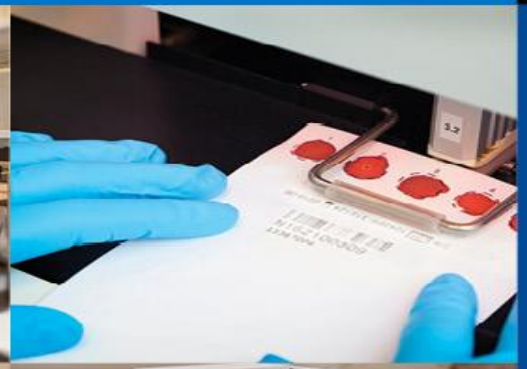
DCLS

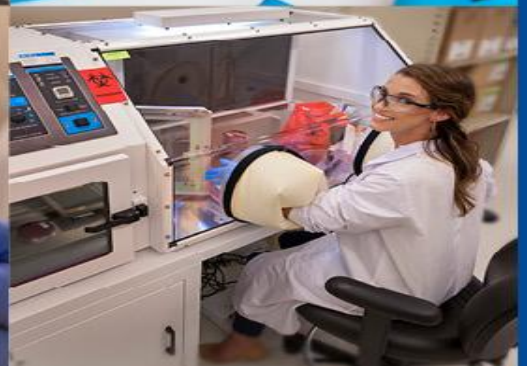
Division of

Consolidated Laboratory Services

DGS

DEPARTMENT OF GENERAL SERVICES





Piloting Wastewater-Based Surveillance of SARS-CoV-2 Variants in Virginia, 2021 – 2022

Lauren Turner, PhD

Learning Objectives

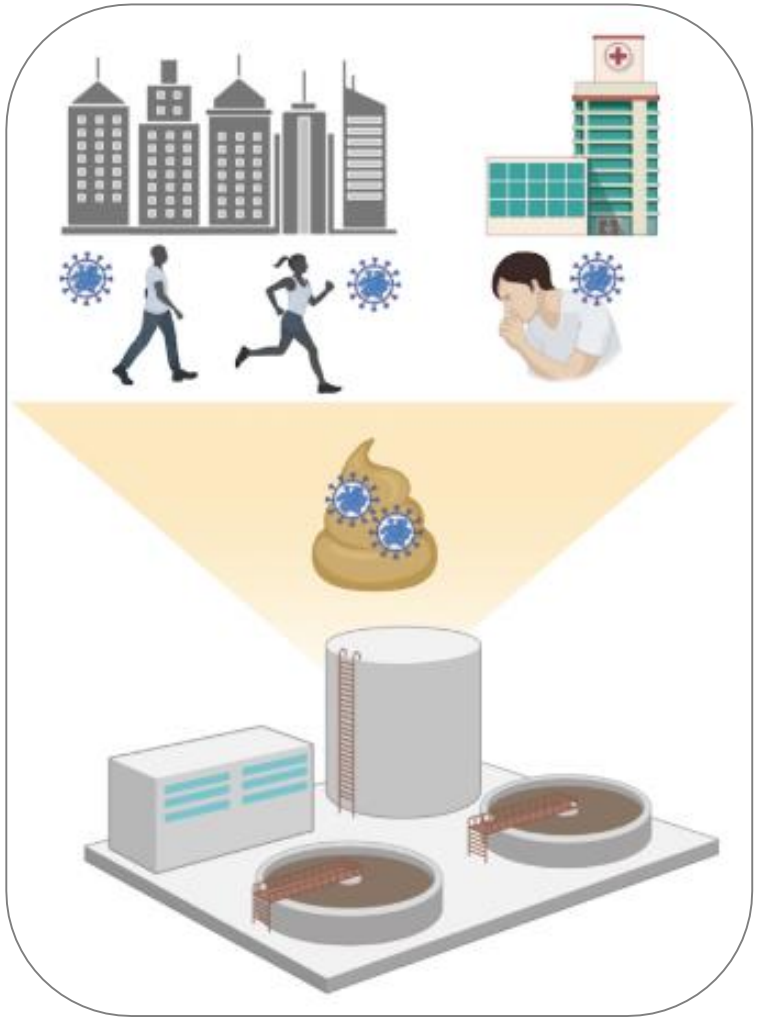
- ✓ Understand advantages and limitations of wastewater sequencing for SARS-CoV-2 variant surveillance
- ✓ Describe how the aggregation of surveillance data for wastewater differs from clinical testing data
- ✓ Describe a laboratory workflow for wastewater sequencing and additional tests that precede sequencing analysis

Presentation Outline

- Wastewater surveillance and programs overview
- Surveillance site selection for FDA project
- Detection and characterization testing workflows
- SARS-CoV-2 monitoring trends 2021 – 2022
- Trending variant proportions in wastewater
- Future directions

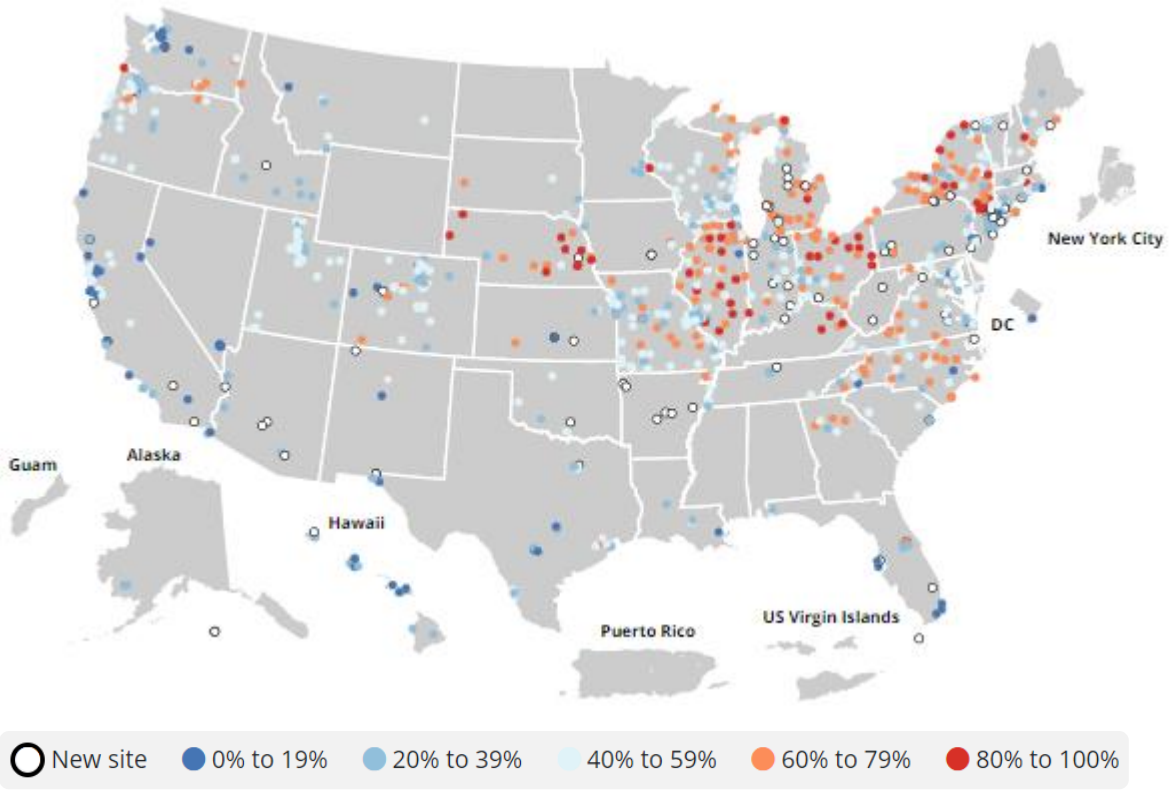
Why Wastewater (WW) Surveillance?

- Up to 80% people with COVID-19 shed virus in their feces
- WW can serve as an early warning system 4 – 6 days prior to clinical case increases
  - Captures asymptomatic infections
  - Independent of healthcare-seeking behavior and testing access
  - An efficient pooled sample of community infection levels
  - Data is available quickly
  - Flexible testing platform that can be leveraged for multiple pathogens



National Wastewater Surveillance System

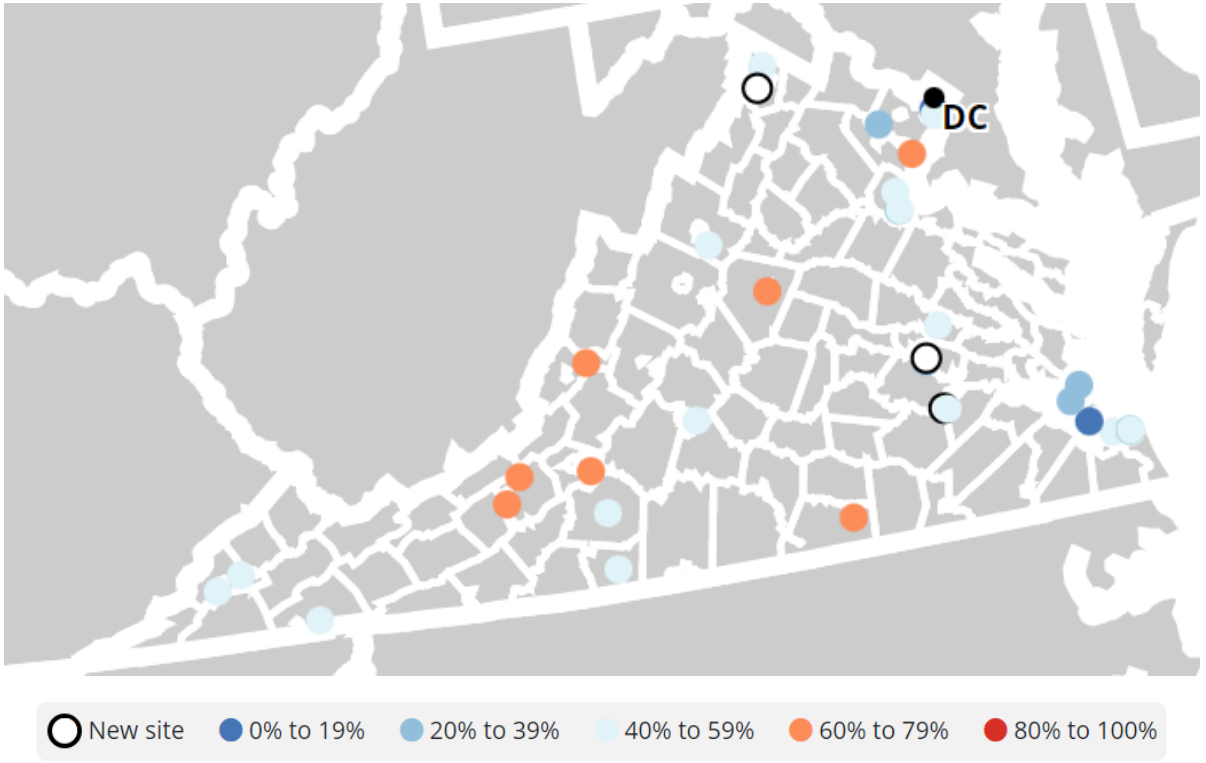
- Established in 2020
- Tracking the presence of SARS-CoV-2 in US wastewater
  - 46 states, 5 major cities, 2 territories
  - Commercial testing contract– 335 sites nationally
  - >83,000 unique WW samples representing more than 130 Million people – ~50% of Americans on a sewer system



CDC COVID Data Tracker: Wastewater Surveillance

Virginia Wastewater Surveillance

- SARS-CoV-2 Wastewater Surveillance Sentinel Monitoring Program
  - Initiated 9/21/2021
  - 25 sentinel monitoring facilities performing once weekly collection
  - Aggregate data reported to CDC
  - Site-specific data reported to utilities



CDC COVID Data Tracker: Wastewater Surveillance

FDA GenomeTrakr WW SARS-CoV-2 Variant Surveillance Project

- Monitor circulating SARS-CoV-2 variants in US populations with an emphasis on food production areas
- Leverage national sequencing capacity established by the FDA
  - GenomeTrakr network collects and uploads genome data to public repositories in real-time
  - Apply to other pathogens of food safety significance (Norovirus, Hepatitis A)

SARS-CoV-2 Sequencing Project: Virginia Site Selection & Sample Collection

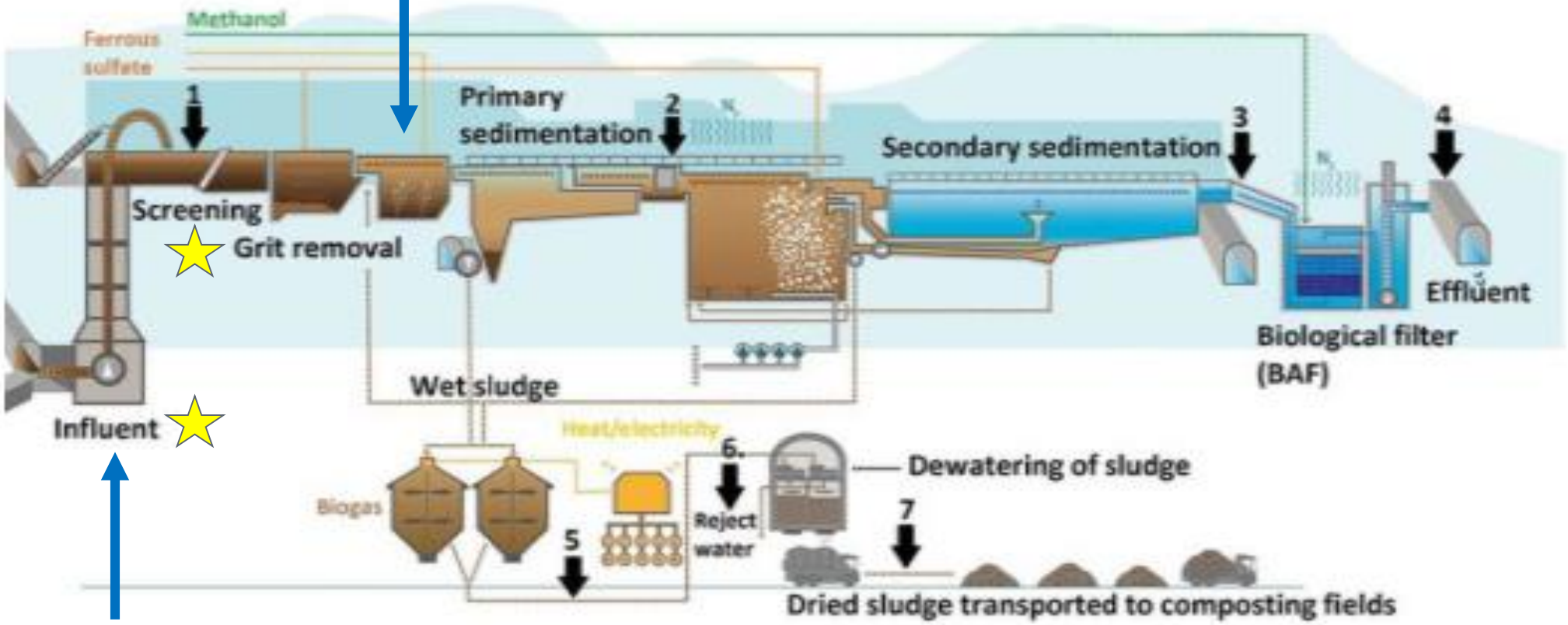
- Three sites in Northwest Virginia
  - **SMP-WW12** (population ≈ 16,500)
    - 5 million gallons per day
  - **SMP-WW26** (population ≈ 16,500)
    - 5 million gallons per day
  - **SMP-WW27** (population unknown)
    - Food processing facility

- 100 mL 24-hour composite samples collected once or twice weekly and transported to Virginia DCLS for testing
- Samples stored at 4°C until processing (up to 2 days)

https://freevectormaps.com/united-states/virginia/US-VA-EPS-02-8001?ref=atr

Wastewater Sampling

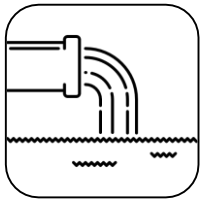
Post Grit Removal



Raw Wastewater

Source: Talvitie, et al. 2015

Laboratory Workflow



- Sample collection
- Virus concentration
- RNA extraction
- ddPCR detection
- SARS-CoV-2 controls

RNA QC

- cDNA
- Library prep
- Sequencing

Sequence QC

- Data submission
- BioSample
- SRA

NCBI
- Human read scrubbing

FDA
- Analysis pipeline
- Reports

DCLS Sample Processing Workflow

<u>Sample Preparation</u>

- Homogenization by mixing
- Centrifugation at 8,500 x g for 10 minutes
- Concentration of the supernatant with the InnovapPrep Concentrating Pipette (0.05 µm filter)
- Elution ~ 400 µL concentrate

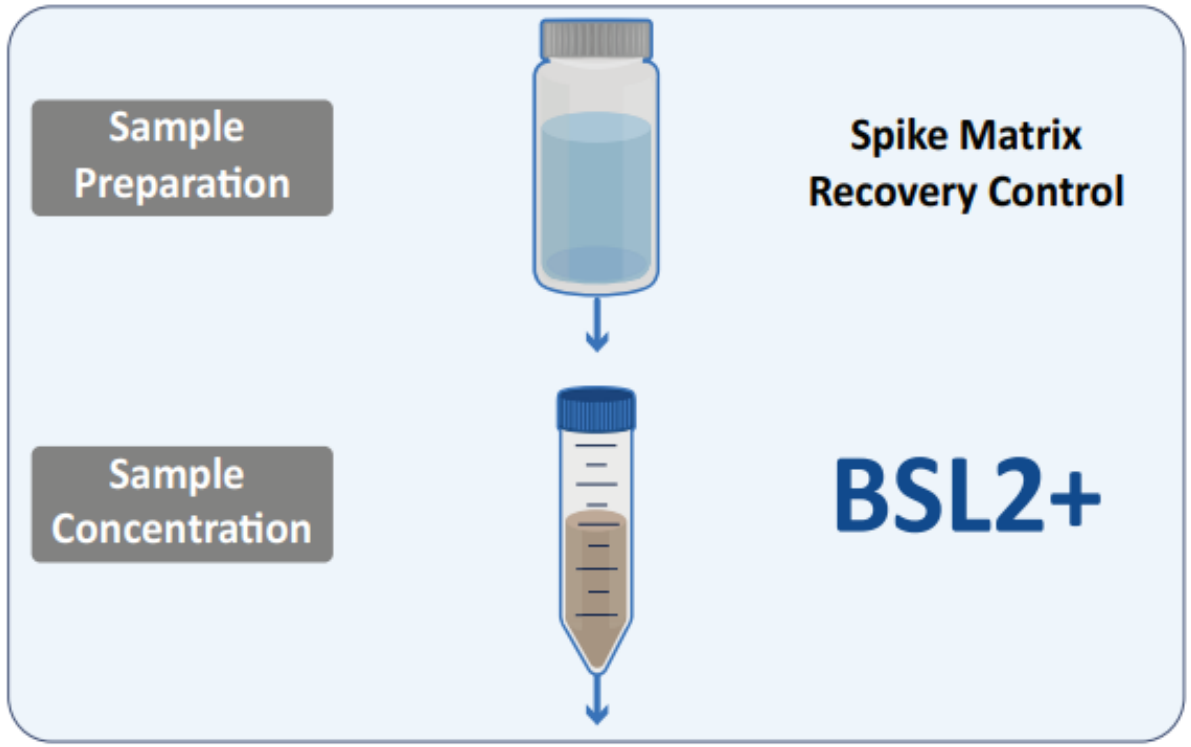


Image Source: [Developing a Wastewater Surveillance Sampling Strategy | Water-related Topics | Healthy Water | CDC]

DCLS Sample Processing Workflow

<u>Extraction and Quantification</u>

- RNA extraction by the Zymo Research Quick-RNA Viral Kit
- Quantification with the BioRad PREvalence ddPCR SARS-CoV-2 Wastewater Quantification Kit on the BioRad QX One instrument
- Yields viral copies per liter
  - Yields viral copies per liter
  - Recovery assessed with matrix spike
  - Extraction and PCR controls
    - SARS-CoV-2 Standard
    - Blank negative control

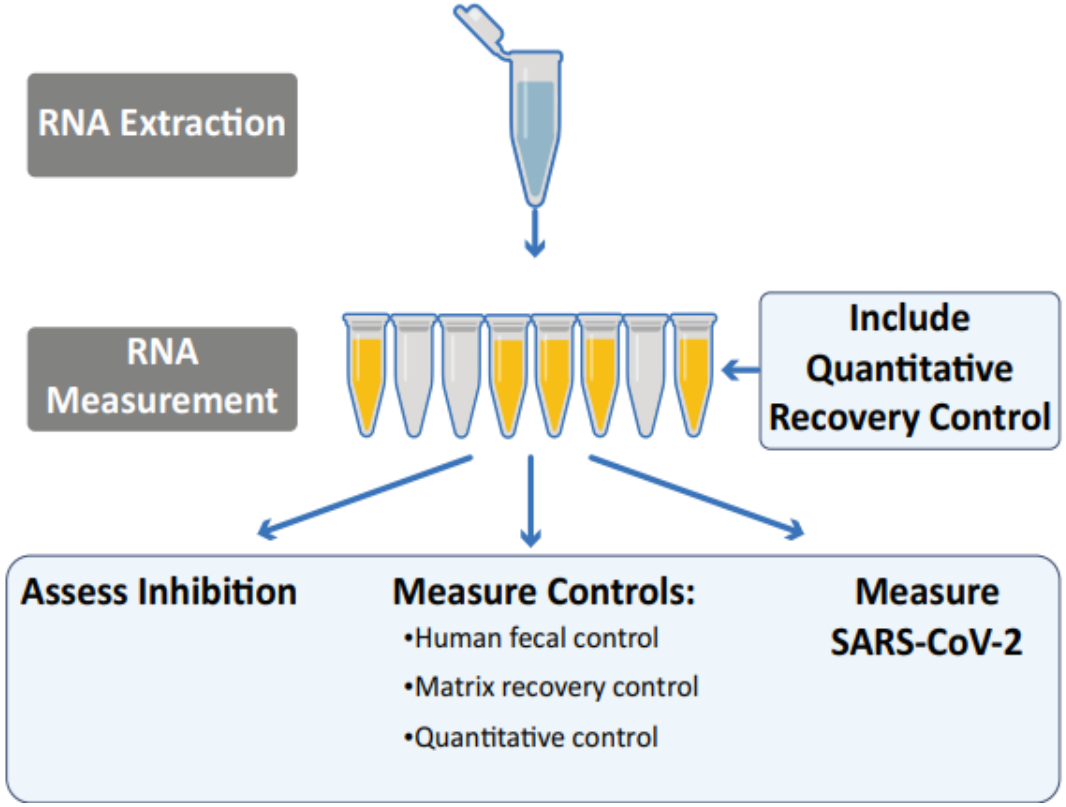


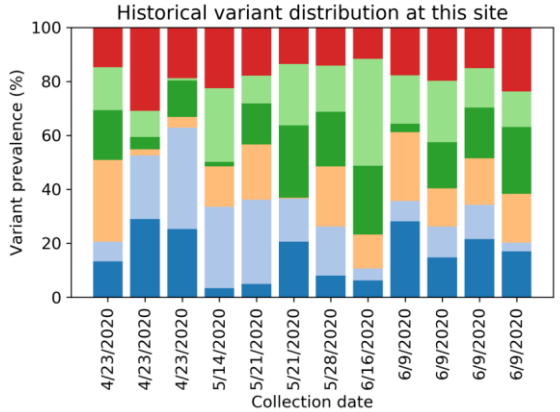
Image Source: [Developing a Wastewater Surveillance Sampling Strategy | Water-related Topics | Healthy Water | CDC]

SARS-CoV-2 Quantification and Variant Typing

ddPCR Detection
- Target= ddCoV_N (N2)
- Limit of detection= 6,200 copies/L
- Concentrations < LOD are assigned a value half the LOD (3,200 copies/L)

Data QC and Analysis
- Monroe Pipeline
- Freyja (version)

SARS-CoV-2 Sequencing
- NEB VarSkip Short SARS-CoV-2 v2 (VSS v2)
- DNA Prep Library Prep
- MiSeq or NextSeq 550 2 x 150 paired end



Northwest Facilities

Select Region
Northwest Virginia

Select Sewershed
All

Figure 1a. SARS-CoV-2 Viral Load in Wastewater

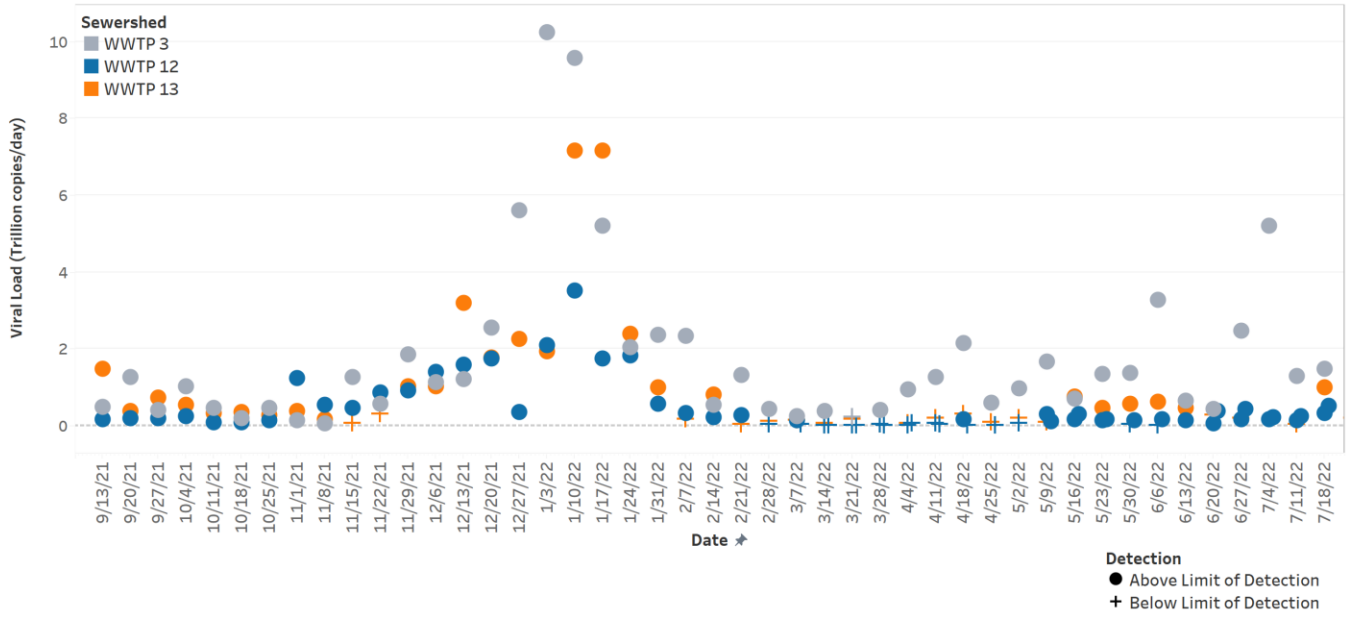
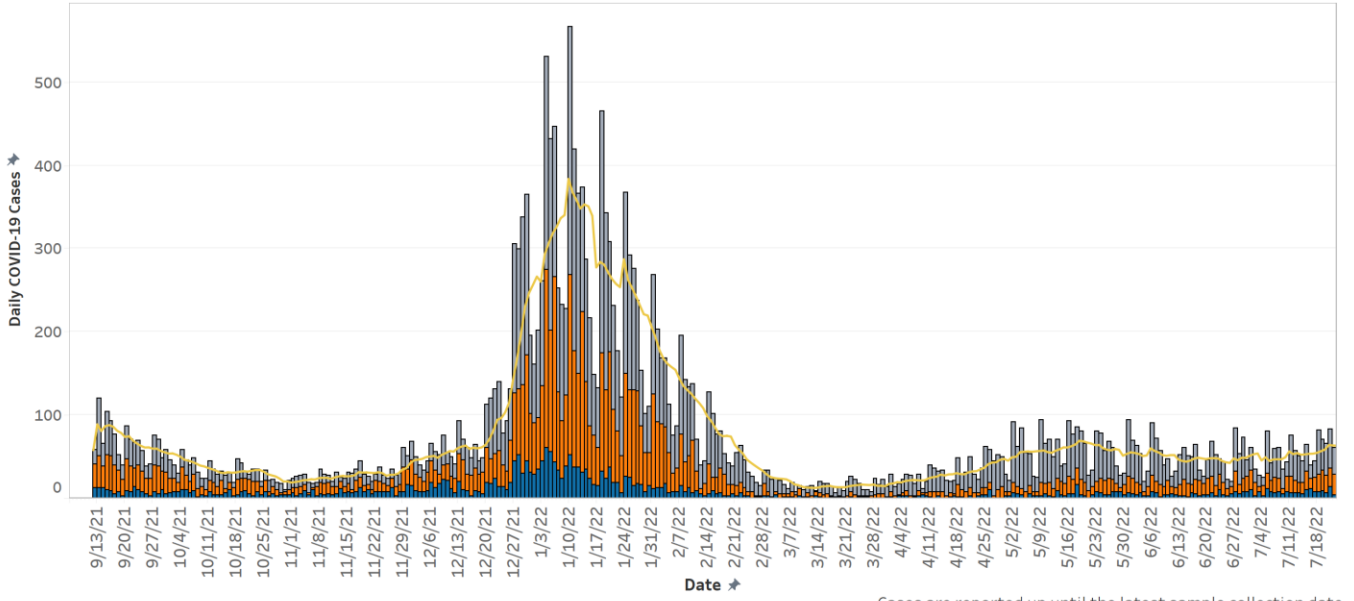


Figure 1b. Reported COVID-19 Cases within Sewershed



Cases are reported up until the latest sample collection date.

WWTP 12

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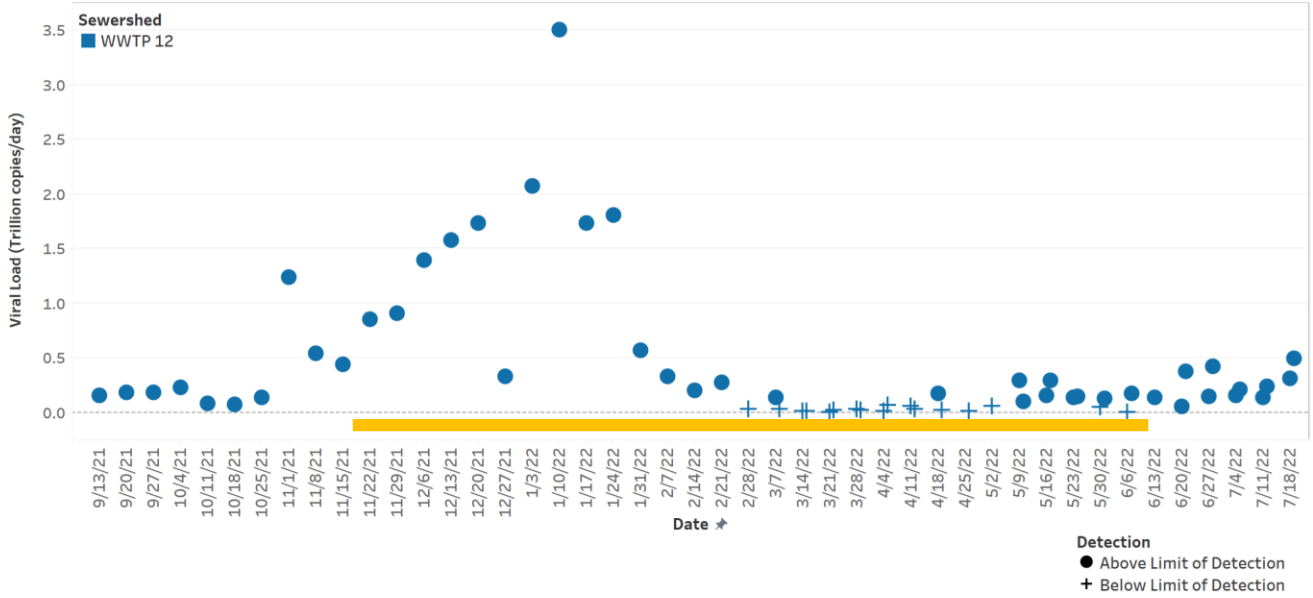
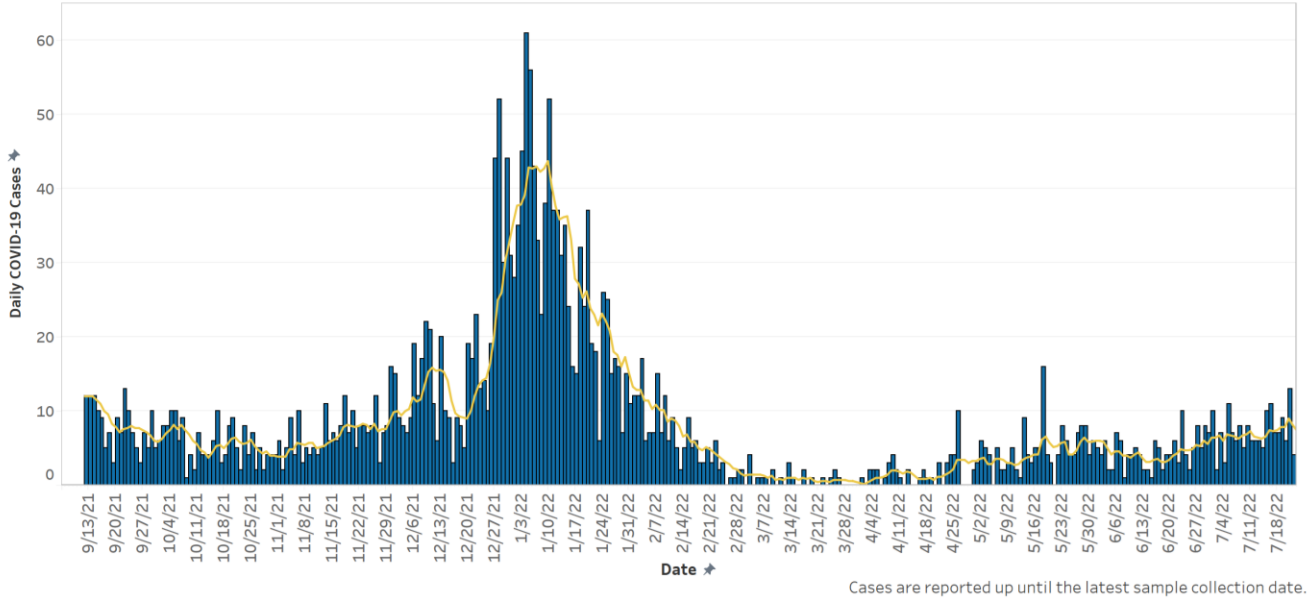


Figure 1b. Reported COVID-19 Cases within Sewershed



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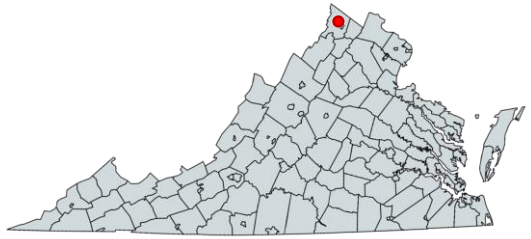
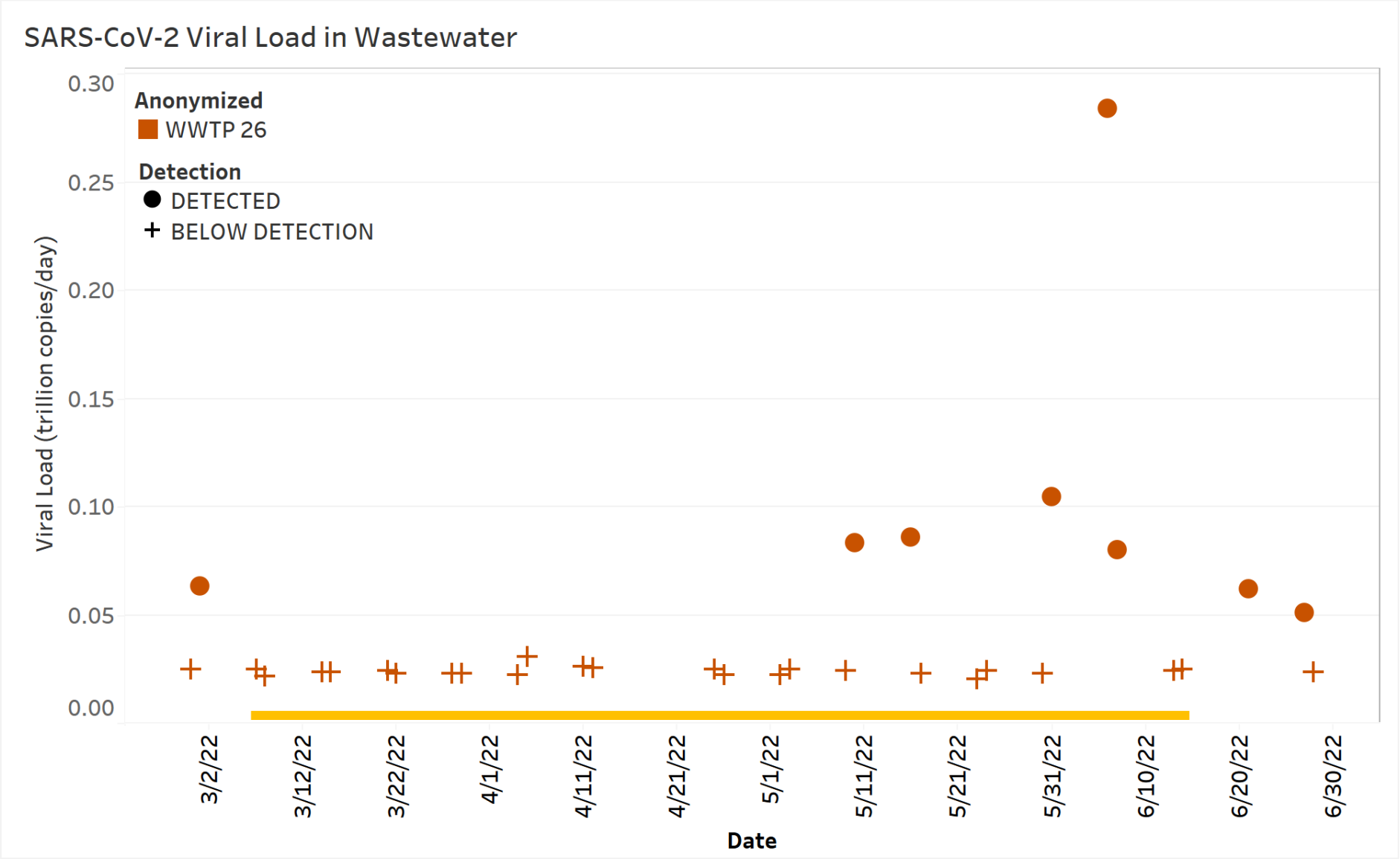
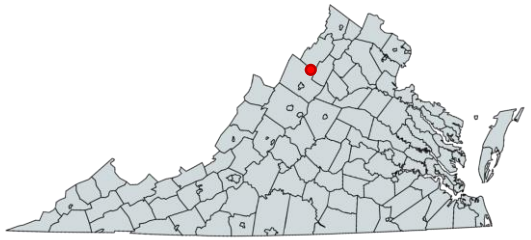


Facility capacity: 5 MGD
Population served: ~16,500

WWTP 26





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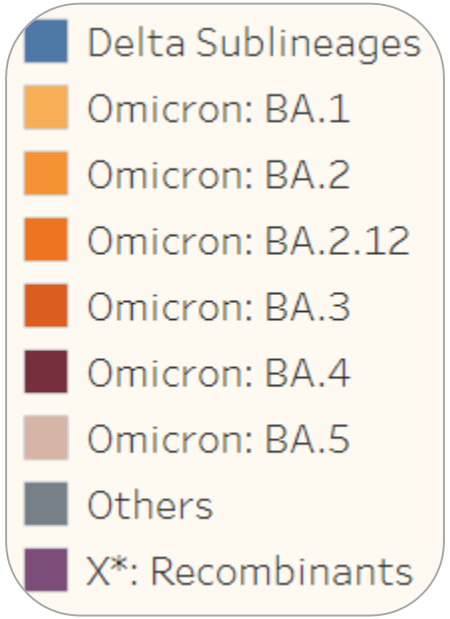
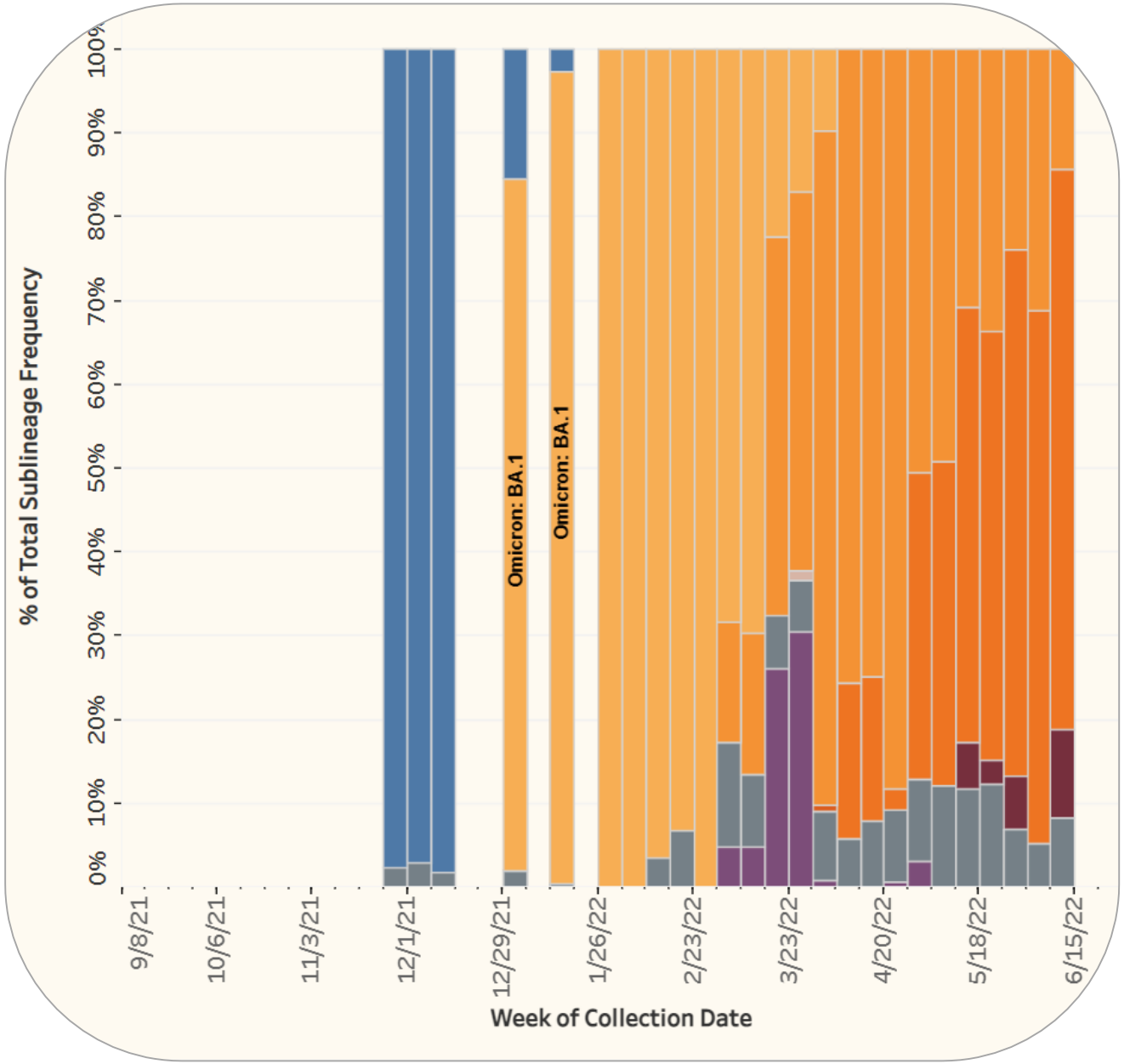
VA Site Trends

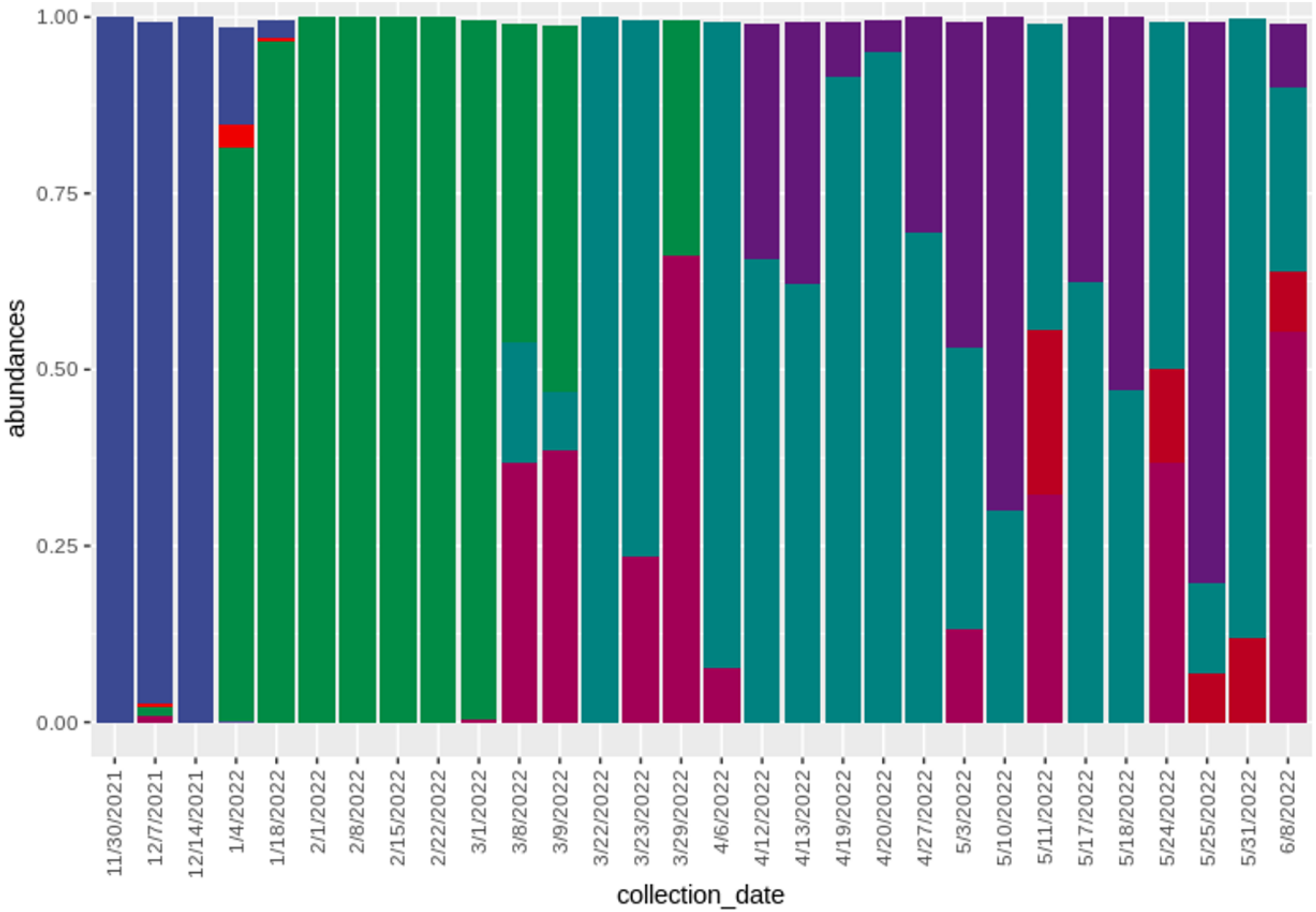


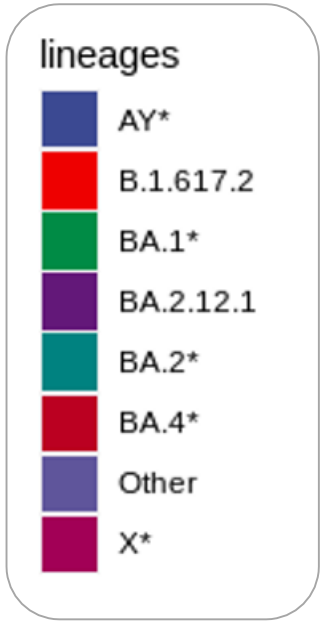


Methods

- Data was processed with C-WAP, which includes the estimation of relative abundances of SARS-CoV-2 with Freyja v.1.3.5
- “Others” is a catch-all for sub-lineages present below 1%

WWTP 12

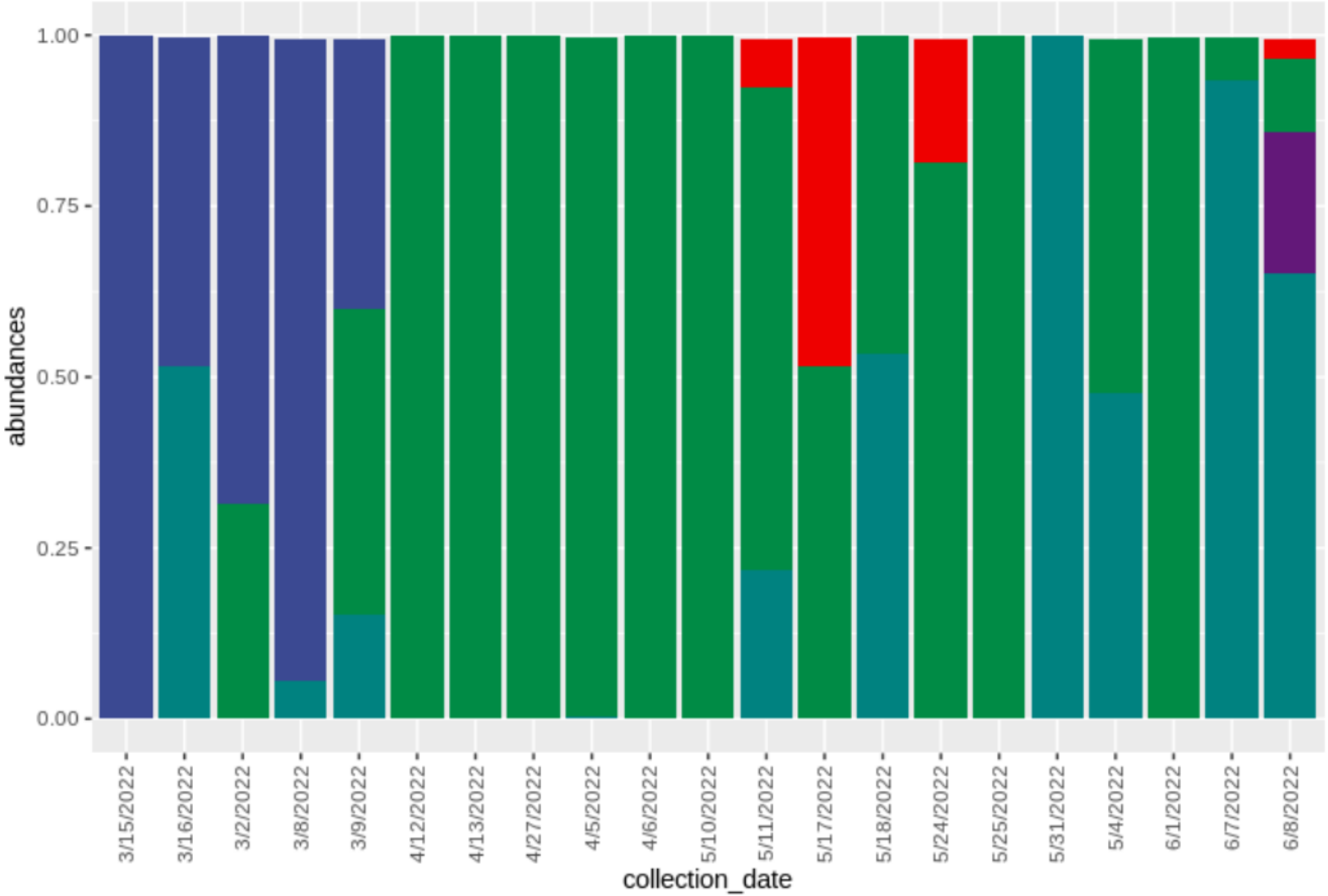




Methods

- SPLiNTER with Freyja v1.3.9 was used to estimate SARS-CoV-2 relative abundances
- Figures were made using R v4.2.0 and ggplot2 v3.3.6.
- “X*” denotes potential recombinant lineages

WWTP 26



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Challenges

- Two significant COVID-19 surges in 2021/2022
- Completely new system needed to support WW testing – partnerships, instrumentation, laboratory infrastructure, work team, quality assurance, data analysis and reporting
- Variability in measurement
  - Sampling location, methods, sewer transit time, industrial waste and storm water, flow rates, etc.
  - Methods vary from collection to result generation
- Dependent on classified variant lineages
- Genetic drift can cause gaps in genome coverage and missed data, sequencing tests will require continues updates
- Data interpreting and action

Future Directions

- Improve efficiency in sample processing and sequencing through automation
- Incorporate total nucleic acid extraction methods to adapt to multiple pathogens
- Broaden surveillance sampling to additional VDH surveillance sites
  - Sequencing surveillance for approximately 20 samples per week
  - Two sequencing runs per month
- Implement and refine variant surveillance data sharing with VDH
- Expand surveillance to emerging pathogens (ddPCR first)

Acknowledgements

DCLS

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Questions?

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