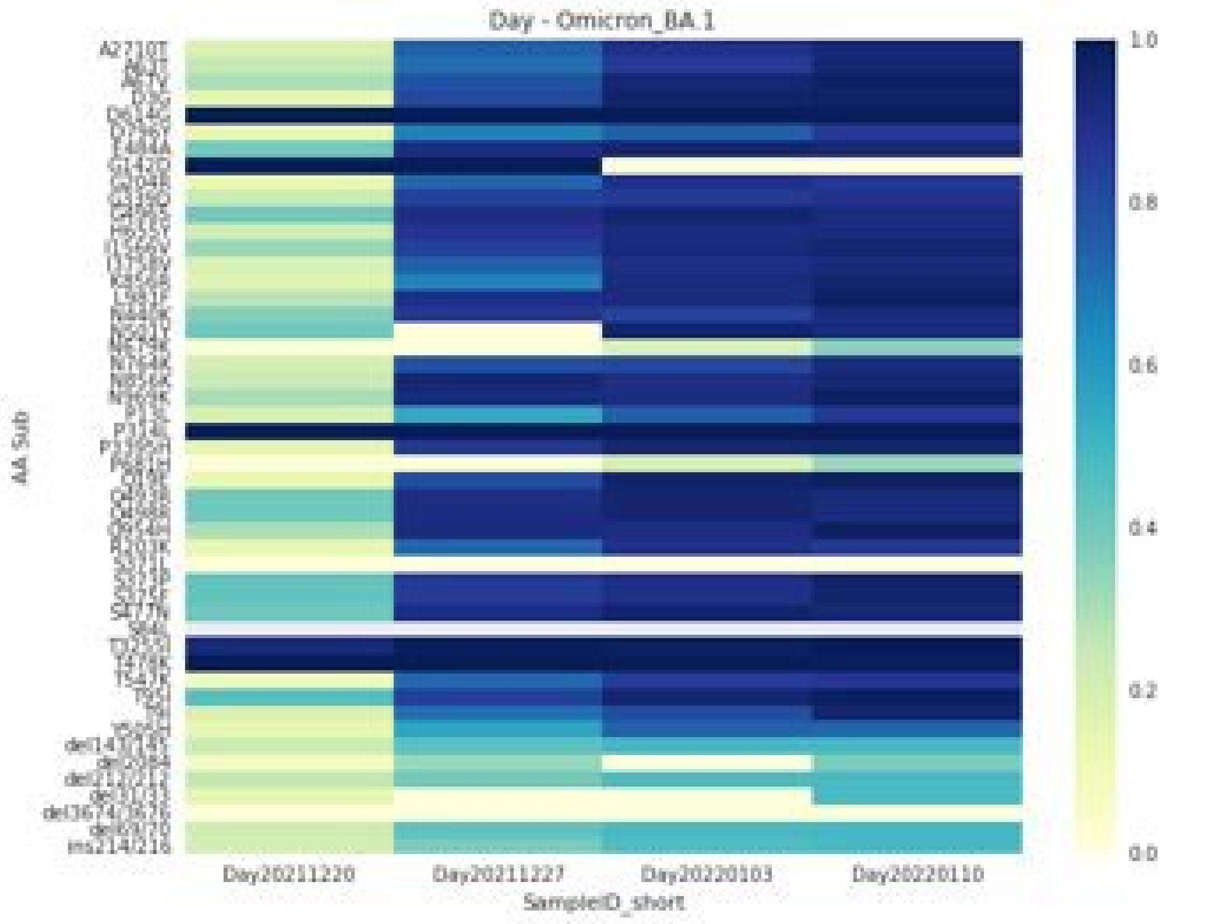
Delta (B.1.617.2) and Omicron (BA.1) Signatures in Wastewater

Tiled amplicon sequencing from November 2021 through January 2022

Dayton



Delta
(B.1.617.2)



Omicron
(BA.1)

Summary and Conclusions

- Wastewater sampling has enabled large-scale monitoring of SARS-CoV-2 dynamics and has successfully enabled the Ohio Health Department to notify local communities when interventions are needed
- Understanding the relationship between 2 imperfect data sets is complex
- SARS-CoV-2 RNA concentrations adjusted to account for recovery efficiency and human fecal input increased correlation with new case data in a large sewershed with stormwater and industrial intrusion, but not in a small sewershed with no outside intrusion
- ddPCR mutation assays show that the ratio of the 69/70 deletion and N501Y alternative allele (associated with Alpha variant) increasing over time and corresponds with increase in B.1.1.7 proportion of clinical data in US Region 5
- Sequencing confirms presence of amino acid changes associated with Alpha and Delta variants in wastewater samples and is consistent with proportion of clinical data in US Region 5