Overview of the Ohio Wastewater Monitoring Network

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Ohio Wastewater Monitoring Network (OWMN)

- ## Goal
  - Monitor trend of SARS-CoV-2 RNA at specific locations (vs compare sites)
  - Serve as early indicator of COVID-19 community spread
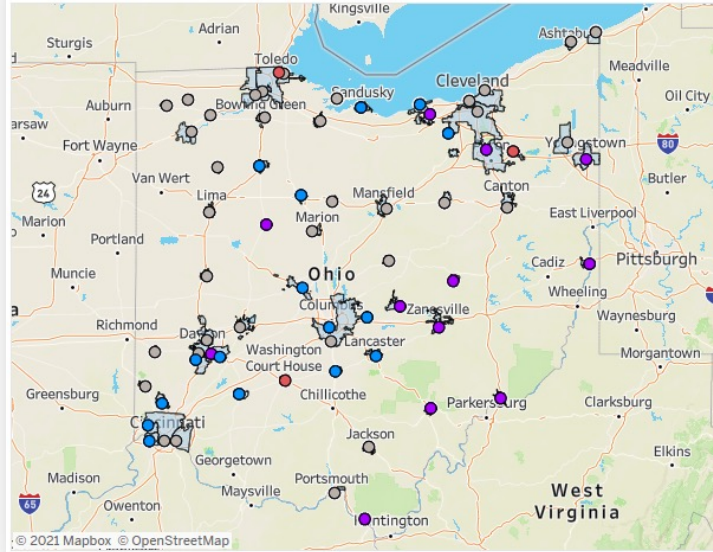 - Prioritize resources
- ## Statewide network
  - Started July 2020
  - leveraged expertise and resources
    - Ohio Universities
    - US EPA-ORD
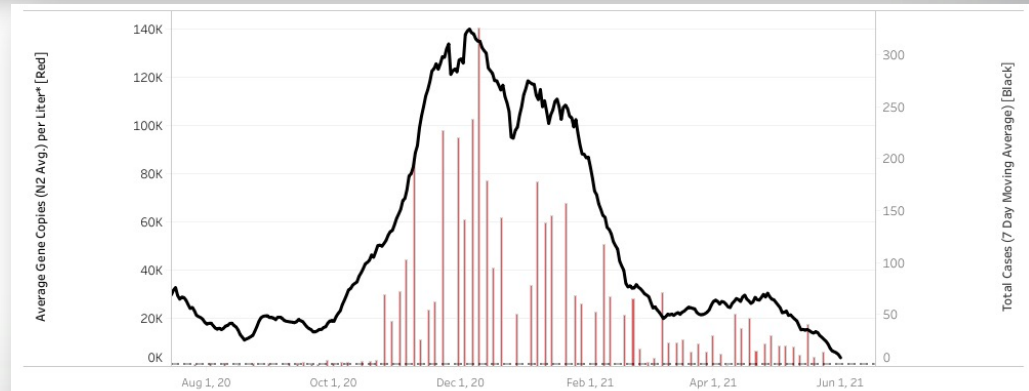- ## 67 locations twice a week
- ## Sequencing to screen for possible presence of SARS-CoV-2 variants
  - Variants of Concern (VOC)
  - Variants of Interest (VOI)

Wastewater Treatment Plant Locations and Boundaries

Athens WTP	⬆
Eastern Regional WRF	⬆
Newark WWTP	⬆
Zanesville WWTP	⬆
Mount Vernon WWTP	⬆
Washington Court House WWTP	⬆
Pataskala WWTP	⬆
Franklin Hills WWTP	⬆
Village of Ottawa WWTP	⬆
City of Sidney WWTP	⬆
City of Wilmington WWTP	⬆
City of Eaton WWTP	⬆
Lorain Black River WWTP	⬆
Conneaut WWTP	↗
Elyria WWTP	↗
NEORSD Southerly WWTC	↗
Toledo Bay View Park WWTP	↗

© 2021 Mapbox © OpenStreetMap

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

Public Health Application

- **To serve as an early warning of infection in communities and an understanding of case trends**

- **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)**

- **State actions when increases are observed:**
  - 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
  - Provide case data by sewershed to local health district (this extraction to be provided soon)

- **Participation in the CDC National Wastewater Surveillance System**

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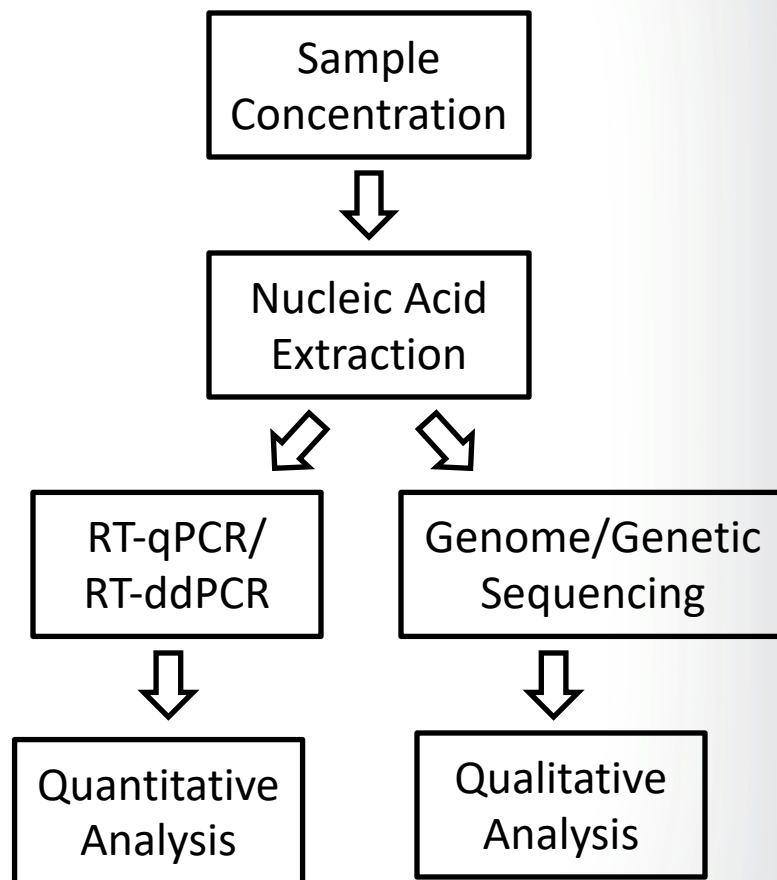
Accomplishments

- **Built statewide network that represents wastewater flow from nearly 5 million residents**

- **Almost 1 year of weekly data collected**

- **All data is publicly available on the Ohio coronavirus dashboard and is updated daily**

- **Provided nearly 500 warnings to local health communities**

- **Expanded to include genomic sequencing of wastewater to pair with clinical data and inform public health decisions**

Lab Method Logistics

- **Twice weekly samples**

- **Report data within 2 days of sample receipt**

- **No prescribed method; labs decide**

- **Supply chain shortages**

- **Low target concentration**

- **Sample hold time: 4°C - 72 hours**

- **No sample pasteurization**

```
Sample Concentration
        ↓
Nucleic Acid Extraction
        ↓                    ↓
RT-qPCR/           Genome/Genetic
RT-ddPCR           Sequencing
        ↓                    ↓
Quantitative       Qualitative
Analysis           Analysis
```

- **Matrix Spike to assess method recovery efficiency**
  - Coronavirus recommended: human (OC43), murine (MHV), bovine (BCoV)
- **Inhibition control to monitor for PCR amplification inhibition**
- **RT-qPCR standards/RT-ddPCR positive control**
- **Human fraction measurements**
  - crAssphage
  - Pepper mild mottle virus
- **Monthly Interlaboratory Method Validation**
  - Pick a site with sufficient concentration of SARS-CoV-2
  - Each lab gets 0.5 L
  - Each lab processes and analyzes sample
  - Report data to Project Coordinator

OH Network Lab Methods

| LAB	LOD (copies/L)	Processing Method	Nucleic Acid Extraction	Quantitative Analysis Method	RT-PCR Standard Curve/ Control	Inhibition Control	Matrix Spike	Fecal Indicator
A	850	Centrifugation, filtration	Qiagen RNeasy PowerWater Kit	RT-qPCR	DNA plasmid	Dilution	MHV	crAssphage
B	135	Centrifugation, filtration	Qiagen Allprep DNA/RNA Kit	RT-qPCR	DNA plasmid	Dilution	BCoV	crAssphage
C	133	Tween, solids removal hollow fiber ultrafiltration (InnovaPrep)	Qiagen PowerMicrobiome Kit	RT-ddPCR	DNA plasmid	Luciferase Control RNA	OC43	crAssphage
D	7,440	Filtration	Trizol, garnet bead beating, alcohol precipitation	RT-qPCR	DNA plasmid	Luciferase Control RNA	BCoV	PMMoV
E	500	Centrifugation, filtration	Trizol and RNA purification kit	RT-qPCR	Synthetic RNA	Luciferase Control RNA	BCoV	crAssphage
F	3,000	Promega, add protease, supernatant through GFA/silica column	Promega Wastewater Large Volume TNA Capture Kit	RT-qPCR	DNA plasmid	Promega probe	OC43	PMMoV
G	231	Centrifugation, filtration	Qiagen RNeasy PowerWater Kit/Trizol-chloroform	RT-ddPCR	SARS-CoV-2 genomic RNA	Luciferase Control RNA	OC43	crAssphage
H	891	Acidification, Filtration, extract filter	Qiagen Allprep PowerViral DNA/RNA Kit	RT-qPCR	Synthetic RNA	Mouse lung RNA	OC43	PMMoV

OH Network Lab Methods

LAB	LOD (copies/L)	Processing Method	Nucleic Acid Extraction	Quantitative Analysis Method	RT-PCR Standard Curve/ Control	Inhibition Control	Matrix Spike	Fecal Indicator
A	850							
B	135							
C	133							
D	7,440							
E	500	5	8	2	3	4	3	2
F	3,000							
G	231							
H	891							

Number of different procedures employed at the various processing/analysis steps
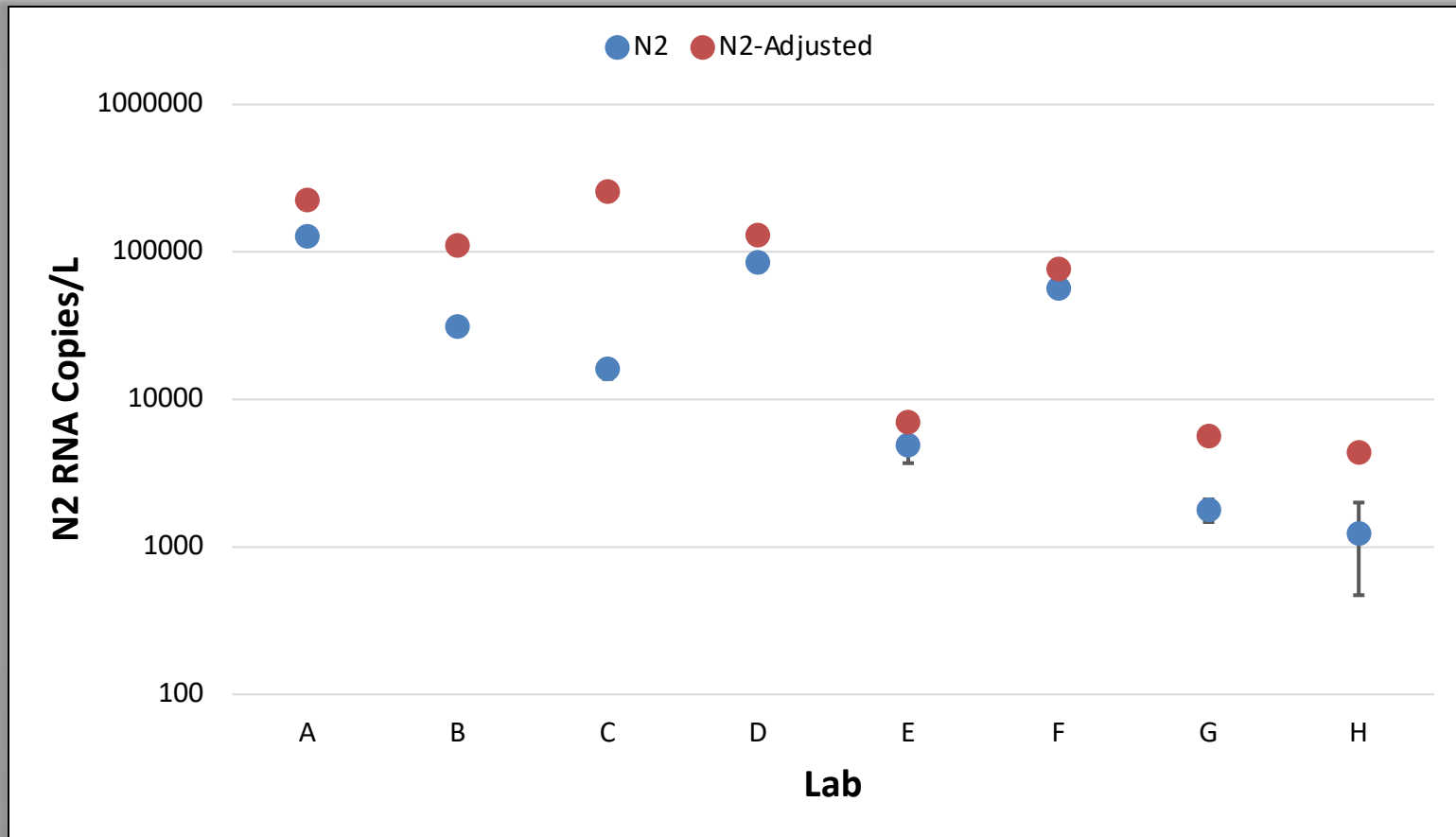
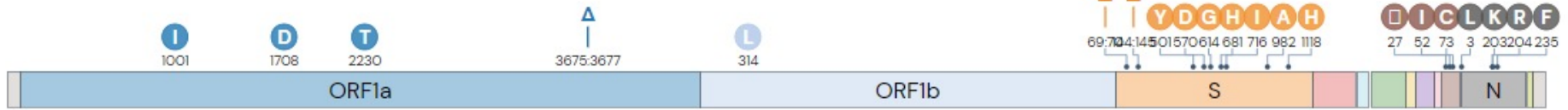N2 RNA concentrations from 8 labs span >2 orders of magnitude

RT-ddPCR Mutation Assays
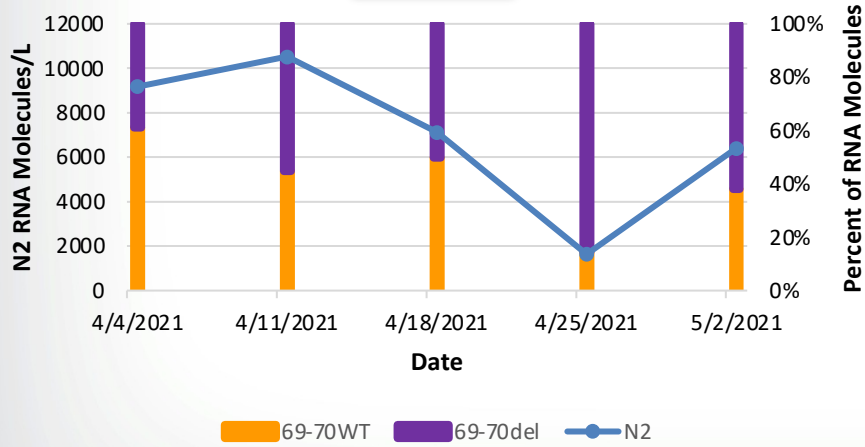
EPA

B.1.1.7
first identified in United Kingdom
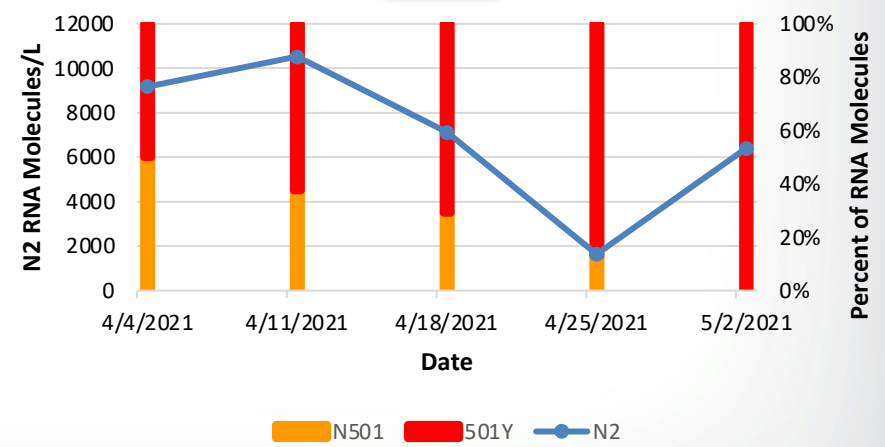a.k.a. **Variant of Concern 202012/01, VOC-202012/01, 20B/501Y.V1, 20I/501Y.V1**

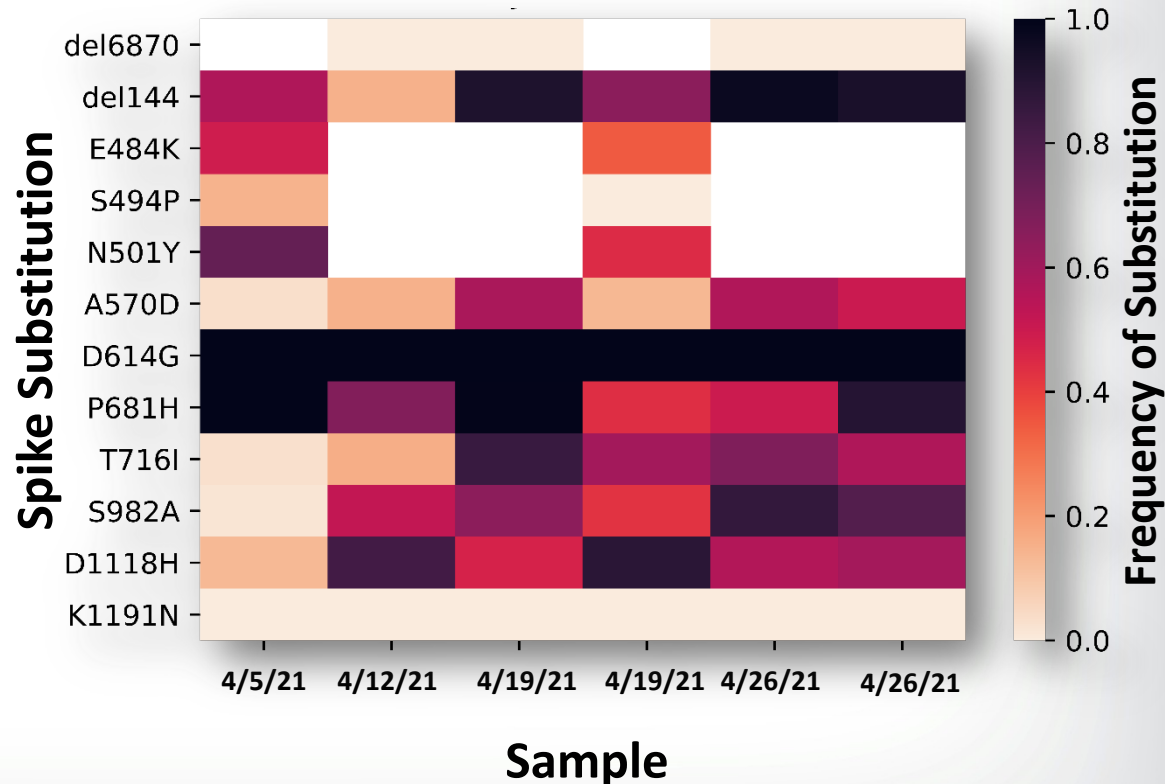VARIANT OF CONCERN

https://outbreak.info

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

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

EPA

- **Pooled sample**
  - Cannot assemble a genome
  - Focus on mutations that cause amino acid substitutions, signatures of VOC/VOI
- **Genome/Genetic Sequencing**
  - 3 labs, different methods
  - Tiled amplicon approach
  - Short read seq via Illumina
  - Short term - spike amino acid changes for CDC's VOC/VOI
  - Report (for each site)
    - Read depth
    - Number of alternative alleles

- **How do current practices (eg. methods, protocols, technologies, best practices, etc.) successfully contribute toward comparable, high quality data/results/decisions?**

  – Using a consistent method, trends of SARS-CoV-2 RNA in a sewershed can be evaluated
  – Implementation of Quality Control parameters allow for confidence in lab measurements
  – Frequent communication/regular meetings facilitate interlab discussion and troubleshooting

- **How do current practices compromise efficiency and reduce confidence in data/results/decisions?**

  – Too many labs/methods result in measurement variation
  – Varied experience leads to measurement variation
  – Supply shortages lead to method changes

- **What is needed to increase comparability and confidence in data and results?**

  – Standardized methods/procedures
  – Standardized quality control samples/reagents
  – Statistical models to quantify uncertainty

- **What types of standards could potentially help to fill these needs?**

  – Matrix Spike
  – Extraction controls
  – RT-qPCR standards
  – RT-ddPCR controls
  – Inhibition controls
  – Sequencing controls

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