

SARS-CoV-2 Variant Tracking in Ohio Wastewater

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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
- 2021: Screen for presence of SARS-CoV-2 variants of concern/interest (VOC/VOI) by sequencing and RT-ddPCR

Department Ohio of Health

Coldwate

Fort Wayn

Portlar

Owentor

Sturgis

24

Marion

•

Muncie

Greensbur

Coronavirus (COVID-19)

COVID-19 Dashboard

Ohio Coronavirus Wastewater Monitoring Network

Ashtabu

ast Liverpool

Pittsbu

Waynesburg

Clarksburg

State of Ohio | COVID-19 Wastewater Treatment Plant Locations and Boundaries

> Marietta WWTP 1 1 Kent WRF Put In Bay WWTP 1 Oil City NEORSD Easterly WWTP 1 NEORSD Westerly WWTC 1 Beavercreek WRRF 1 Hamilton WRF 3 . Newark WWTP . City of Eaton WWTP . Ironton WWTF Athens WTP \leftrightarrow Morgantow Lancaster WPCF \leftrightarrow Little Miami WWTP \leftrightarrow Marysville WRF \leftrightarrow Elkins Muddy Creek WWTP \leftrightarrow Portsmouth Lawson Run WWTP \leftrightarrow Springfield WWTP \leftrightarrow

Last Updated:02-25-2022

Updated Weekdays

Legend: Red arrow ↑= substantial increase (>100%), Yellow arrow / = increase (50% to 100%), Gray arrow \leftrightarrow = steady (-49% to 49%), Blue arrow \searrow = decrease (<=-50%)

West

Virginia

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

Charleston

Portsmout

SEPA

OWMN Dashboard



Application

- The focus is on <u>trends or significant</u> <u>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 10fold (1 log)

Accomplishments

- Statewide network represents wastewater flow from nearly 5 million residents
- ~2 years of twice weekly data collected
- Provided nearly 1000 warnings to local health communities

Bohrerova, et al. in preparation



Lab Method Logistics

- Report quantitative data within 2 days of sample receipt
- No prescribed method; labs decide
- Sample hold time: 4°C 72 hours
- No sample pasteurization
- QA/QC (informed by NWSS)
 - Matrix spike to assess method recovery efficiency
 - Coronavirus recommended: human (OC43), murine (MHV), bovine (BCoV)
 - PCR Inhibition control
 - RT-qPCR standards/RT-ddPCR positive control
 - Human fraction measurements
 - crAssphage
 - pepper mild mottle virus (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	Library Preparation	Sequencing
А	1200	Centrifugation, filtration	Qiagen RNeasy PowerWater Kit	RT-qPCR	DNA plasmid	Dilution	MHV	crAssphage	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
В	170	Centrifugation, filtration	Qiagen Allprep DNA/RNA Kit	RT-qPCR	DNA plasmid	Dilution	BCoV	crAssphage	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
С	133	Tween, solids removal, hollow fiber ultrafiltration (InnovaPrep)	Qiagen PowerMicrobiome Kit	RT-ddPCR	DNA plasmid	Luciferase Control RNA	OC43	crAssphage	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
D	4050	Filtration	Trizol, garnet bead beating, alcohol precipitation	RT-qPCR	DNA plasmid	Dilution	BCoV	PMMoV	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
Е	500	Centrifugation, filtration	TRIzol and RNA purification kit	RT-qPCR	Synthetic RNA	Luciferase Control RNA	BCoV	crAssphage	SWIFT/IDT SARS- CoV-2 Panel	NextSeq550
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	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
G	231	Centrifugation, filtration	TRIzol-chloroform	RT-ddPCR	SARS-CoV-2 genomic RNA	Luciferase Control RNA	OC43	crAssphage	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
н	891	Acidification, Filtration, extract filter	Qiagen Allprep PowerViral DNA/RNA Kit	RT-qPCR	Synthetic RNA	Mouse lung RNA	OC43	PMMoV	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000



Sequence Analysis

- Lab specific analytical pipelines
- Shared list of substitutions*; focus on spike region for Variants of Concern
- Depth of sequence read coverage
- Presence of variant-specific substitution = Alternate allele frequency > 1%
- Lineage presence = 50% of variant-specific substitutions



SEPA

These WWTPs combined represent 1,658,094 (14%) OH residents



Alpha: OH Wastewater and Case Data





Delta in OH Wastewater



OH Wastewater and Case Data



Omicron in OH Wastewater

SEPA

WWTP B WWTP A WWTP C A67V 🗕 D614G -D796Y XX X del143 -Substitutions del211 xxxxxxx del69 🗕 E484A -G142D - $\times \times$ \times \times G339D -G4465 -G4965 -H655Y -ins214 = K417N = Acid L981F - \times N440K = N501Y -N679K -Amino . N764K = N856K N969K -P681H - \times Q493R = Spike Q498R -Q954H -X \$371L -S373P -S375F -Omicron S477N -T478K -T547K -T951 -.... ******* \times \times \times \times Y145D = Y505H 4125 8/15 9/19 10/10 NT 215 2220 1/18 1112 6% 216 N25 9/26 11/28 22179 222 ALA 6% ANTA . 1128 212 NA 8/1 a130 10m 10131 \$ 10 alle 200 Nº. P Sr. 0.2 0.4 0.6 0.8 0 0.2 0.4 0.6 0.8 0 0.2 0.4 0.6 0.8 0 1.0 1.0 1.0

Omicron: OH Wastewater and Case Data





WWTP A

Alpha



Delta



Omicron



Omicron Sublineage BA.2 in OH

Sporadic appearance of BA.2 signatures at a couple OH WWTP



Case data: BA.2 Lineage Report.outbreak.info, (available at https://outbreak.info/situation-reports/pango=BA.2). Accessed 3 March 2022.

0.20 WWTP D T19I L24S -0.15 del25/27 V213G -0.10 \$371F -T376A -0.05 D405N -R408S -0/12 10124 12/19 1/Nº 8/15 1122 216 6121 \$ 0.20 **WWTP E** T19I L245 -0.15 del25/27 • V213G -0.10 S371F -T376A -0.05 D405N -R4085 -10124 12119 1121 1118 216 5 a12

⇒EPA

Summary and Data Usage

- Wastewater surveillance has enabled large-scale monitoring of SARS-CoV-2 trends
- The Alpha, Delta and Omicron variant surges were observed by wastewater sequencing and reflects the confirmed case data
- Presence of minority sublineage (BA.2) was observed sporadically in wastewater
- Ohio Department of Health is using wastewater sequencing data in conjunction with clinical samples to confirm and monitor the spread of the variants across communities in Ohio
- Clinical data is not readily available from all OH locations; wastewater sequencing data was able to identify the presence of variants in these communities
- Explore other mutations in the full-length genome data set