



Quantitative and Qualitative Environmental Surveillance of SARS-CoV-2 in Ohio

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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

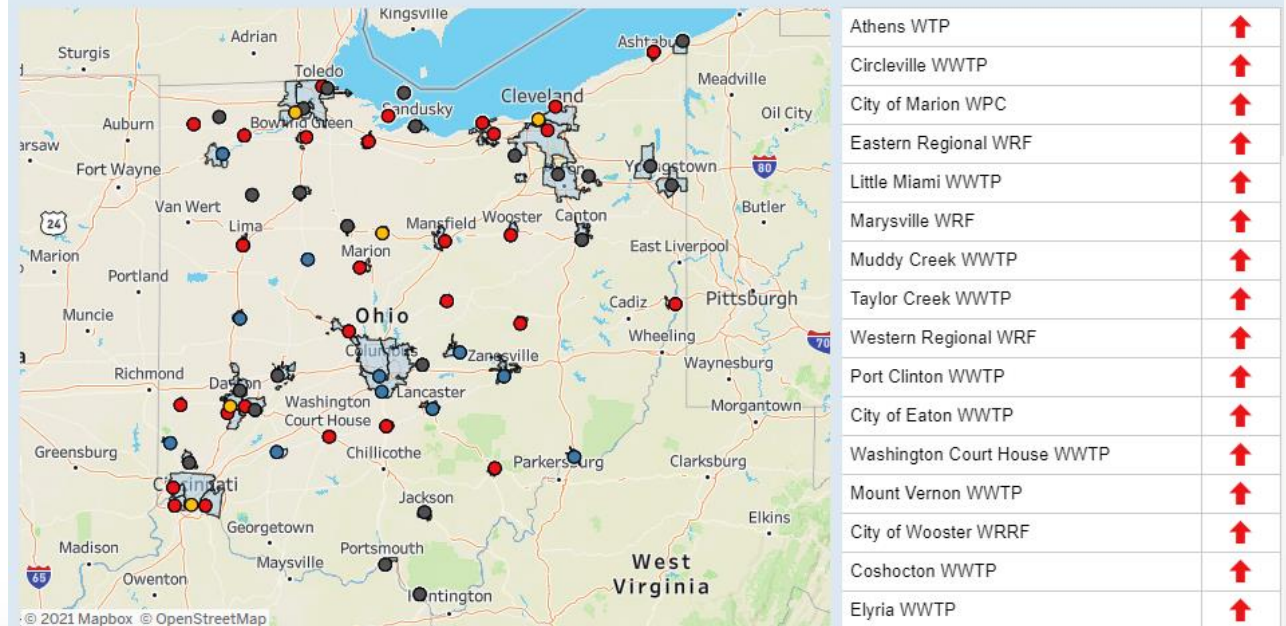
- **Goal**

- 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

COVID-19 Dashboard

Ohio Coronavirus Wastewater Monitoring Network

Wastewater Treatment Plant Locations and Boundaries



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>



OWMN Dashboard

Mill Creek WWTP

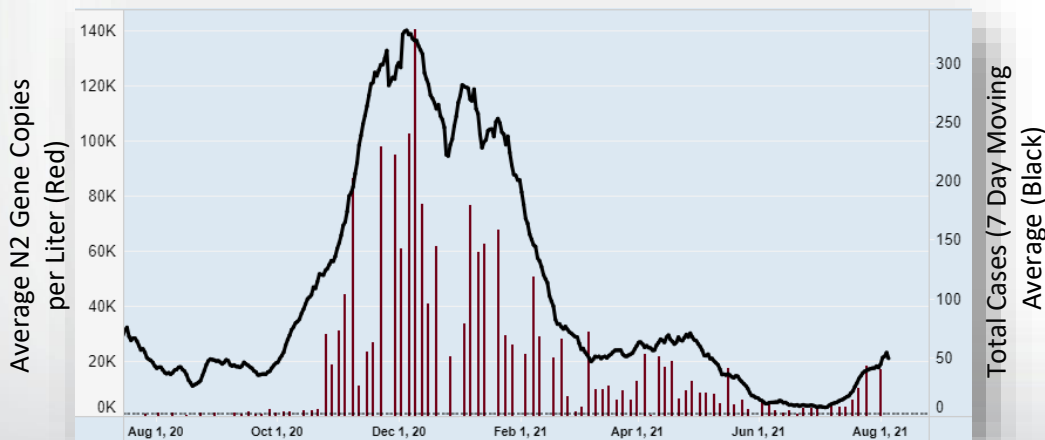
Levels Increasing

Calculated based on: 7/18/2021 - 8/1/2021

Viral Load



Wastewater and Clinical Data



- **Application**

- The focus is on trends or significant changes 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



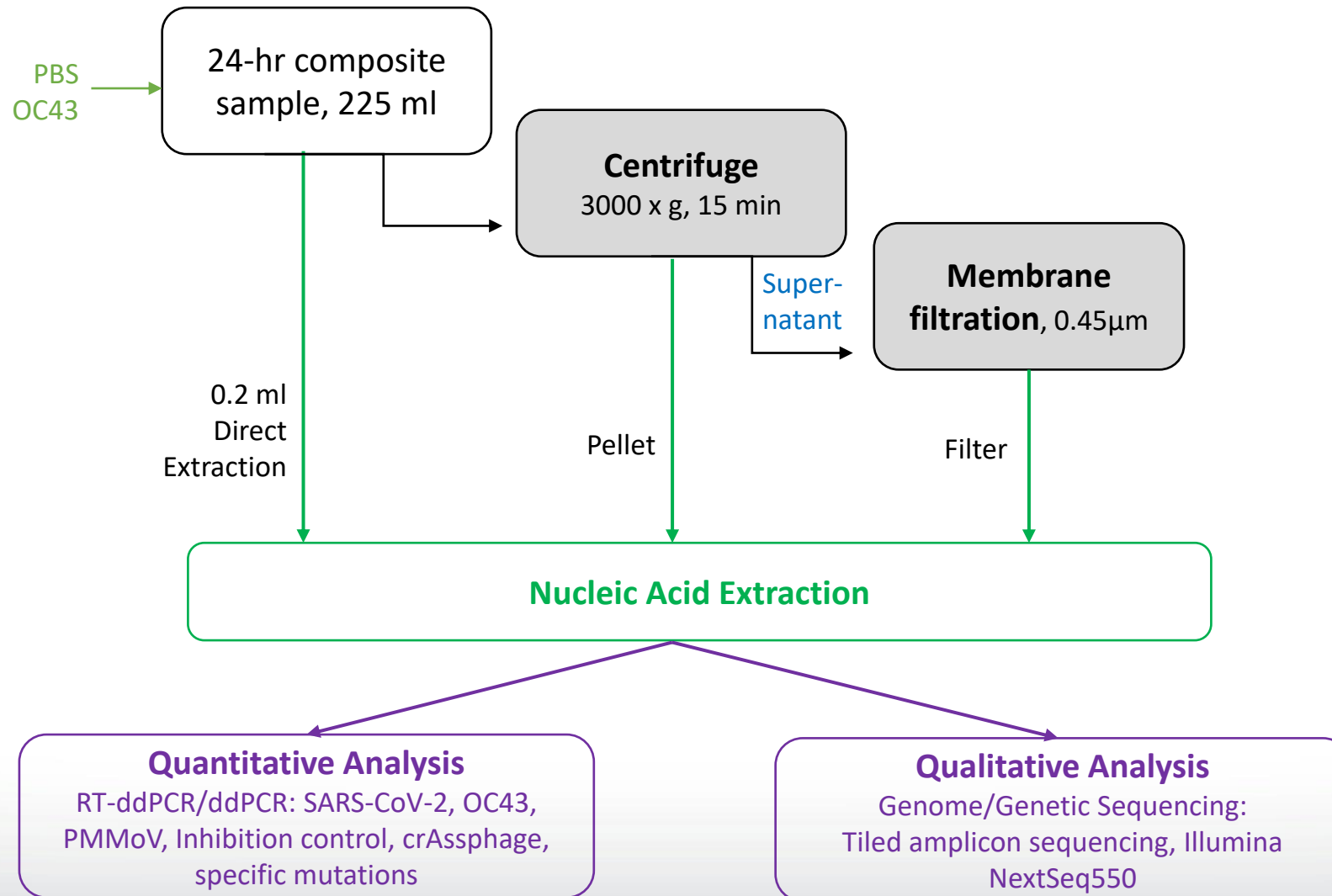
US EPA Support to OWMN



- **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**

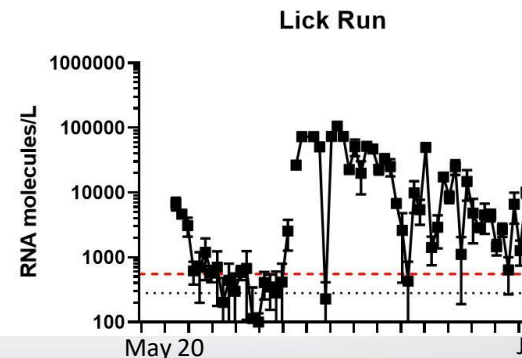
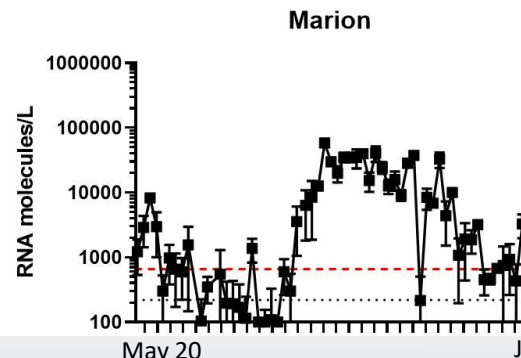
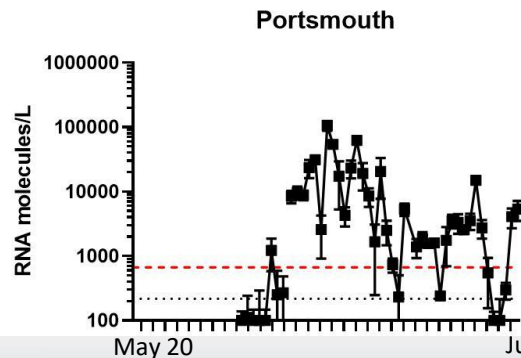
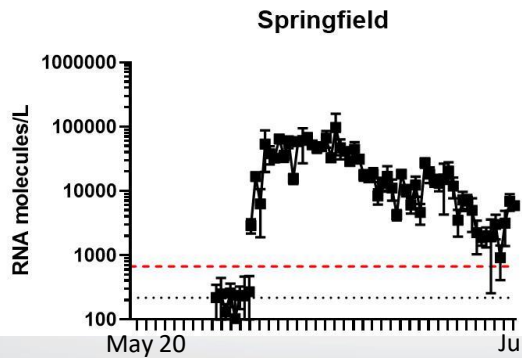
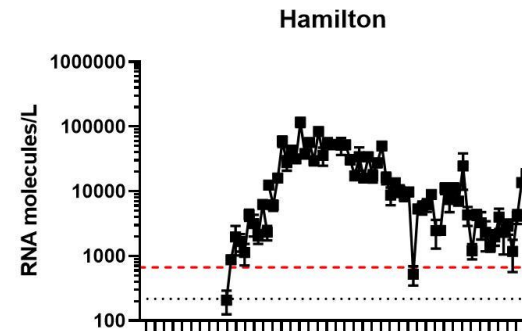
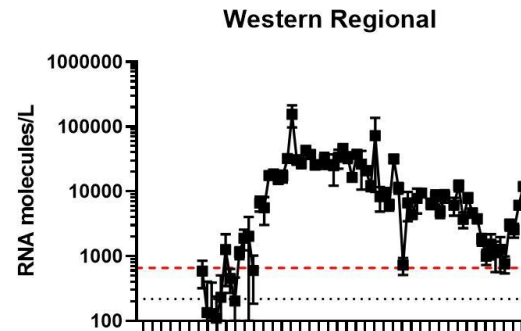
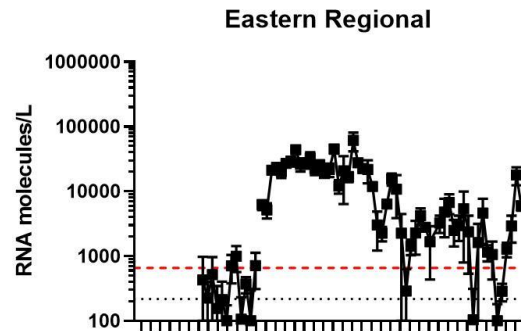
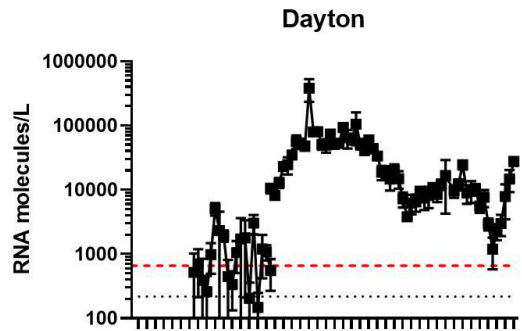
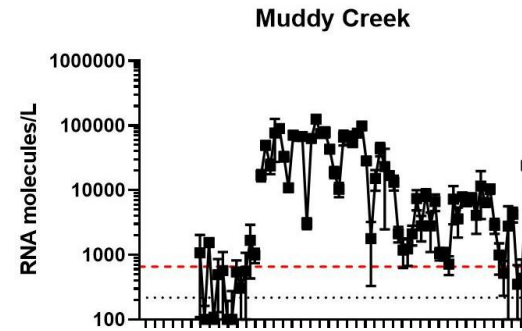
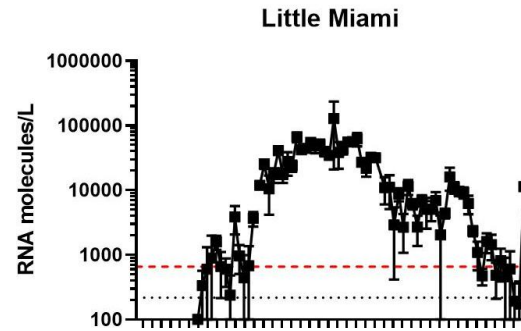
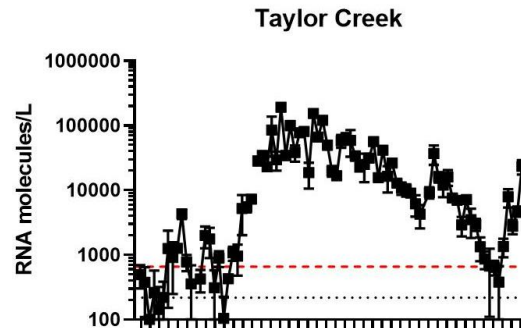
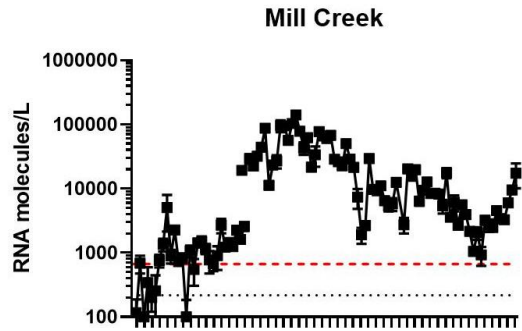


USEPA Sample Processing and Analysis





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





Factors that influence the relationship between wastewater and clinical data

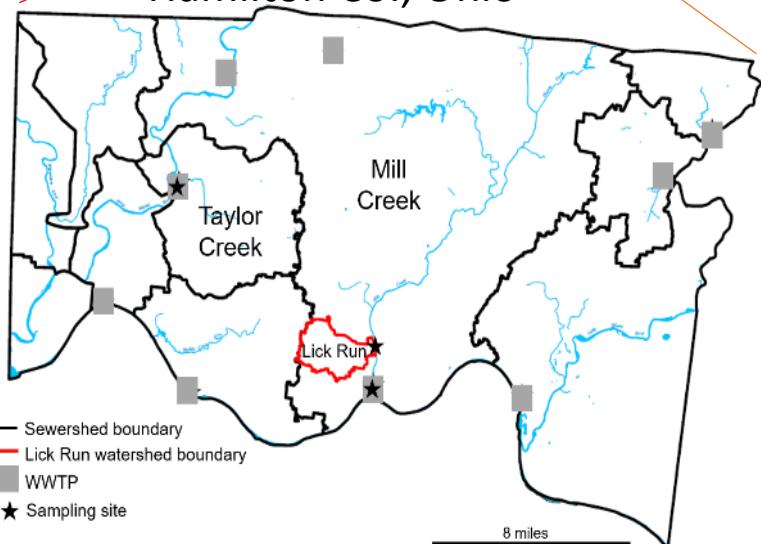
Factor	Factor Details	Data Parameters
Fecal shedding	Load, duration	Variable, up to 10^7 copies/g
Fate and transport of viral particle	Decay during conveyance	Decay rate
Sewershed Characteristics	Physical data	Flow, pH, temperature, TSS
Sampling Scheme	Frequency Type	Daily, weekly Grab, 24-hour flow-weighted 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



Hamilton Co., Ohio



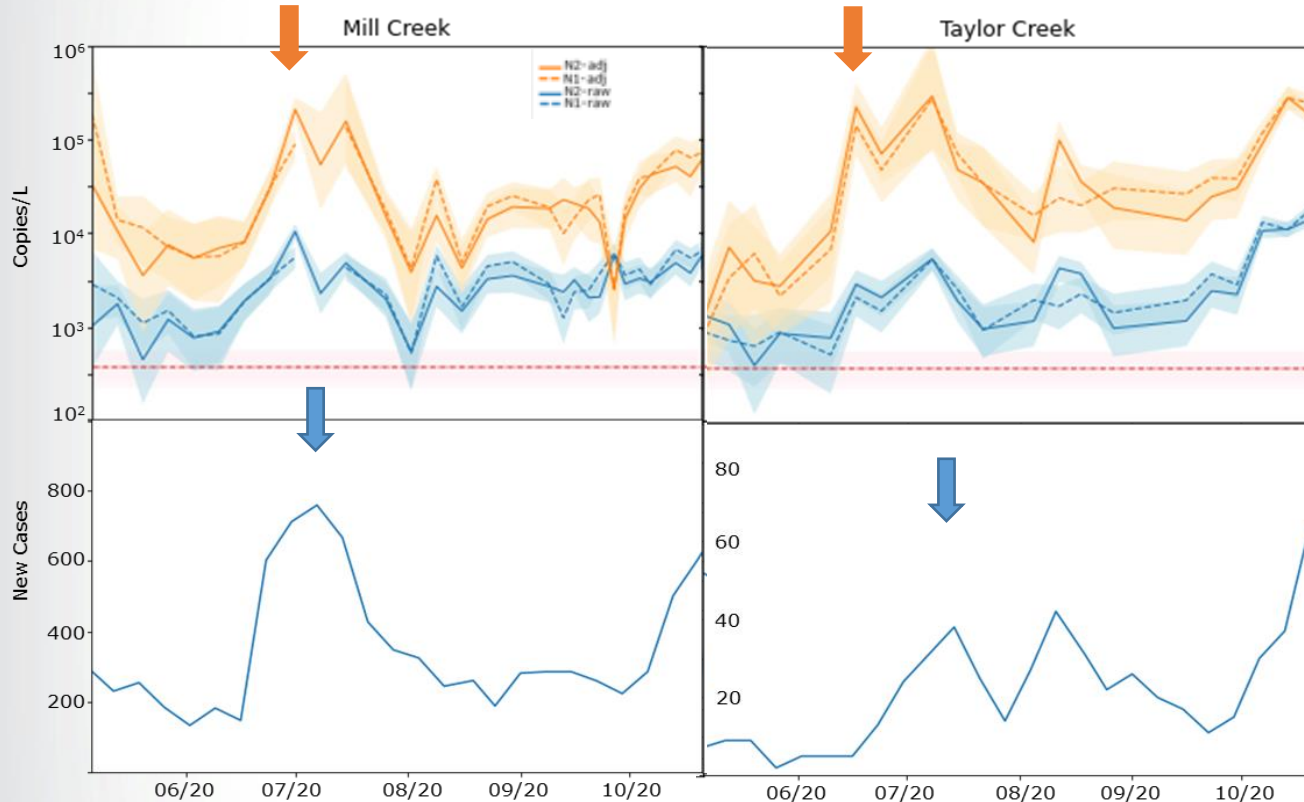
Parameter	Mill Creek	Taylor Creek
Population size	488,000	34,000
Mean MGD	93.17	2.79
MGD range	55.12-350.31	2.11-6.87
% Combined sewers	40	0
% Industrial flow	5	0
pH range	6.04-8.86	6.4-7.38
Mean TSS (mg/L)	247.87	340.96
TSS range	90-640	180-700

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



Wastewater RNA and Case Trend Data

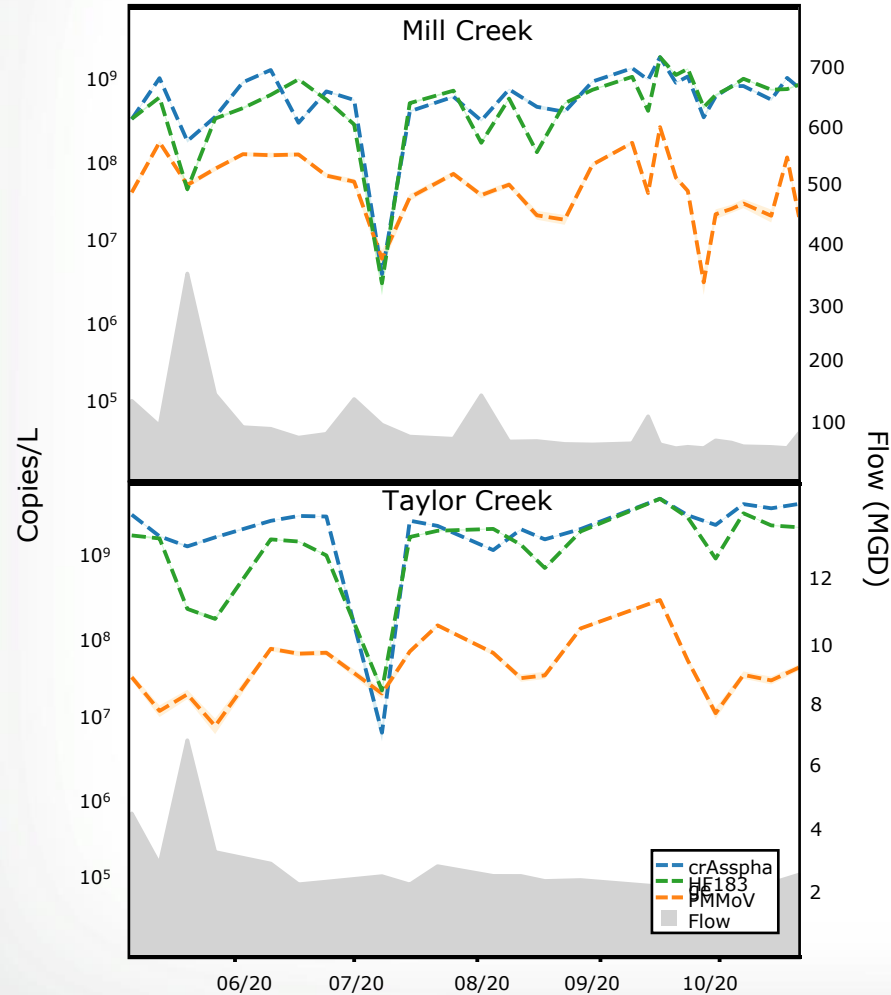
May – October 2020



- NI and N2 (raw and OC43-adjusted) concentrations were highly correlated ($r = 0.87$, $BF_{10} > 100$)
- Raw and OC43-adjusted NI/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



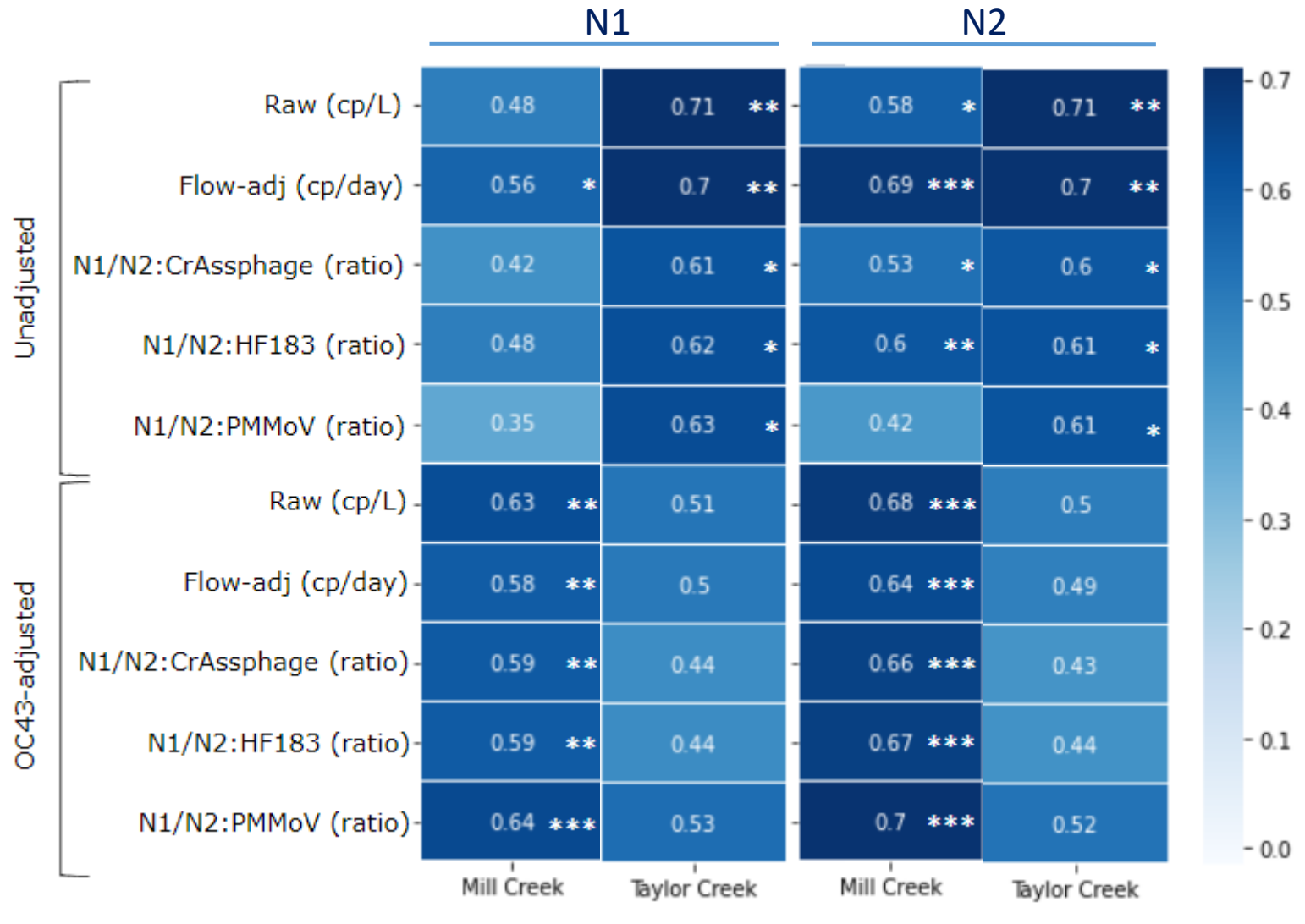
Fecal Indicators, Flow and TSS



		Mill Creek		Taylor Creek	
		Pearson's r	BF ₁₀	Pearson's r	BF ₁₀
crAssphage	HF183	0.9	***	0.842	***
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	



Correlations of Wastewater and New Case Data



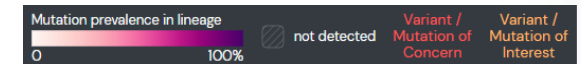
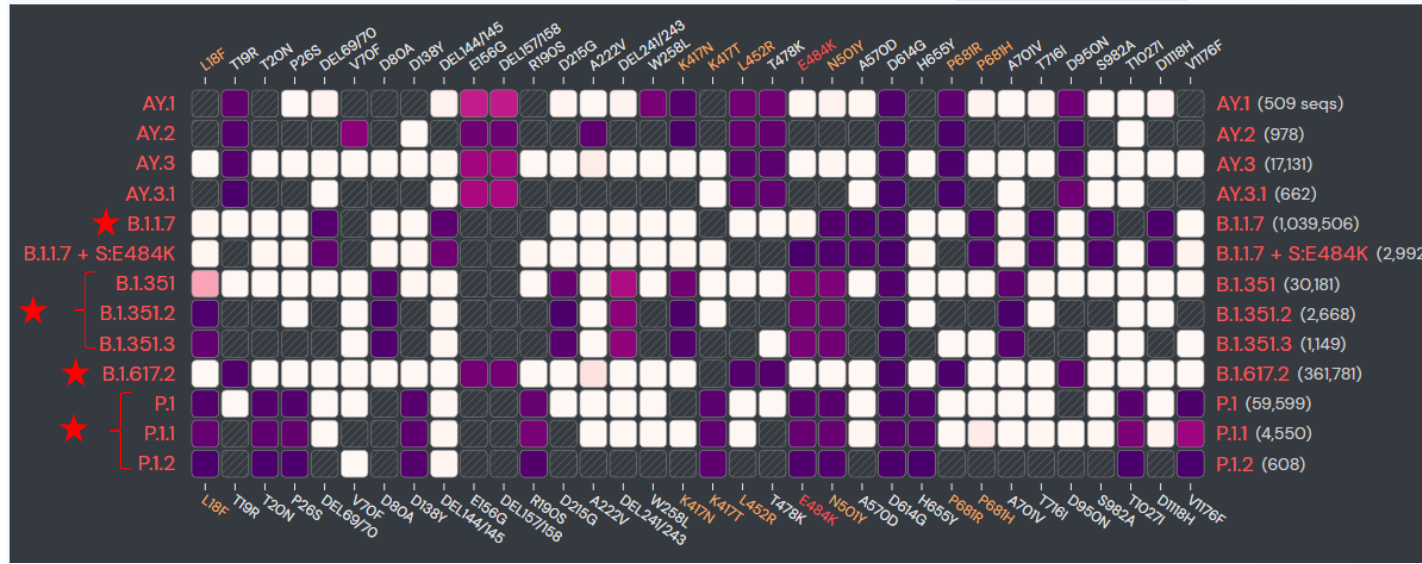
* = Bayes Factor (BF)₁₀ > 10; ** = BF₁₀ > 30; *** = BF₁₀ > 100



Variants of Concern (VOC) in Wastewater

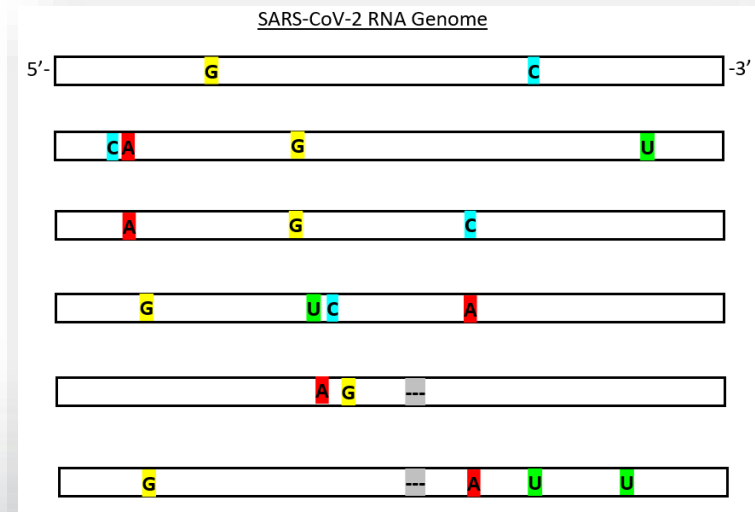
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<https://outbreak.info> [as of Aug 09, 2021]



* = 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

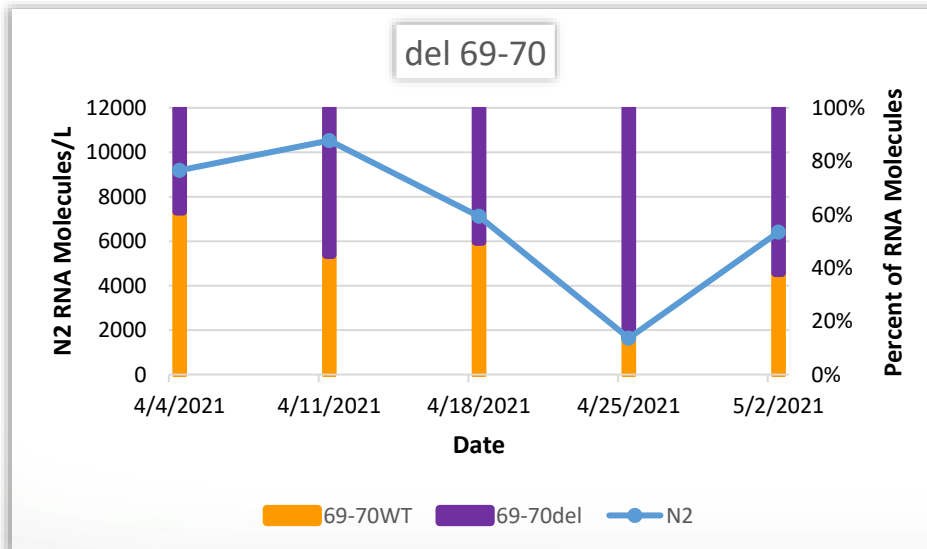
B.1.1.7

first identified in United Kingdom

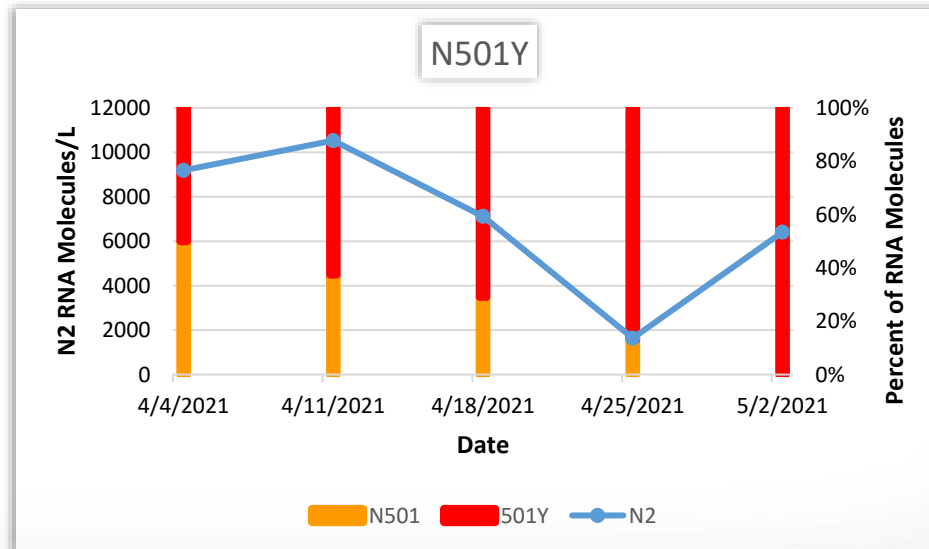
a.k.a. Variant of Concern 202012/01, VOC-202012/01, 20B/501Y.V1, 20I/501Y.V1



<https://outbreak.info>



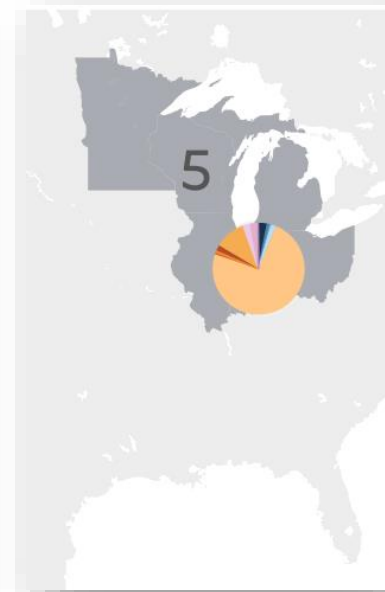
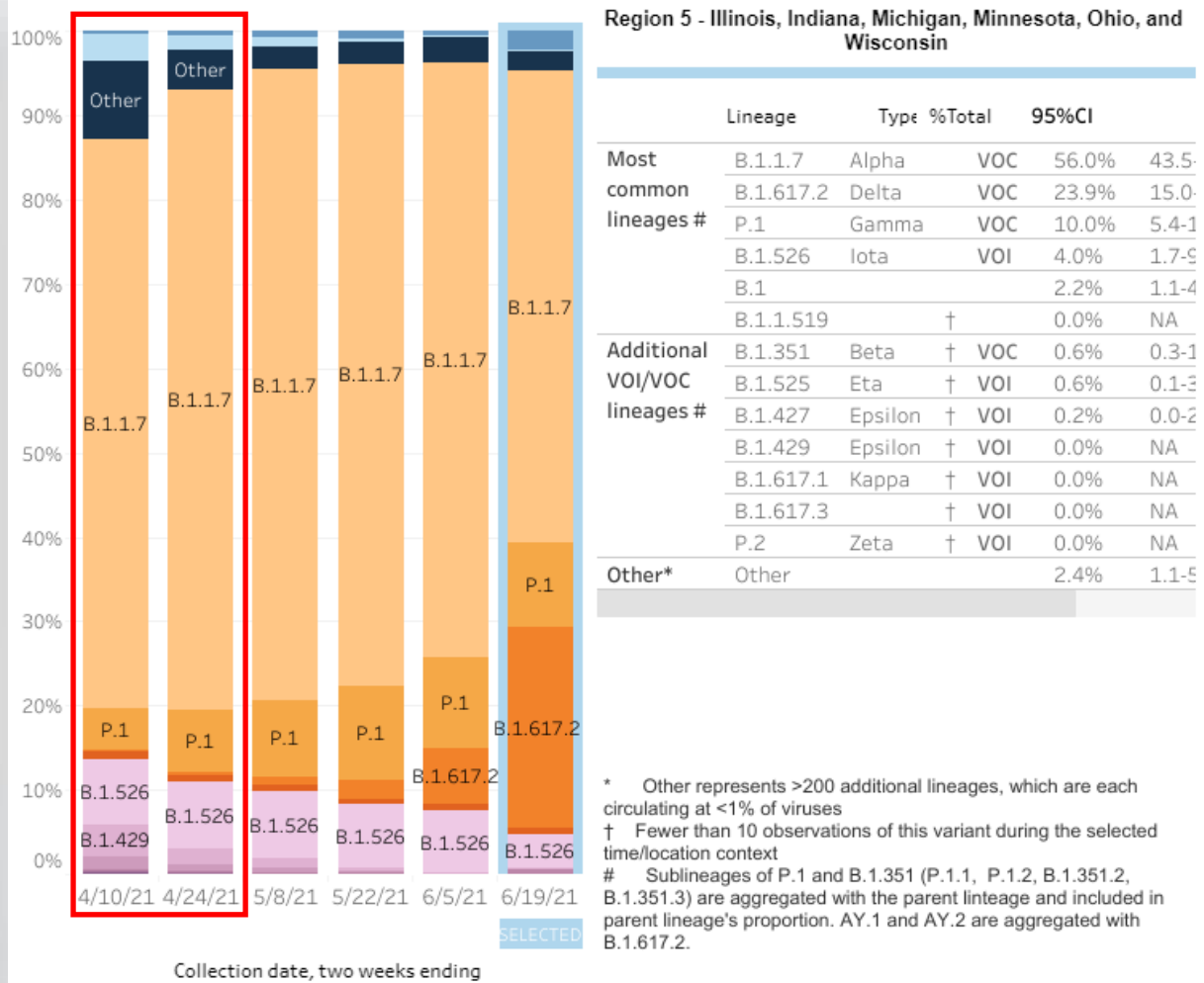
RNA sequences with deletions of nucleotides that result in absence of spike aa 69-70 increase over time



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



US Region 5 Distribution of B.1.1.7



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)

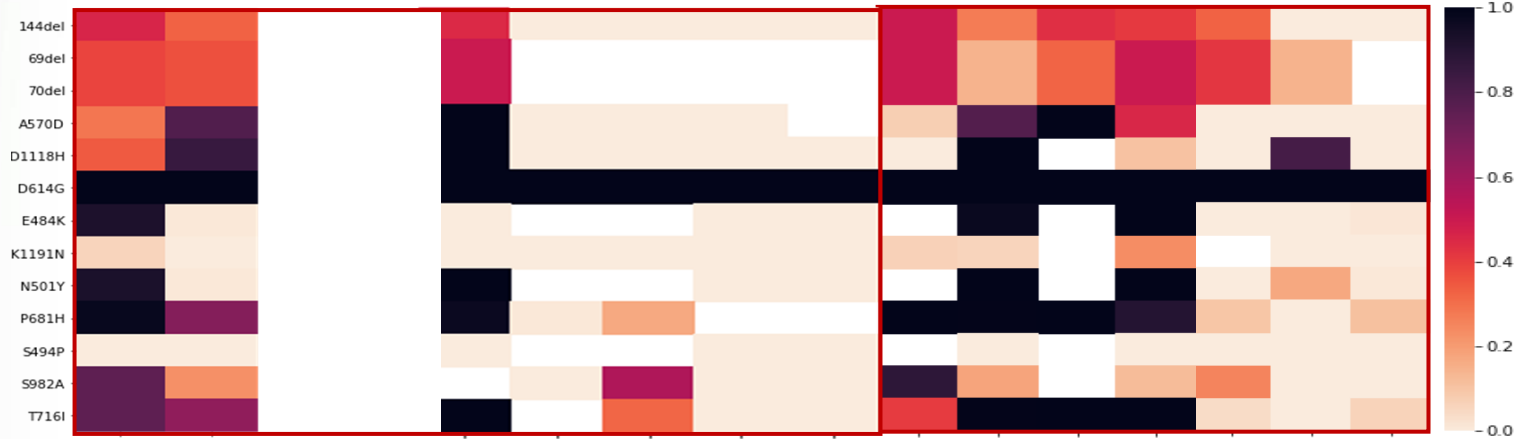
[¶]average of 10 Ohio sewersheds during sample dates April 11,12 and April 25,26 April 4 and 5: 69/70del 30.3(7.8-52.8); 501Y 33.5(13.5-53.5)



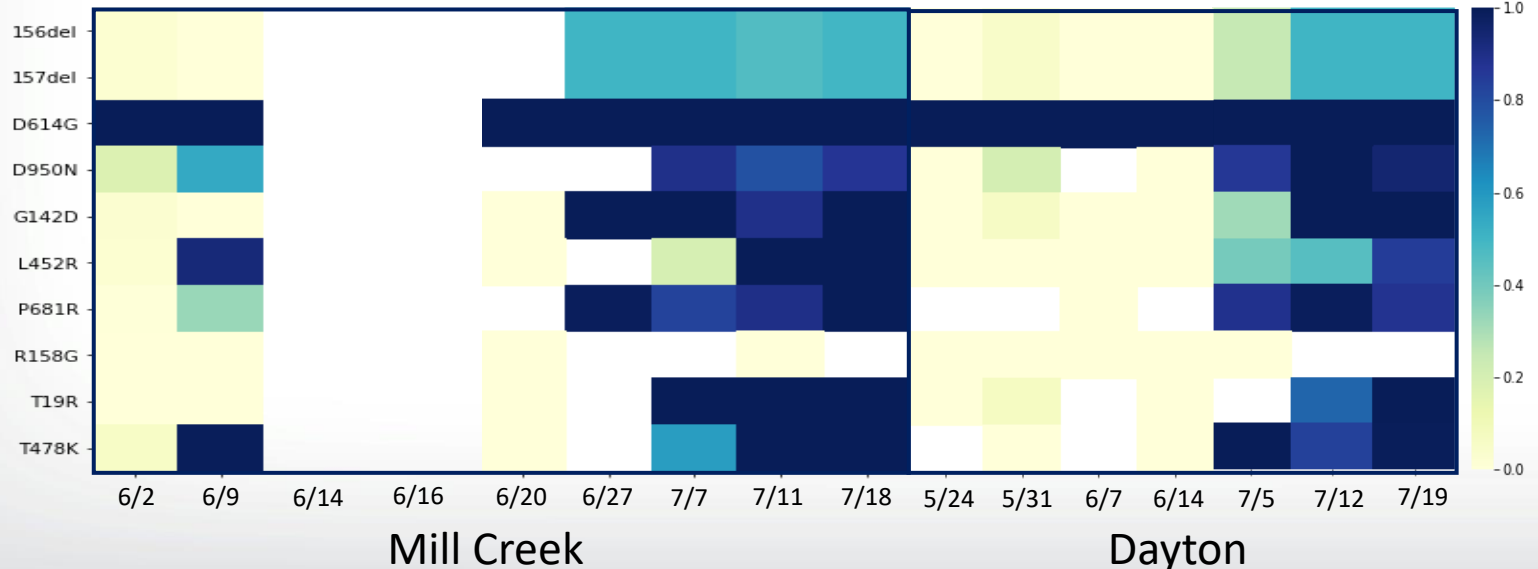
Alpha (B.1.1.7) and Delta (B.1.617.2) Signatures in Wastewater

Tiled amplicon sequencing from June through mid-July

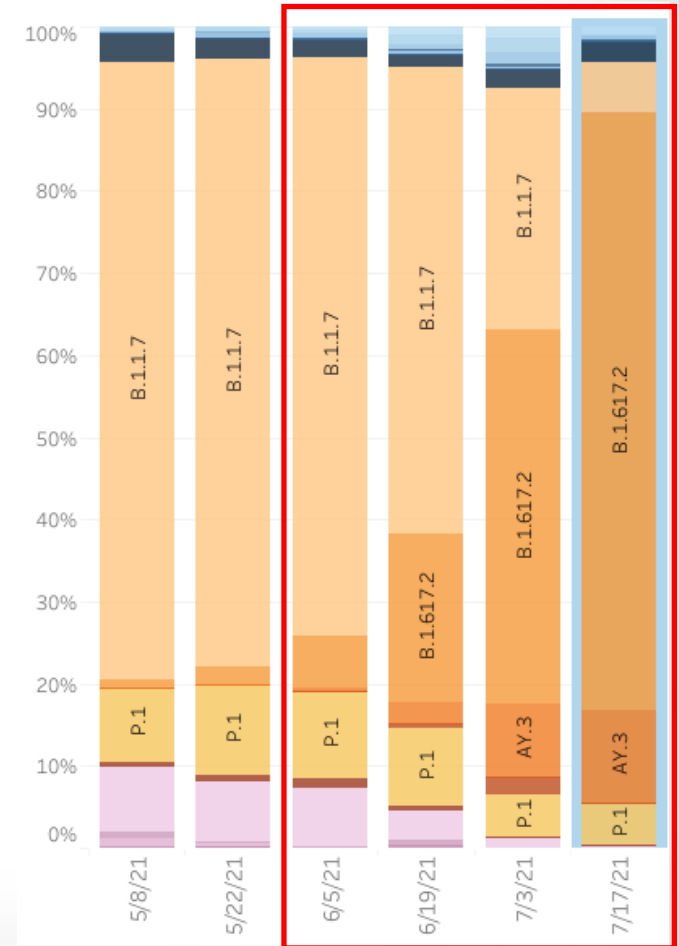
Alpha
(B.1.1.7)



Delta
(B.1.617.2)



US Region 5 VOC Distribution





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