



Division of
**Consolidated
Laboratory Services**

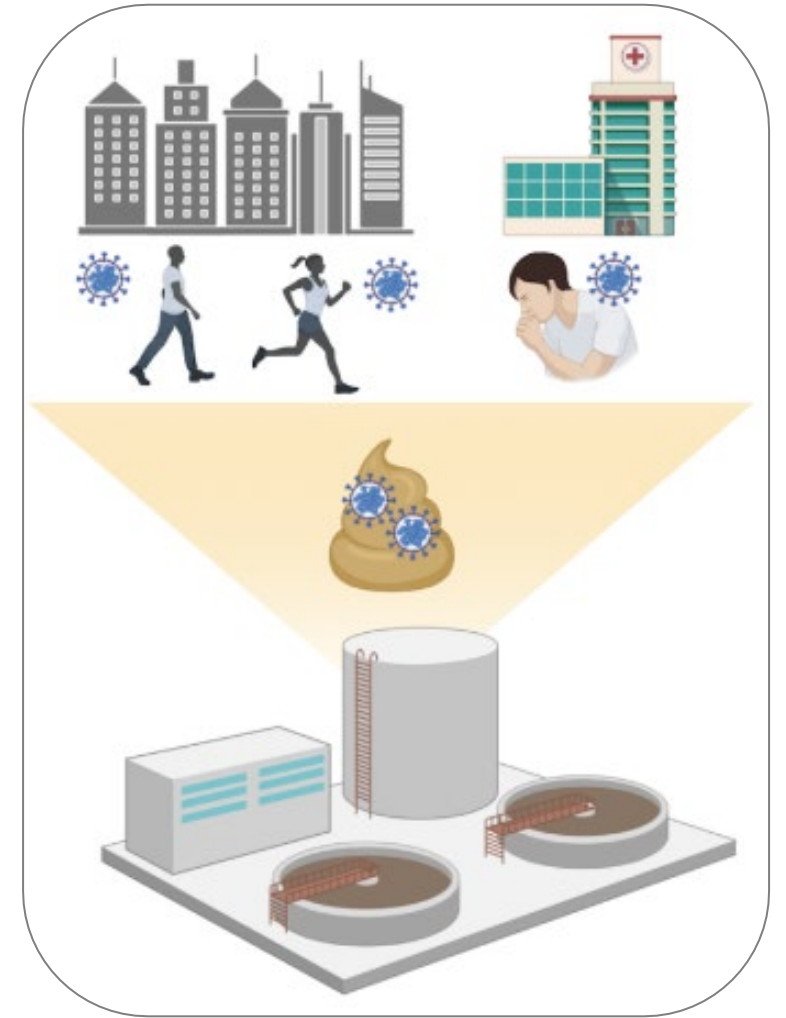


Piloting Wastewater-Based Surveillance of SARS-CoV-2 Variants in Virginia, 2021 – 2022

Lauren Turner, PhD

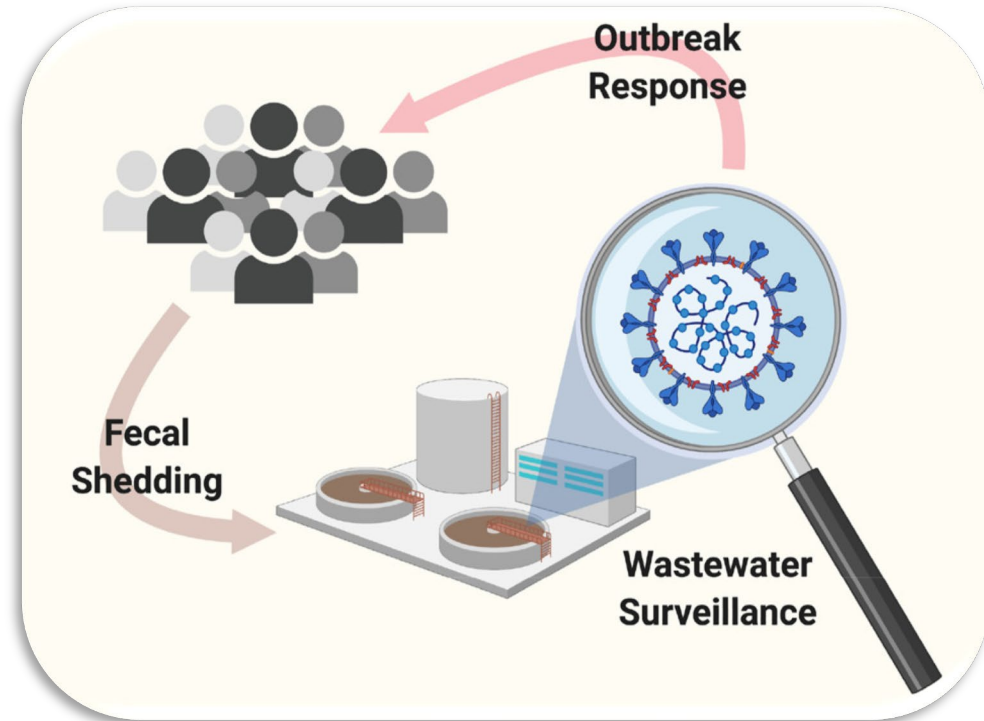
Wastewater (WW) Surveillance

- ~84% US households are connected to a WW treatment plant (WWTP)
- Un-sewered population may still be represented through time spent in sewer areas for work, school, or other activities
- Variable populations served, from 100 to > 1 million
 - Median of ~45,000 people
- Genetic pathogen biomarkers as an indicator of the magnitude of pathogen load in WW
- Used for decades for polio surveillance



SARS-CoV-2 WW Surveillance

- First respiratory virus target at scale
 - Up to 80% people with COVID-19 shed the virus
- WW can serve as an early warning system (days – weeks) ahead of case increases
 - Symptomatic and asymptomatic infections
 - Independent of healthcare and testing access
 - An efficient pooled sample of community infection
 - Data is available quickly, within days of viral shedding



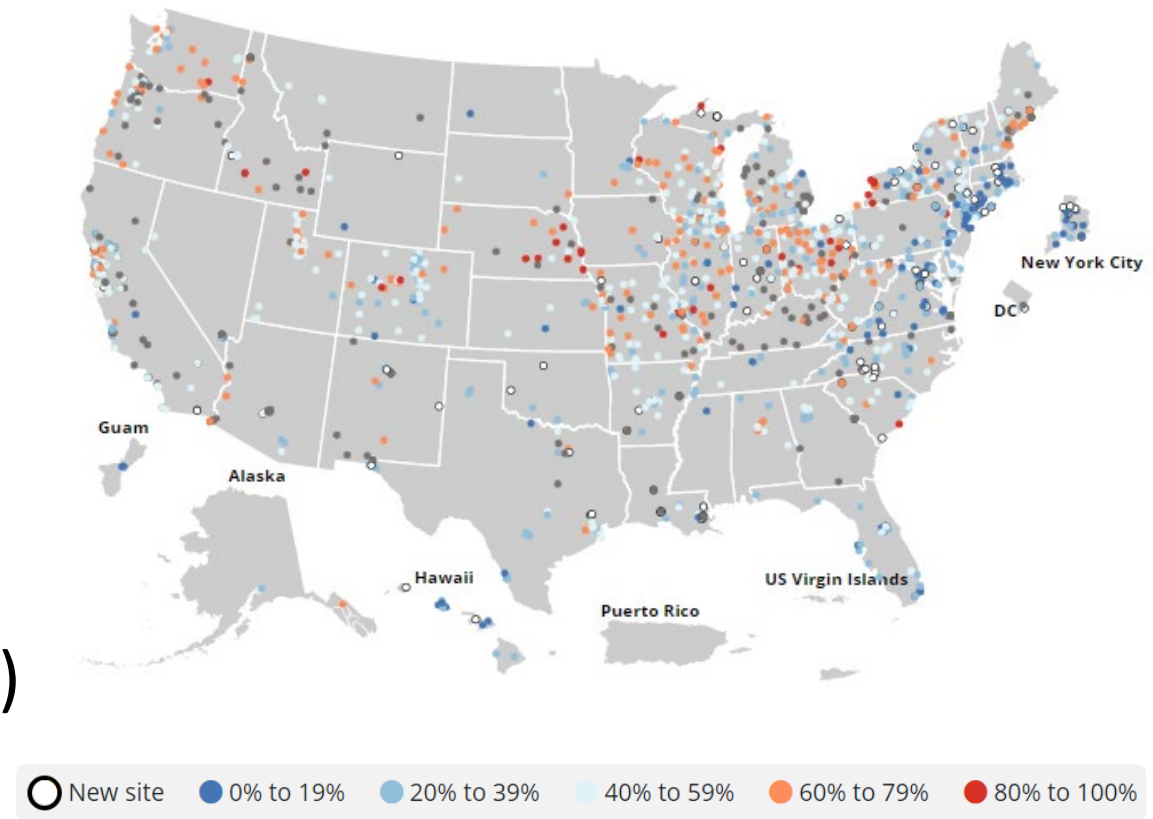
Environ. Sci. Technol. 2020, 54, 13, 7754-7757

Biomarker Surveillance Considerations

- Inherent sample and method variability
 - Lack of standardized methods
 - Location, method, sewer transit time, addition of industrial waste or storm water to the sewer, wastewater flow rates, fecal shedding rate
- Inhibition
 - Poor virus recovery due to complex matrix composition
 - Biological oxygen demand, suspended solids, metals, industrial contributions, disinfectants, etc.
 - Numerous controls needed
- Normalization
 - Population size and sewage flow rate data, etc.

National Wastewater Surveillance System (NWSS)

- Established in 2020 by CDC
- Tracking the presence of SARS-CoV-2 in US wastewater- ~1,500 sites
 - All 50 states, major cities, US Territories
 - Commercial testing contract
 - State and Territorial Laboratories
 - Wastewater treatment plants (WWTP)

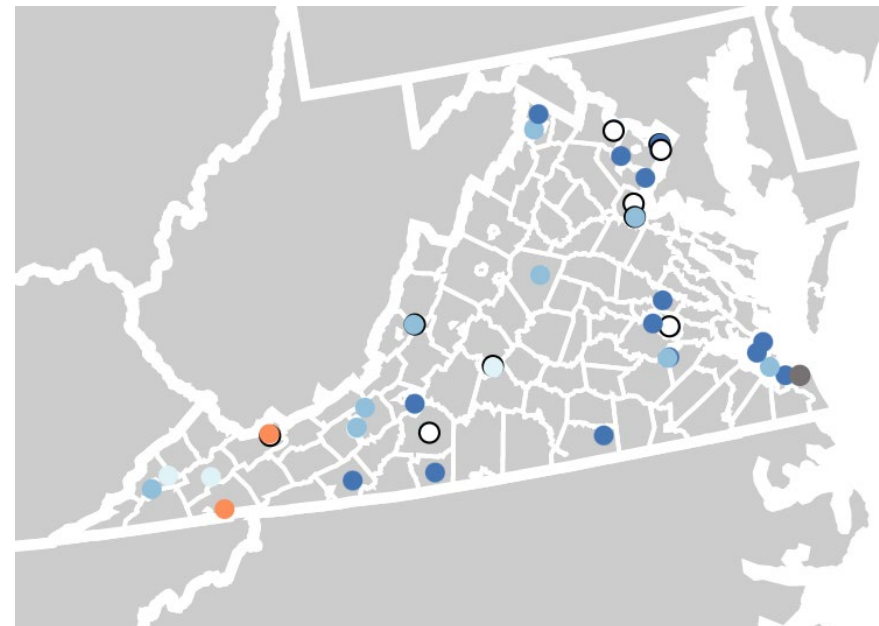


[CDC COVID Data Tracker: Wastewater Surveillance](#)

Virginia Wastewater Monitoring Program

- SARS-CoV-2 Wastewater Surveillance Sentinel Monitoring Program

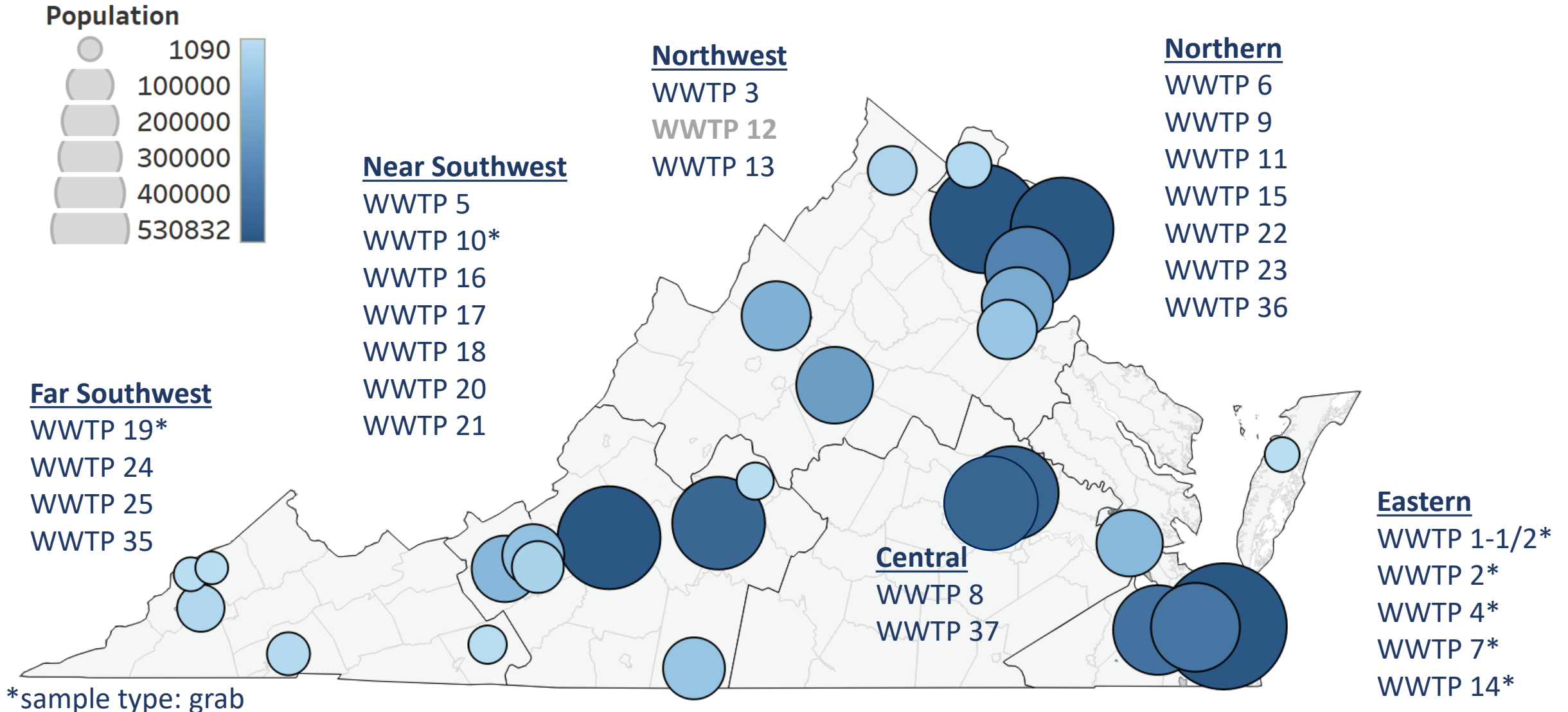
- Initiated 9/21/2021
- Currently **28** wastewater treatment plants
 - **9** sampling *twice* weekly
 - **19** sampling *once* weekly
- Ongoing enrollment (up to 40 sites)
- Wastewater treatment plant influent
- Aggregate data reported to CDC
- Site-specific data shared with WWTP



○ New site ● 0% to 19% ● 20% to 39% ● 40% to 59% ● 60% to 79% ● 80% to 100%

[CDC COVID Data Tracker: Wastewater Surveillance](#)

Virginia Sentinel Monitoring Facilities



Outcomes from Local Surveillance Projects

- Development of actionable strategies to prevent and control local spread
 - Mobilized clinical testing and vaccine offerings
 - Educational campaigns where infection rates are increasing/higher
 - Targeting resources in response to early signals of case increases

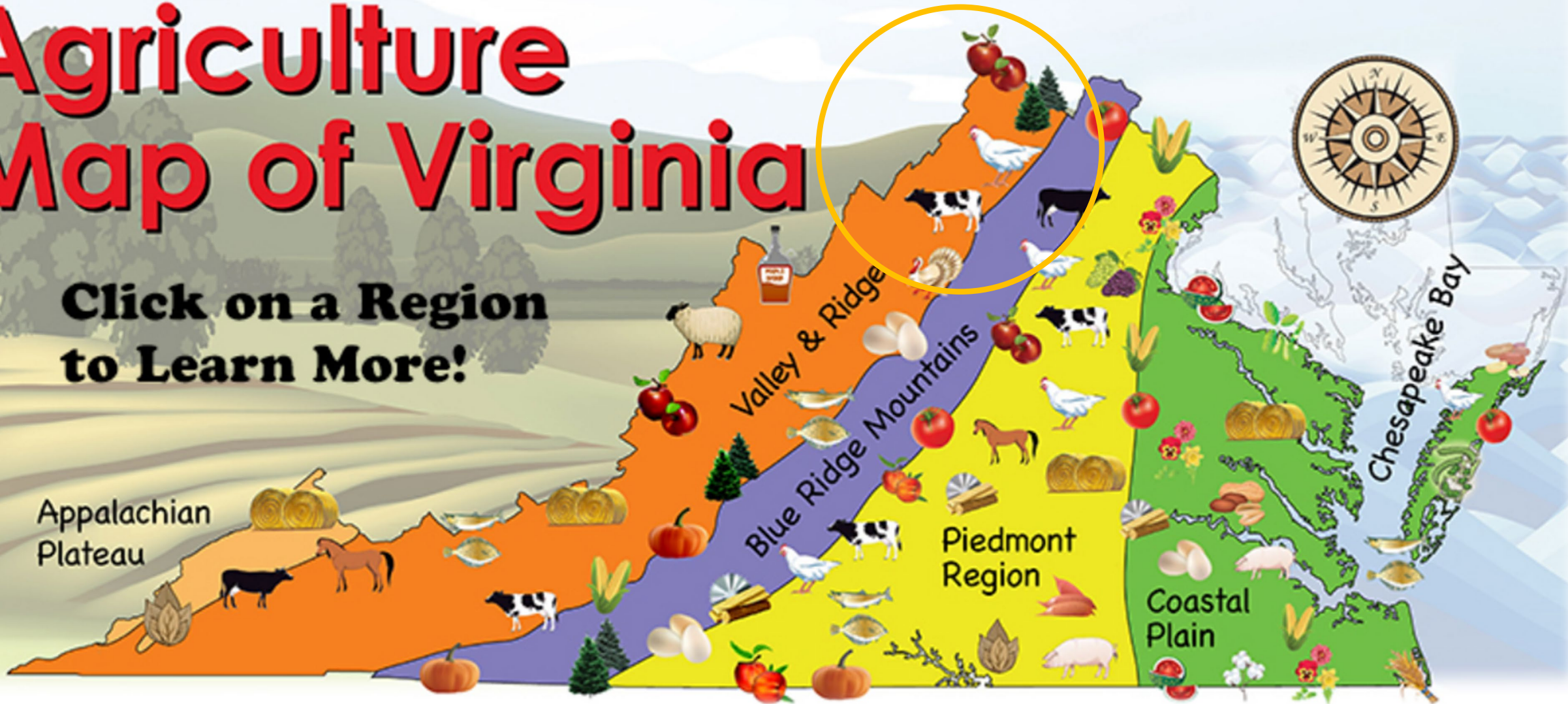
FDA WW SARS-CoV-2 Variant Surveillance Project (2021 – 2022)

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



Agriculture Map of Virginia

**Click on a Region
to Learn More!**



[Virginia Commodities Pavilion \(statefairva.org\)](http://statefairva.org)

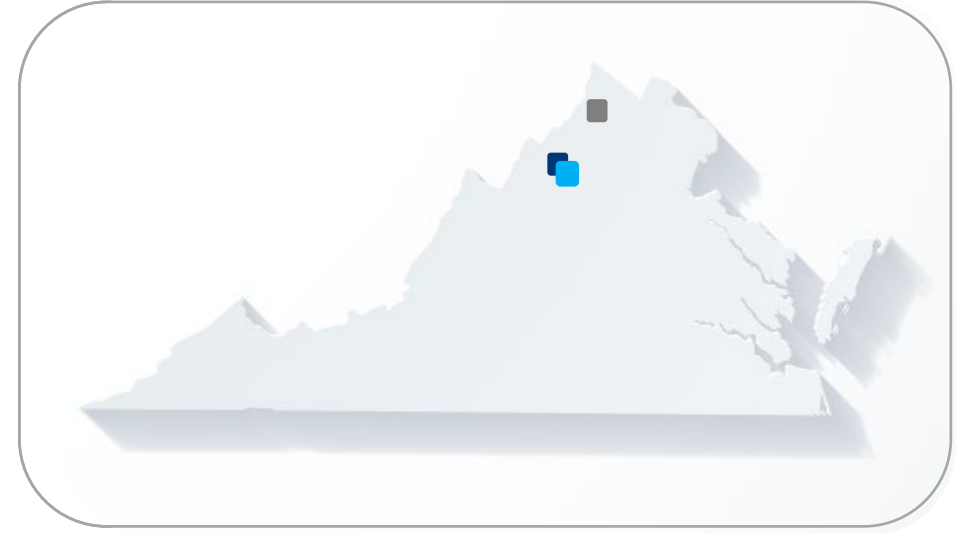


DCLS | Division of Consolidated Laboratory Services



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

- Three sites
 - **WWTP 12** (population ≈ 16,500)
 - 5 MGD
 - Seasonal fruit and tree-nut agriculture
 - **WWTP 26** (population ≈ 16,500)
 - 5 MGD
 - Town adjacent to poultry processing facility
 - **WWTP 27** (population ≈ 500)
 - 0.99 MGD
 - Poultry-processing facility

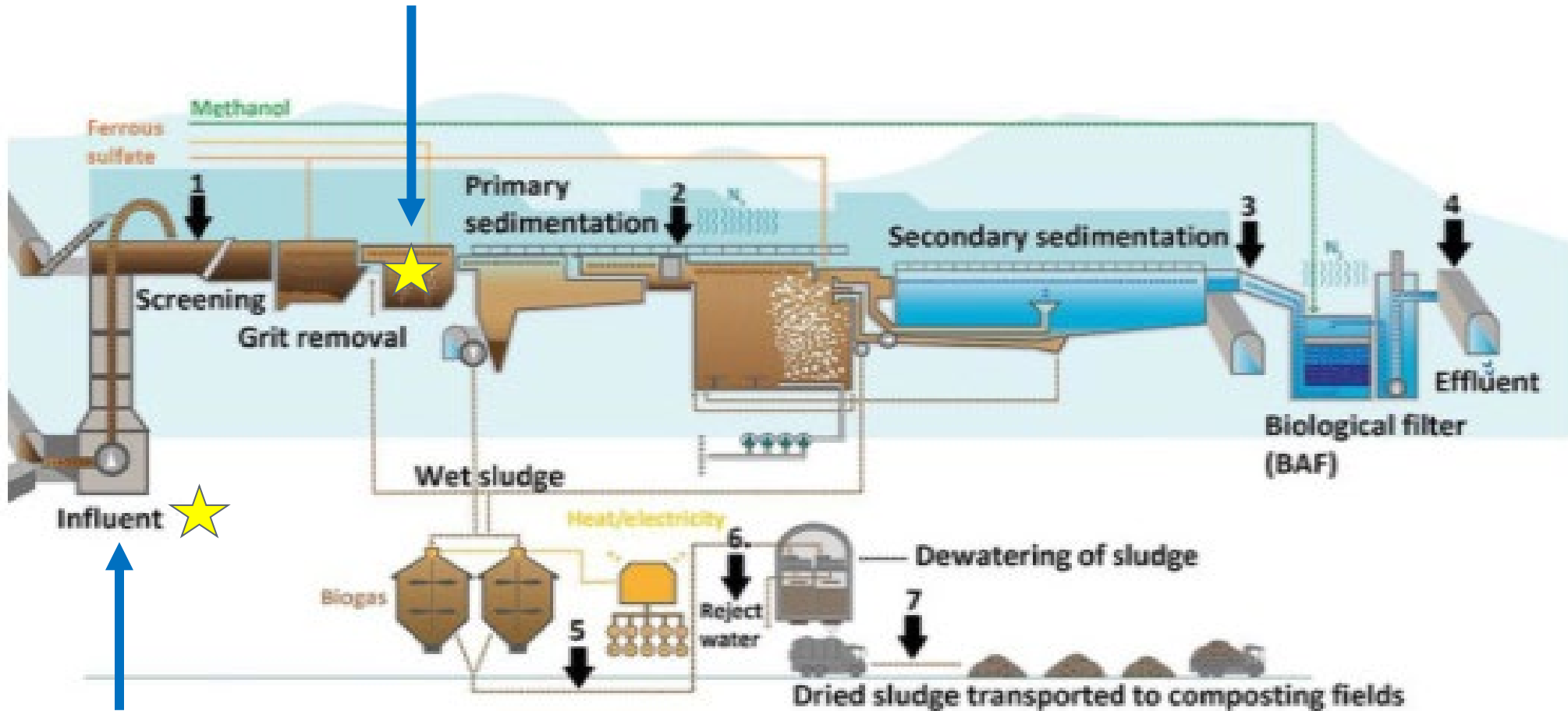


- 100 mL 24-hr composite samples collected 1 – 2x/week
- Transported to DCLS via contracted courier
- Samples stored at 4°C until processing (up to 2 days)

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

WWTP-Assisted Sample Collection

Post Grit Removal



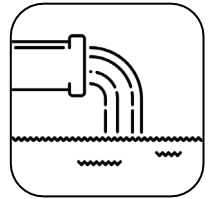
Raw Wastewater

Source: Talvitie, et al. 2015

Proposed Project Laboratory Workflow

Laboratory-developed

FDA-advised methods and processes



- Sample collection
- Concentration
- RNA extraction
- RT-dPCR
- Quantification

RNA

QC



- cDNA
- Library prep
- Sequencing

Sequence

QC



NCBI submission

- Removal human reads
- BioSample
- SRA



FDA

- Analysis pipeline
- Reports

Overview of Sample Submission Process

DCLS Provides Sampling Kit

Prepare Samples for Transport



Collect Samples

Package Samples

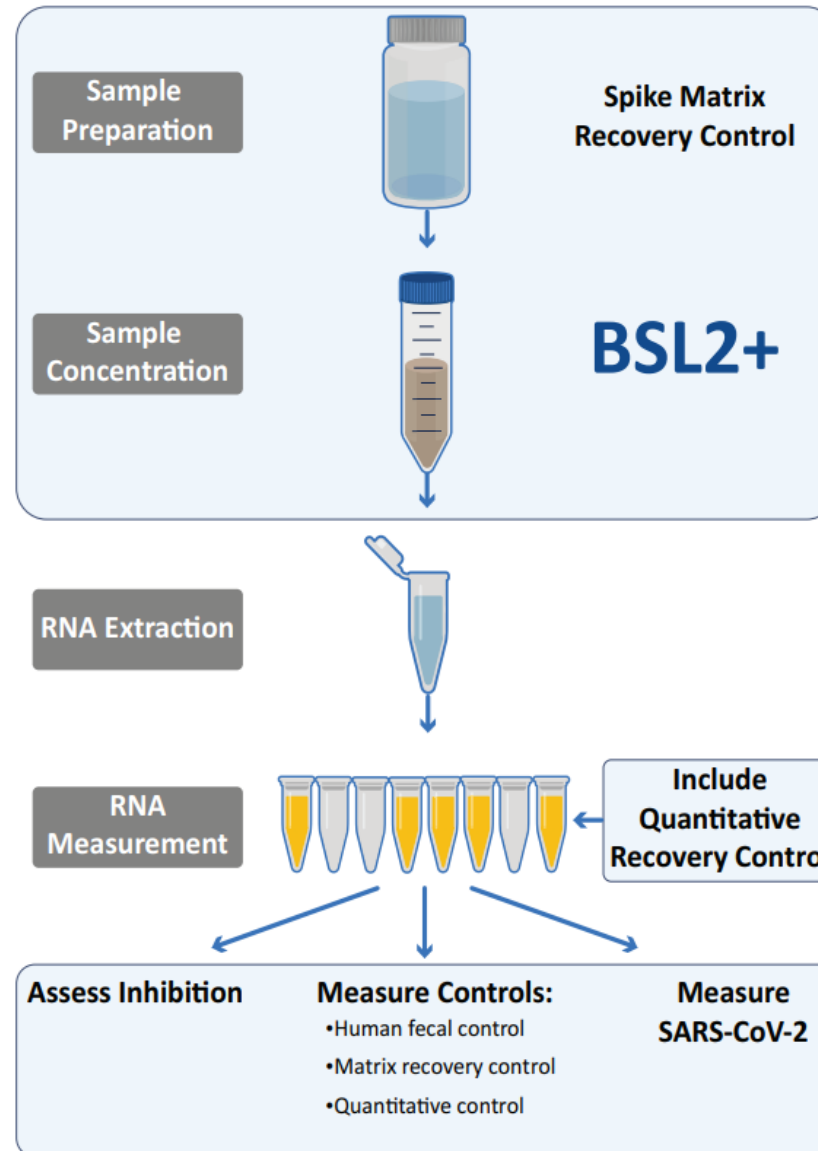


Fill out
Sample
Submission
Form

DCLS Sample Processing Workflow

Sample Preparation

- Homogenization
- Matrix spike
- 5% Tween added to 40 mL
- 8,500xg for 10 min
- InnovaPrep CP Select Concentrating Pipette (0.05 µm filter)
- ~ 400 µL concentrate



Extraction and Quantification

- Zymo Research Quick-RNA Viral Kit extraction
- BioRad PREvalence ddPCR SARS-CoV-2 Wastewater Quantification Kit
- BioRad QX One Droplet Digital PCR System

Measurement

- Yields viral copies/liter
- Matrix spike for recovery
- Extraction and PCR controls
 - Bio-Rad SC2 standard
 - Blank negative control

SARS-CoV-2 Quantification and Variant Typing

RT-dPCR Detection

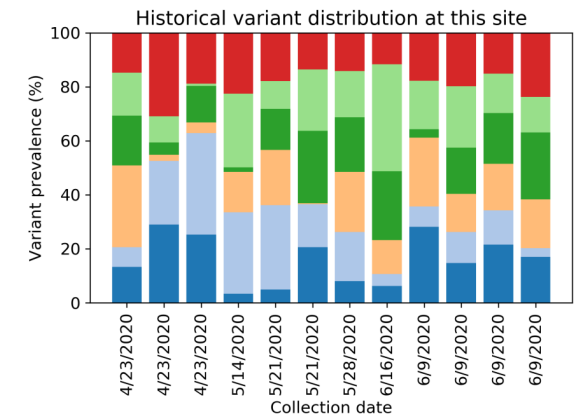
- Target= ddCoV_N (N2)
- Limit of detection= 6,200 copies/L
- Concentrations < LOD are assigned a value half the LOD (3,200 copies/L)



SARS-CoV-2 Sequencing

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

Data QC and Analysis



Primer Scheme Selection – PCR Tiling

Avoiding potential variant drop-out

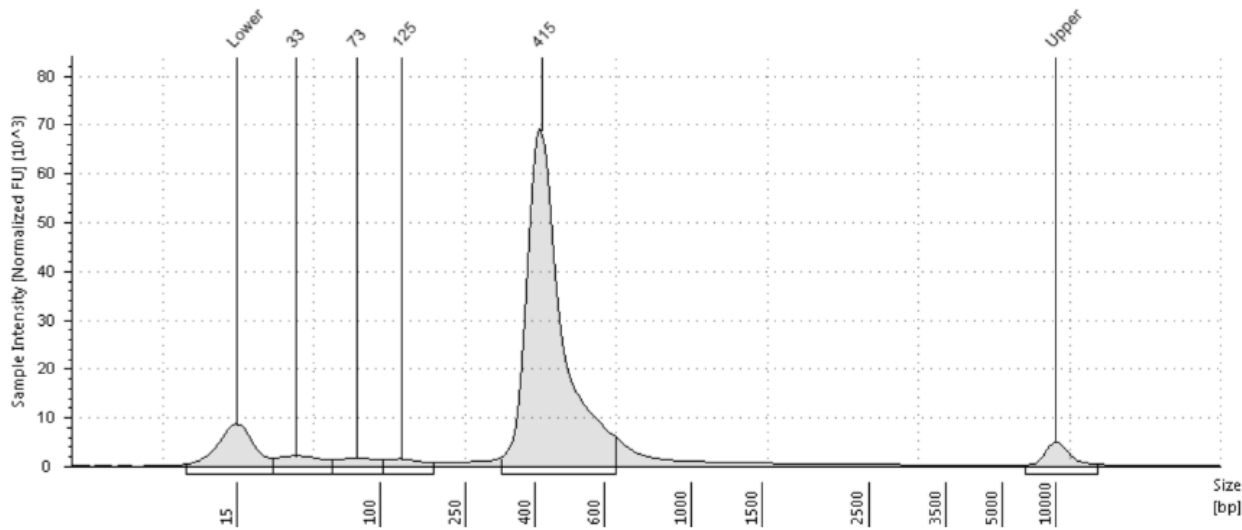


[Primer Monitor \(neb.com\)](https://primer.monitor.neb.com)

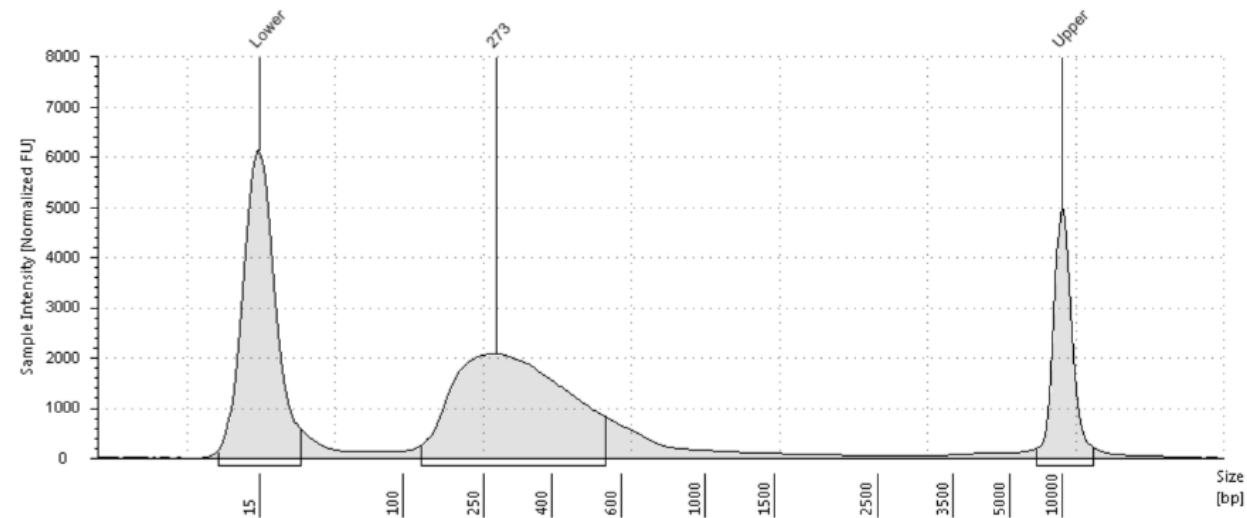
TapeStation Fragment Analysis

- 4200 HM D5000 Tape

cDNA fragments $\approx 400 - 425$ bp



Library fragments $\approx 250 - 300$ bp



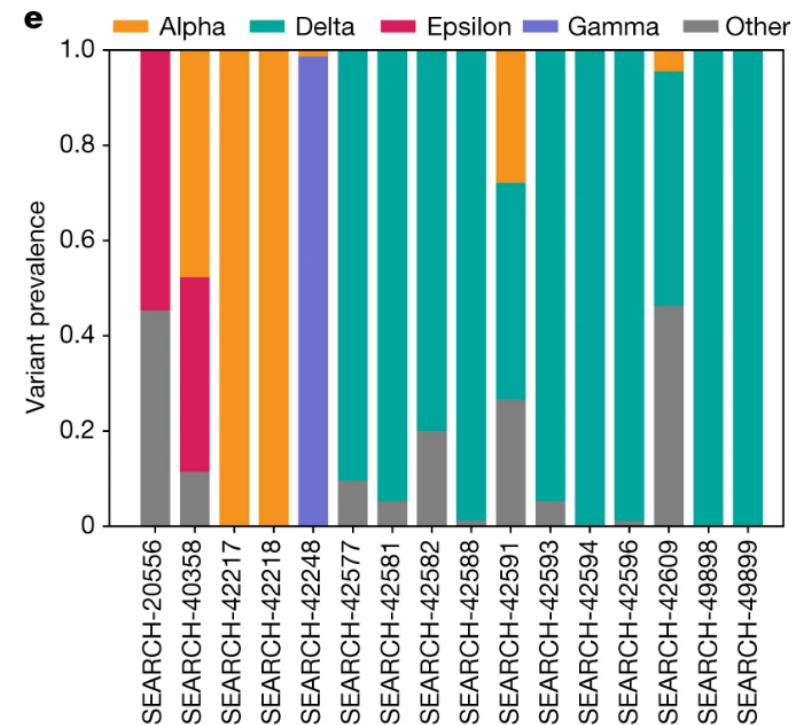
FDA CFSAN Quality Reports

VSS v1a Primers	Sample#	Sample name	Total #reads	% covid hits	Genomic coordinates 0X	Genomic coordinates <10X
	1	Blank-1-020822RB	1025996	0.30	19396	29903
	2	Blank-2-020922RB	2160	7.41	29185	29903
	3	FDA-B-01	2150132	54.62	16536	19752
	4	FDA-C-01	1929908	43.13	14001	16623
	5	FDA-SA-1	3722448	97.05	106	129
	6	FDA-SA-2	6117156	94.93	108	111
	7	Positive-020922RB	6304656	90.24	1834	2146

VSS v2a Primers	Sample#	Sample name	Total #reads	% covid hits	Genomic coordinates 0X	Genomic coordinates <10X
	1	Blank-1-021822RB	1225240	0.77	9989	29884
	2	Blank-2-022222RB	2416	6.71	29404	29903
	3	FDA-B-01	3807738	79.22	6806	9383
	4	FDA-C-01	3869348	83.48	6939	8901
	5	FDA-SA-1	4403204	93.73	100	135
	6	FDA-SA-2	6005726	93.54	108	135
	7	PositiveControl	5620516	86.16	1848	2223

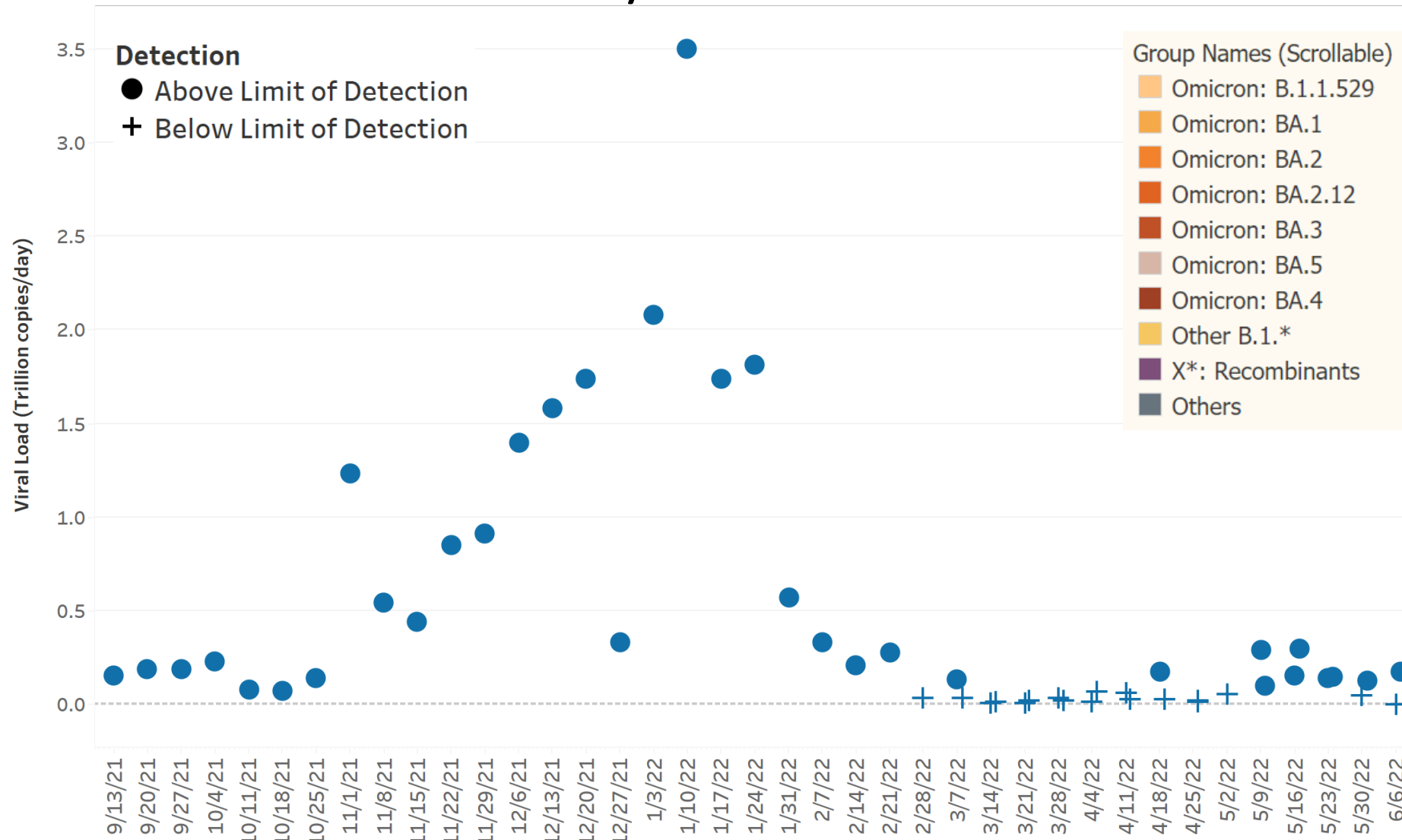
Estimation of Relative Abundance in WW

- Single dominant variant lineage typing tools used for clinical SC2 not appropriate for WW
- Scripps Research Institute Andersen Lab Freyja Tool
 - Estimation of relative abundance of multiple SARS-CoV-2 lineages in a mixed viral sample
 - UShER global phylogenetic tree-derived lineage-determining mutational barcode library
 - Measures single nucleotide variant frequency and sequencing depth at each position in the genome to return an estimate of the true lineage abundances in the sample

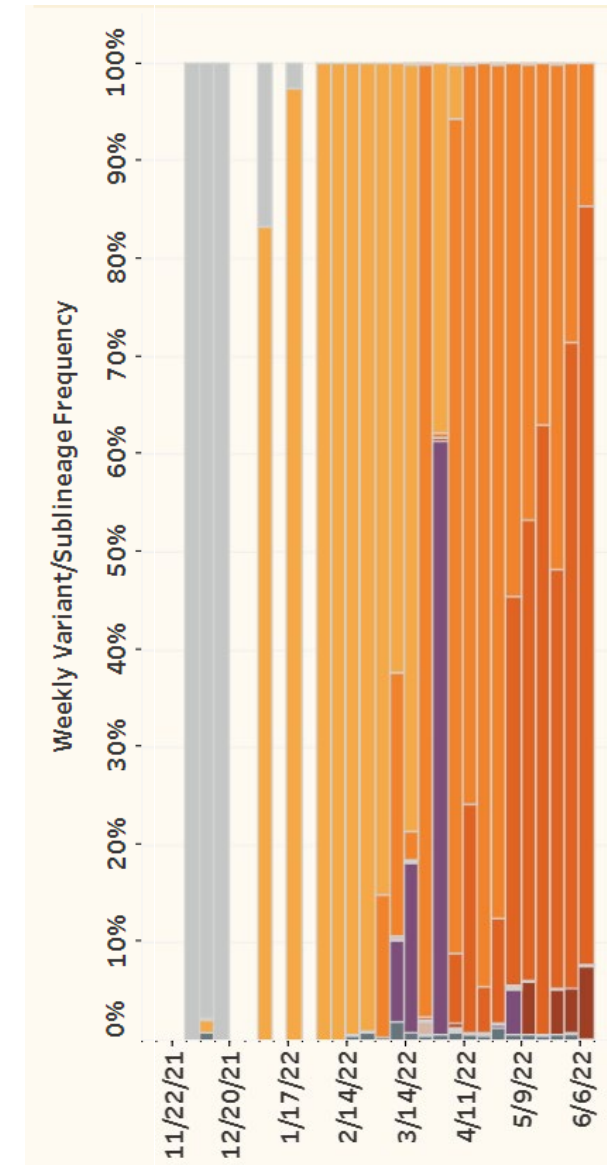


Nature **609**, 101–108 (2022)

WWTP 12 Weekly Viral Load Profile



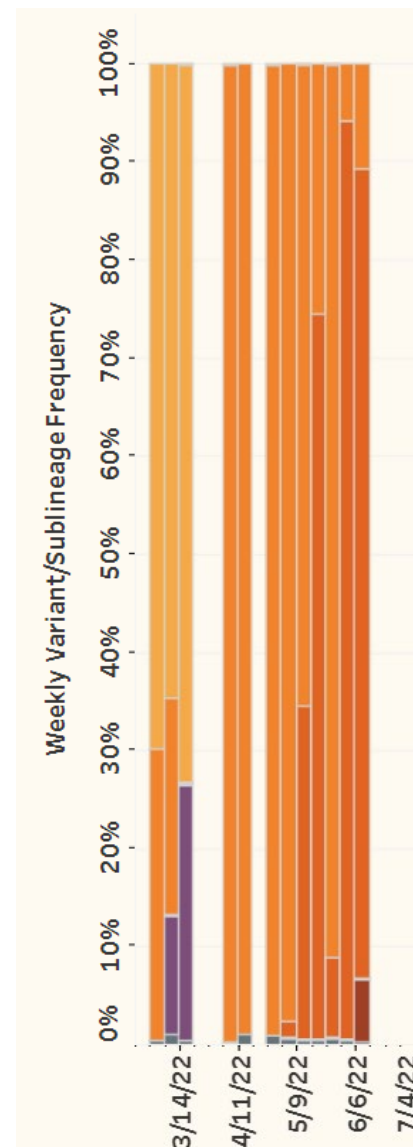
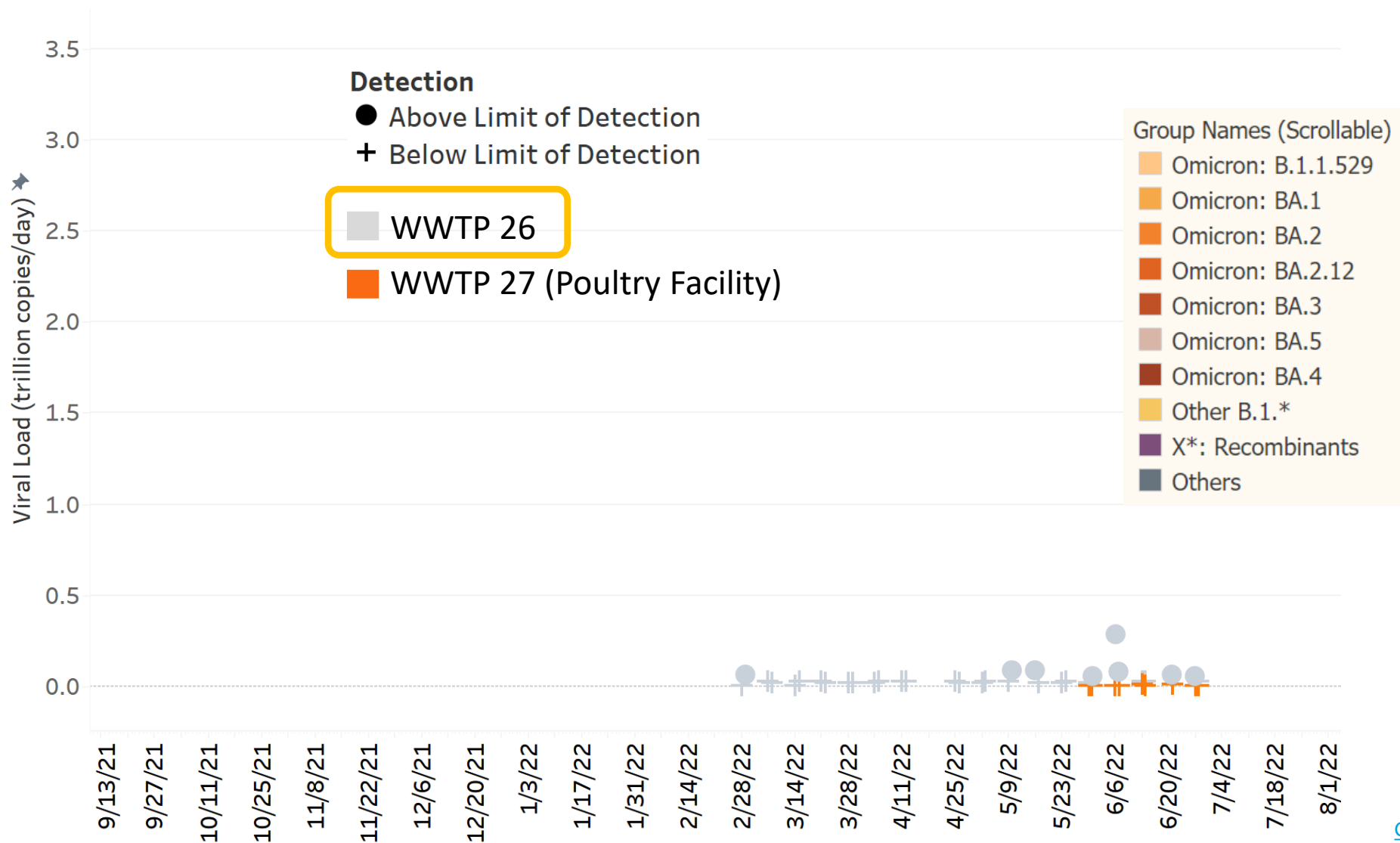
$$\text{Viral load (copies/day)} = \text{Viral concentration (copies/L)} * \text{Flow rate (MGD)} * \frac{3785411.8 \text{ L/d}}{1 \text{ MGD}}$$



[GitHub - CFSAN-Biostatistics/C-WAP: SC2 variant detection and composition pipeline](#)



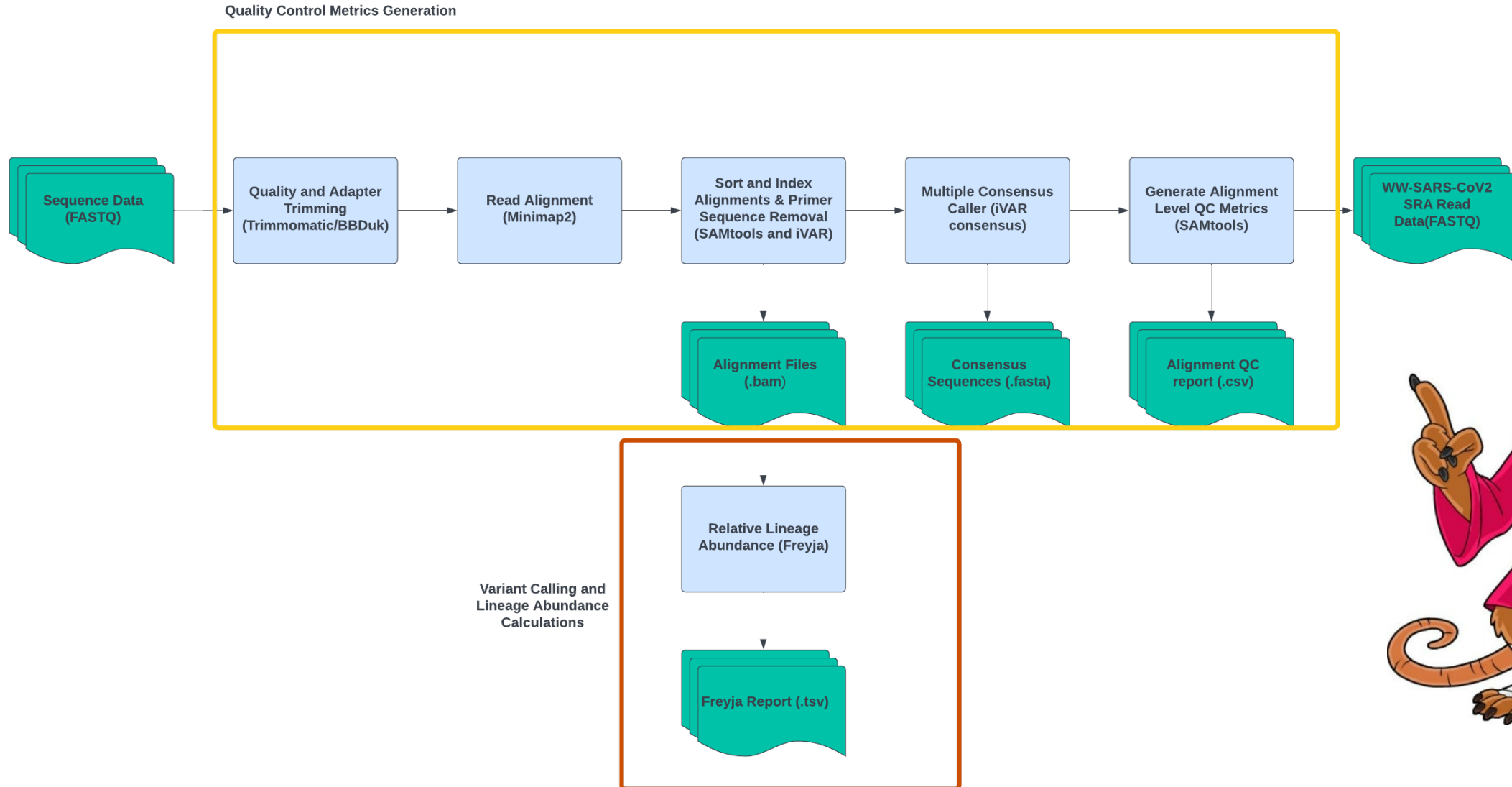
WWTP 26 and WWTP 27 (Poultry Facility)



[GitHub - CFSAN-Biostatistics/C-WAP: SC2 variant detection and composition pipeline](#)



DCLS SPLINTER Pipeline



SPLINTER Quality Acceptance Criteria

- Splinter quality reviewed prior to data acceptance

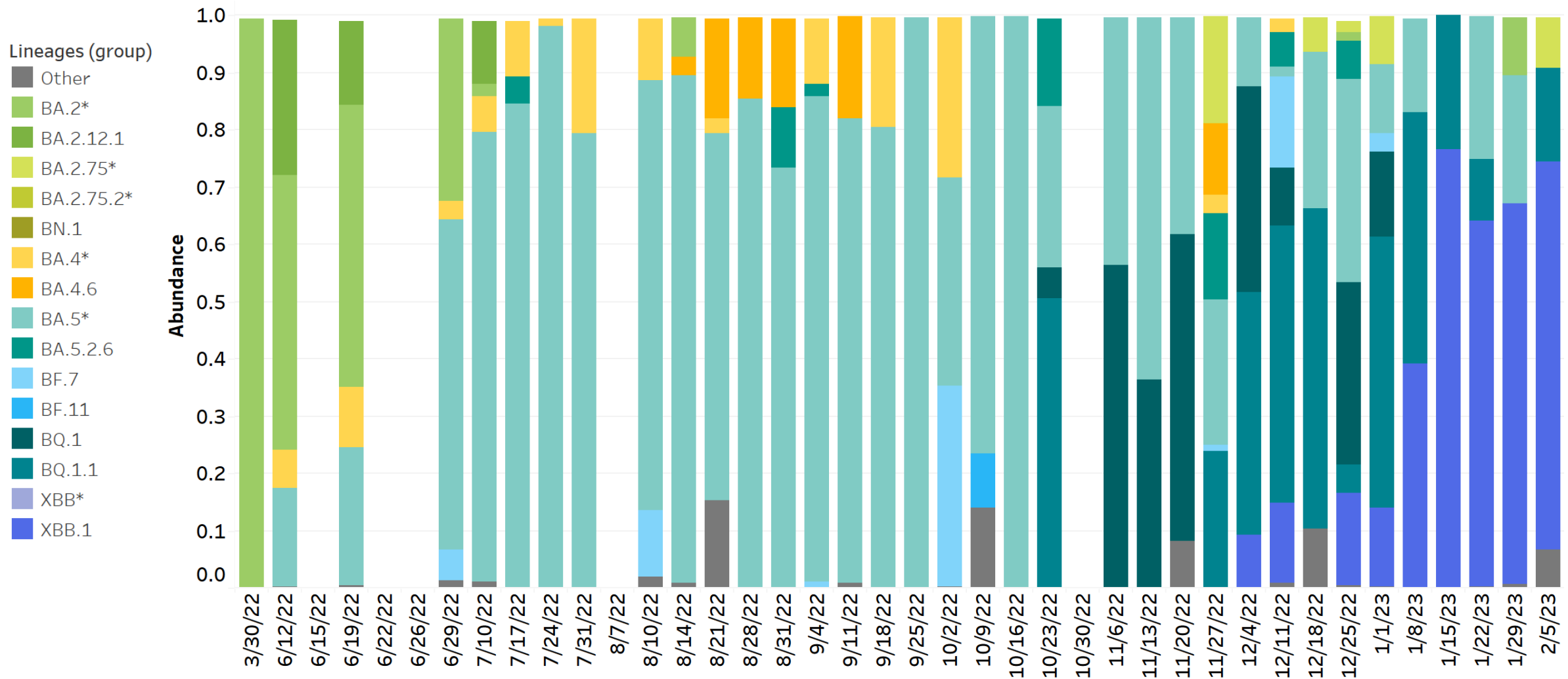
	Aligned Bases	Minimum % Coverage Compared to Reference Genome	Minimum Mean Depth	Minimum average base quality Phred Score	Minimum Mapping Quality	Pango Lineage	
Wastewater Sample	$\geq 17,900^*$	60*	$\geq 10x^*$	35*	30	N/A	} Met for samples < RT-dPCR LOD
Positive Control Material	$\geq 26,900$	90	$\geq 100x$	30	30	B	
Blank NTC	≤ 1500	$\leq 10^*$	$< 10x$	N/A	N/A	Failed Pango Lineage QC	

Comparison of Expected and SPLINTER Freyja Results

- *In silico* dataset received from CDC NWSS for ARTIC V4 primers

	Dataset	WHO Variants	Expected Results	Actual Results
ARTIC V4	TOAST-v4-m	Delta	0.41	0.4
		Eta	0.2	0.2
		Epsilon	0.15	0.15
		Beta	0.1	0.1
		Alpha	0.08	0.08
		Gamma	0.06	0.06
		Other	0	0
	UK-mock-1-v4	Delta	0.41	0.4
		Eta	0.2	0.2
		Epsilon	0.15	0.15
		Beta	0.1	0.1
		Alpha	0.08	0.08
		Gamma	0.06	0.06
		Other	0	0

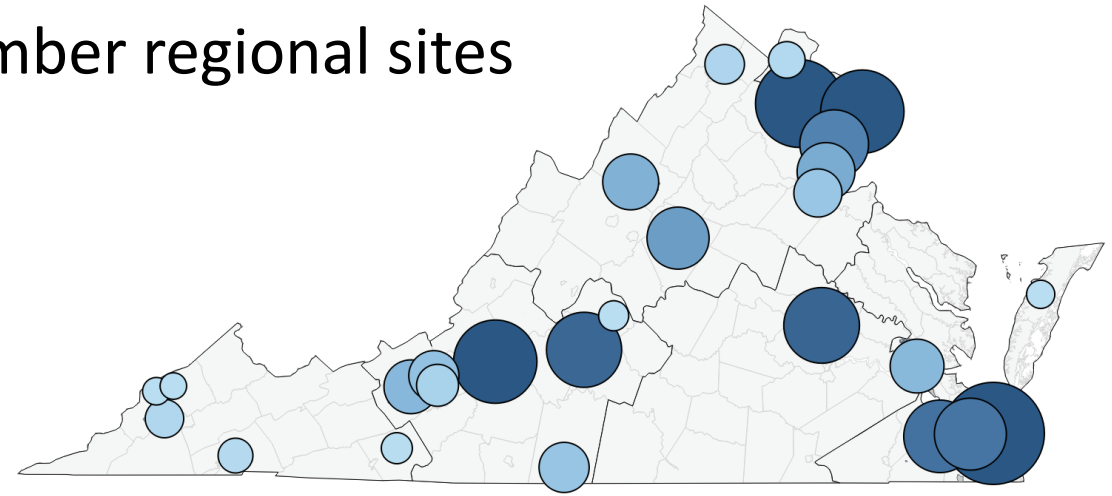
WWTP 12 Sequencing by Date



Study to Implementation

July 2022 - present

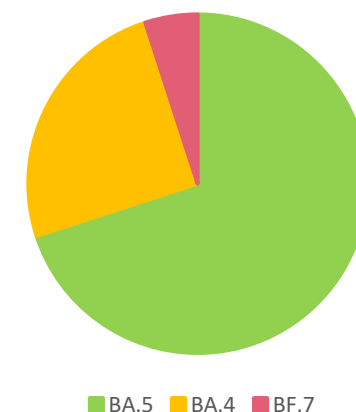
- Subsampling of ~20 samples close to or above LOD weekly
- Biweekly sequencing runs
 - 18 sites sampled weekly (Central, Eastern, far Southwest, and Northwest)
 - 10 sites rotate biweekly (Eastern, near Southwest, and Northern)
- Frequency of selection dependent on number regional sites



Data Sharing and Reconciliation

- Freyja reports shared with HD Team via shared Teams Chanel
- Sub-lineage data collapsed based on abundance in population
 - $\geq 1\%$ global population are represented
 - $\leq 1\%$ collapsed into respective clades

Initial Results	BA.5	BA.5.1	BA.5.1.1	BA.5.2	BA.5.2.3	BA.5.5	BA.4.6	BF.7
	0.05	0.02	0.02	0.35	0.17	0.09	0.25	0.05
Collapsed Results	BA.5						BA.4	BF.7
	0.7						0.25	0.05

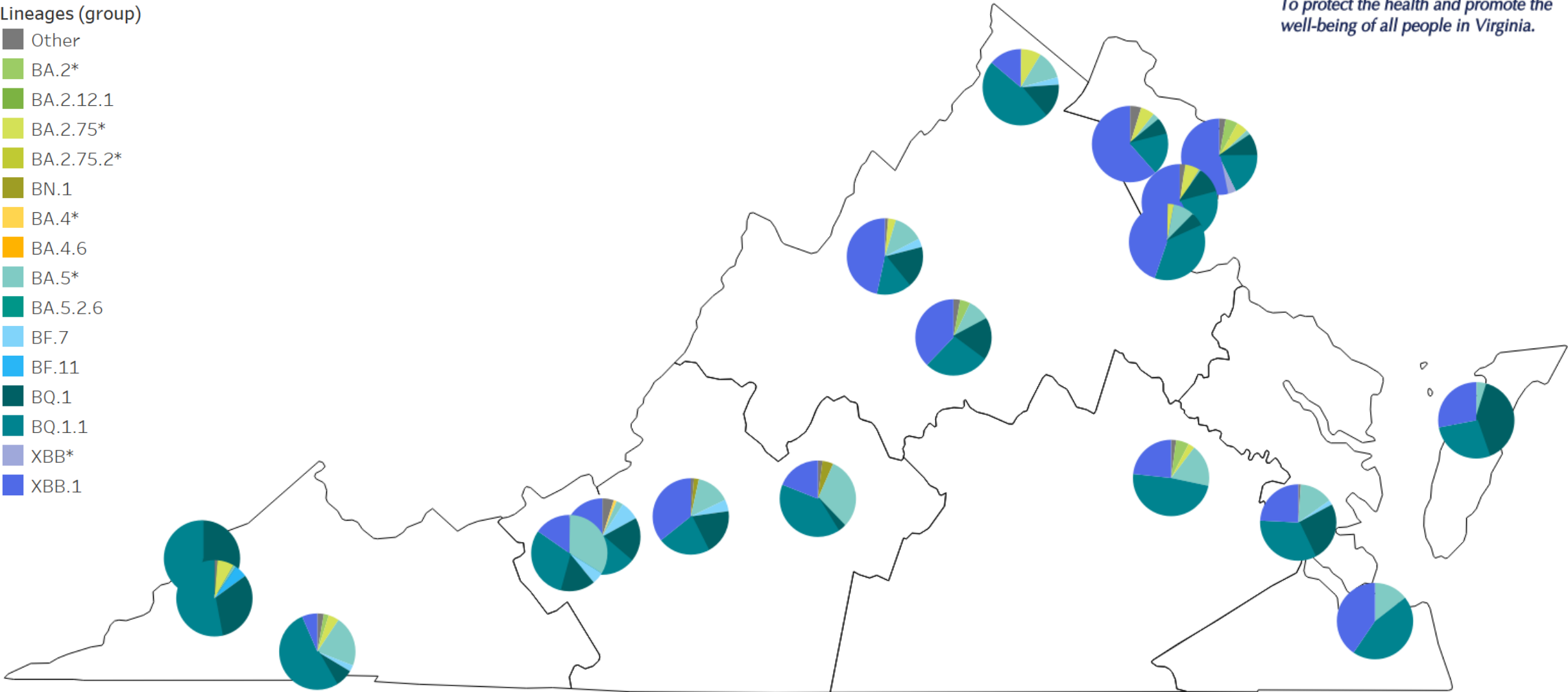


■ BA.5 ■ BA.4 ■ BF.7

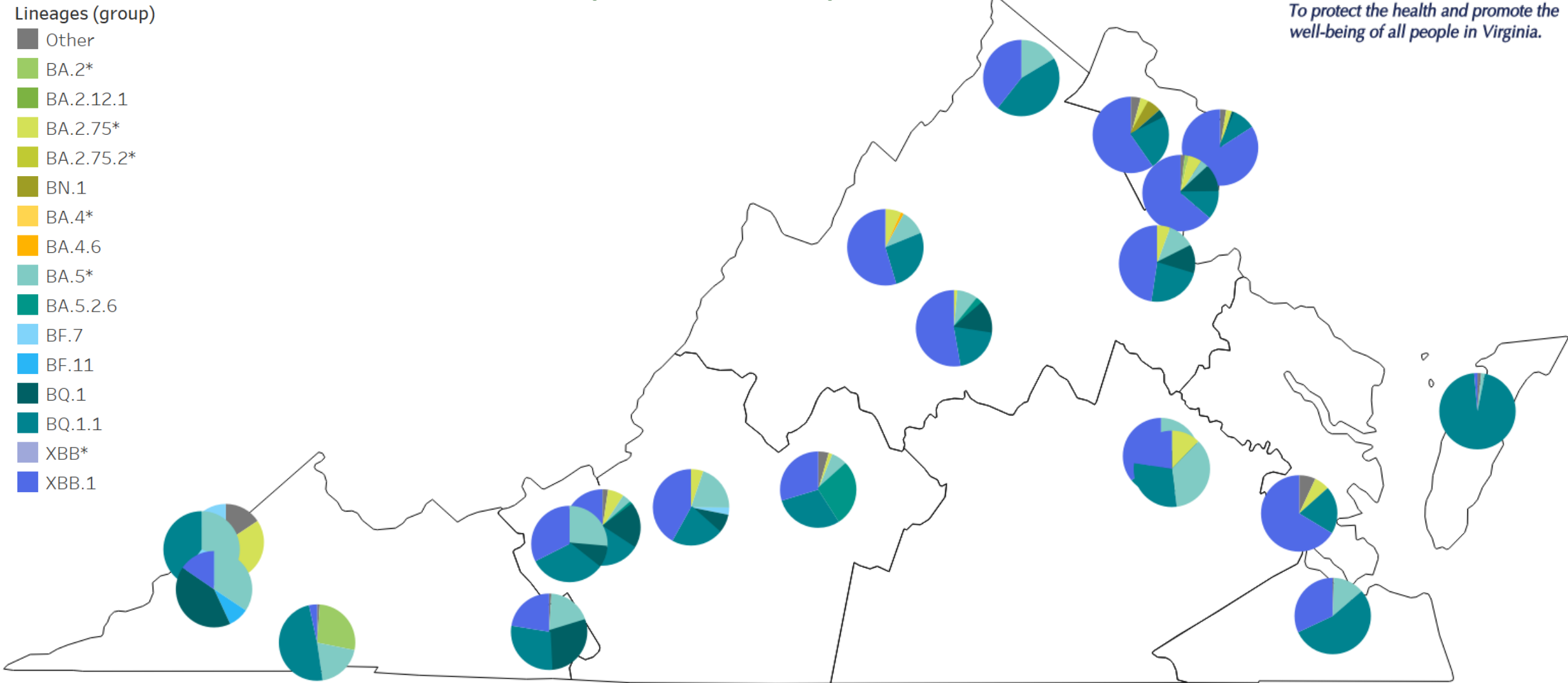
SMP/MMWR Week 69/1 (1/1-1/7)

Lineages (group)

- Other
- BA.2*
- BA.2.12.1
- BA.2.75*
- BA.2.75.2*
- BN.1
- BA.4*
- BA.4.6
- BA.5*
- BA.5.2.6
- BF.7
- BF.11
- BQ.1
- BQ.1.1
- XBB*
- XBB.1



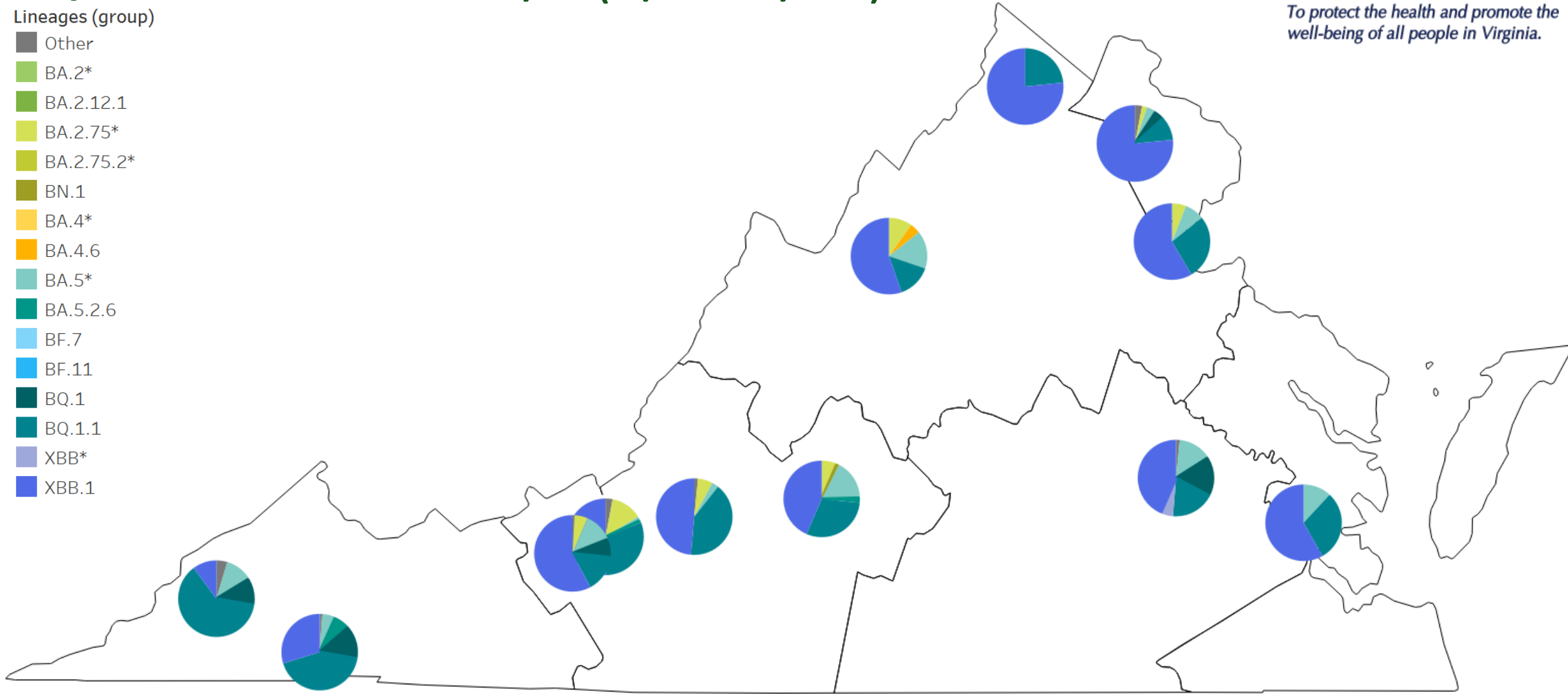
SMP/MMWR Week 70/2 (1/8-1/14)



SMP/MMWR Week 71/3 (1/15-1/21)

Lineages (group)

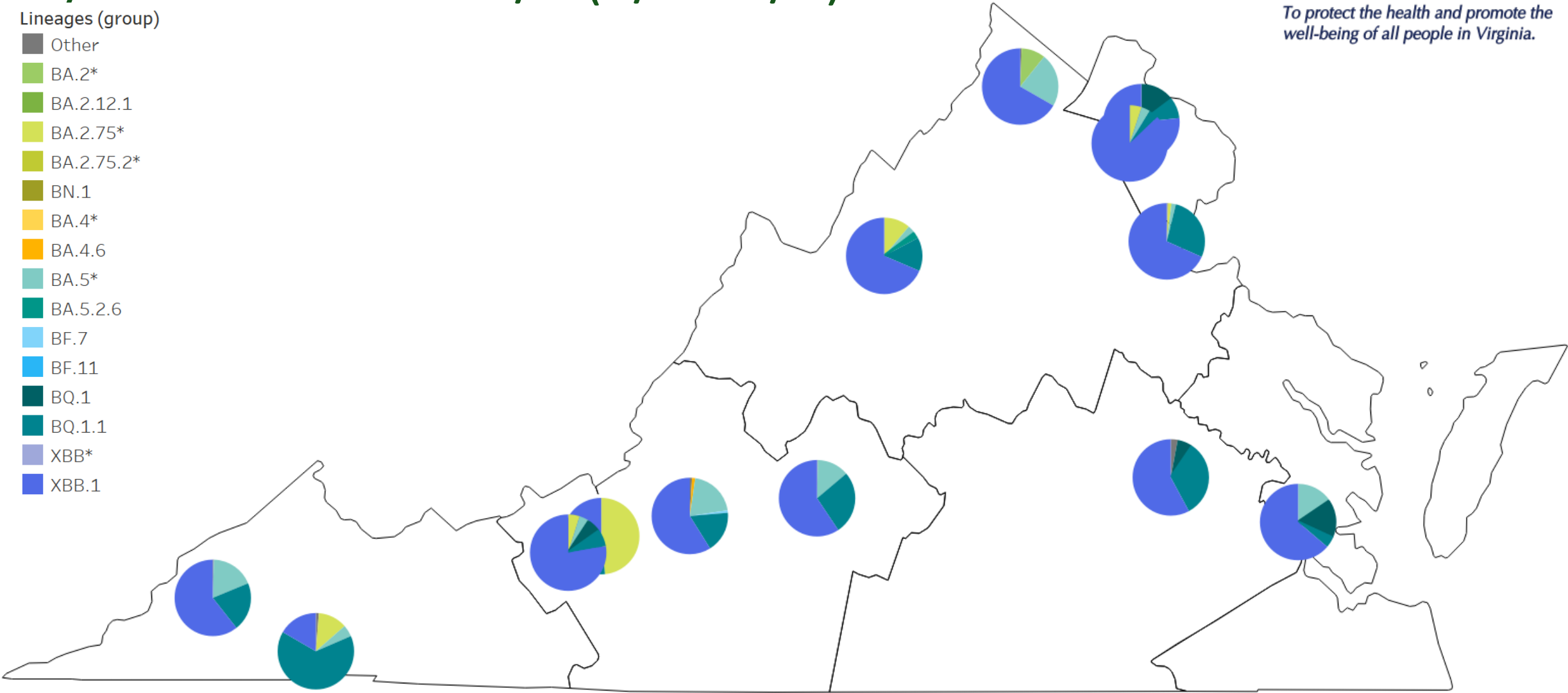
- Other
- BA.2*
- BA.2.12.1
- BA.2.75*
- BA.2.75.2*
- BN.1
- BA.4*
- BA.4.6
- BA.5*
- BA.5.2.6
- BF.7
- BF.11
- BQ.1
- BQ.1.1
- XBB*
- XBB.1



SMP/MMWR Week 73/5 (1/29-2/4)

Lineages (group)

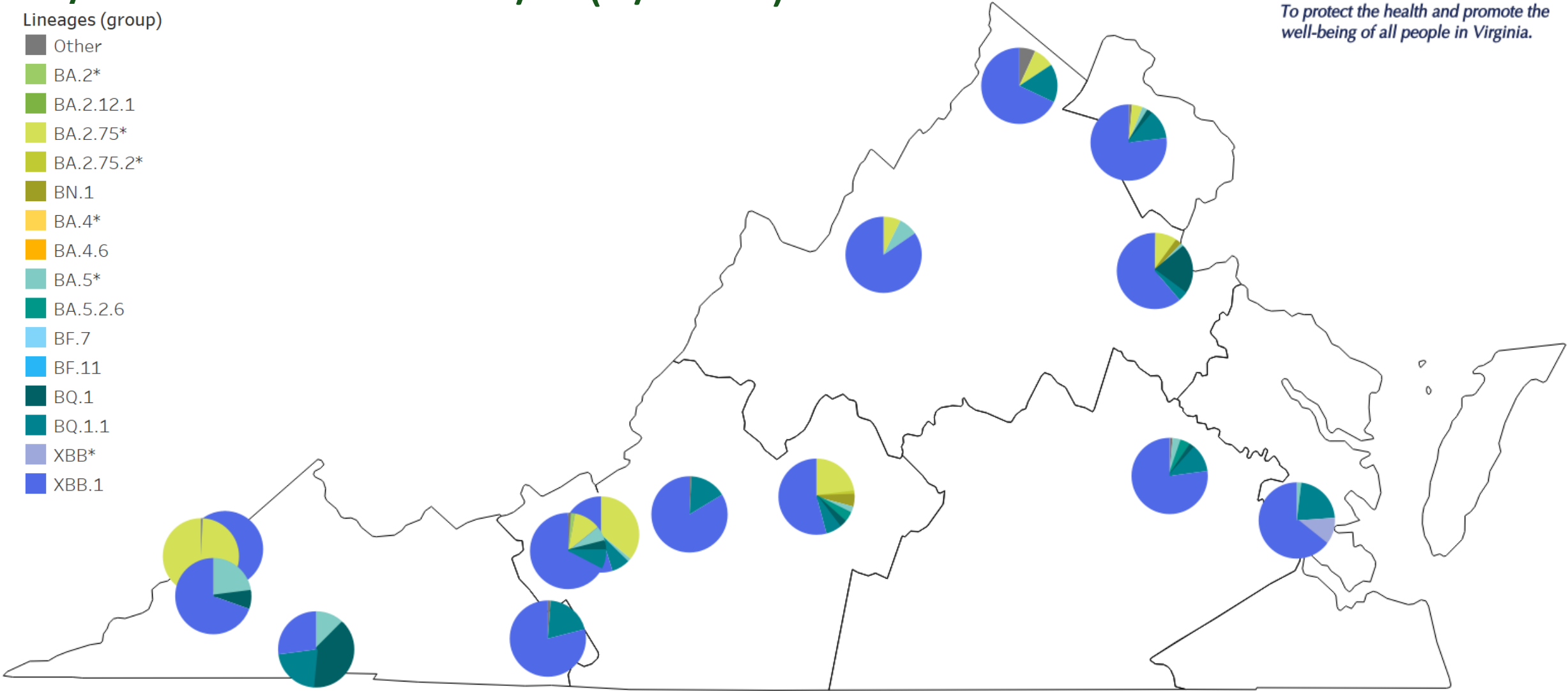
- Other
- BA.2*
- BA.2.12.1
- BA.2.75*
- BA.2.75.2*
- BN.1
- BA.4*
- BA.4.6
- BA.5*
- BA.5.2.6
- BF.7
- BF.11
- BQ.1
- BQ.1.1
- XBB*
- XBB.1



SMP/MMWR Week 74/6 (2/5-11)

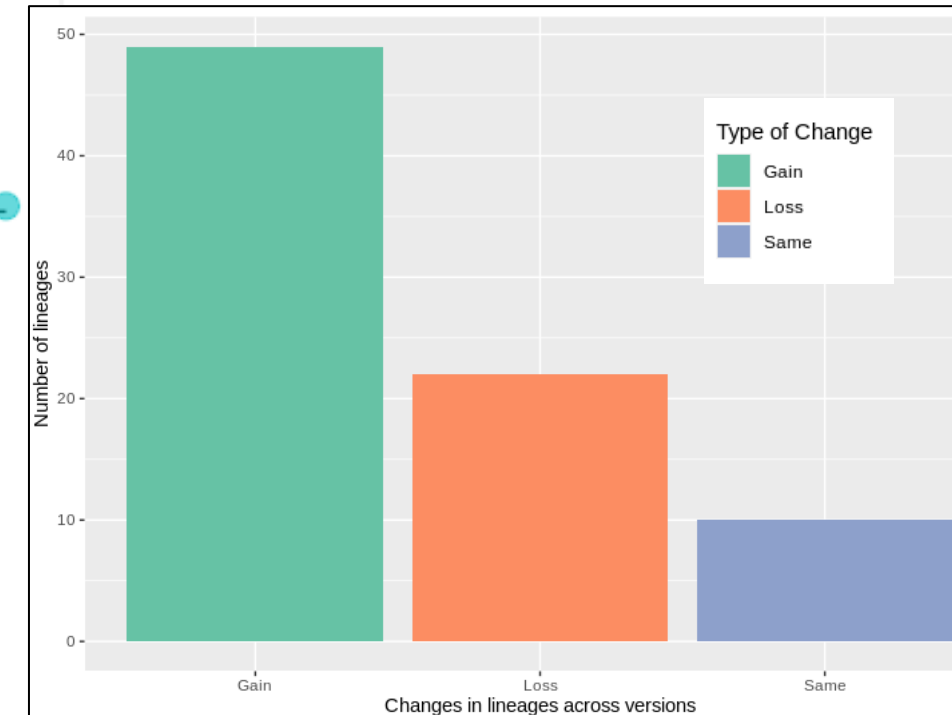
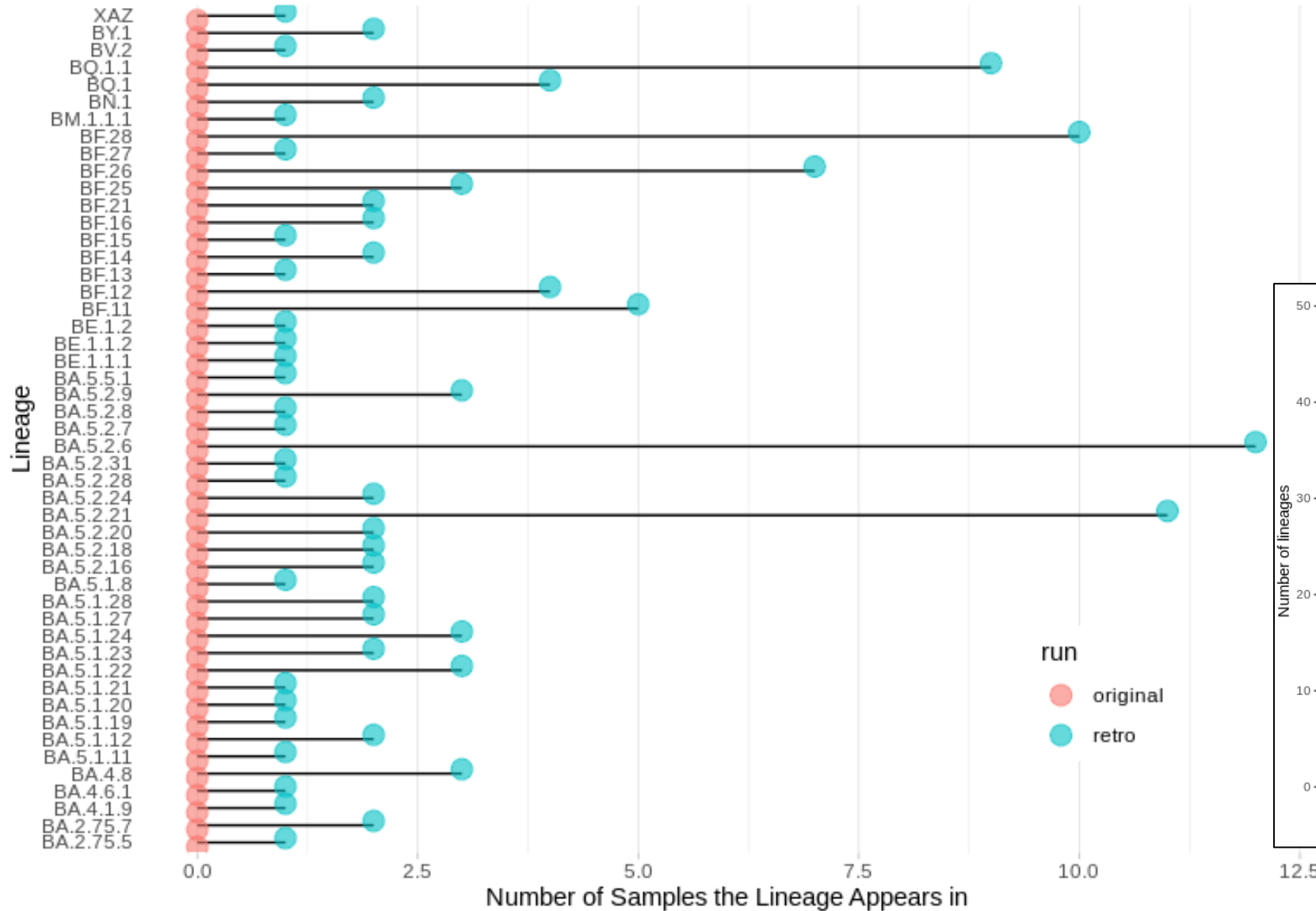
Lineages (group)

- Other
- BA.2*
- BA.2.12.1
- BA.2.75*
- BA.2.75.2*
- BN.1
- BA.4*
- BA.4.6
- BA.5*
- BA.5.2.6
- BF.7
- BF.11
- BQ.1
- BQ.1.1
- XBB*
- XBB.1



Considerations

UShER version lineage changes



Challenges

- Completely new system needed to support WW testing – partnerships, instrumentation, laboratory infrastructure, quality assurance, data analytics, reporting
- Data uncertainty and variability in measurement
- Complex mixed sample analysis
- Dependent on classified variant lineages, retrospective consideration
- Genetic drift can cause gaps in amplification profile and missed data – amplicon scheme will require continuous updates
- Interpreting and determining action from WW data

Future Directions

- Improve efficiency in laboratory through automation
- Incorporate total nucleic acid extraction that can accommodate additional pathogens
- Refine data reporting and sharing with VDH
- VDH program expansion to get equitable representation from Commonwealth of VA
- New targets analysis on all sentinel monitoring sites
- Publication of VDH internal dashboard



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