



Virginia Wastewater Surveillance Program: Community of Practice

WWS Team

VDH | Office of Environmental Health Services

July 28, 2021



Agenda

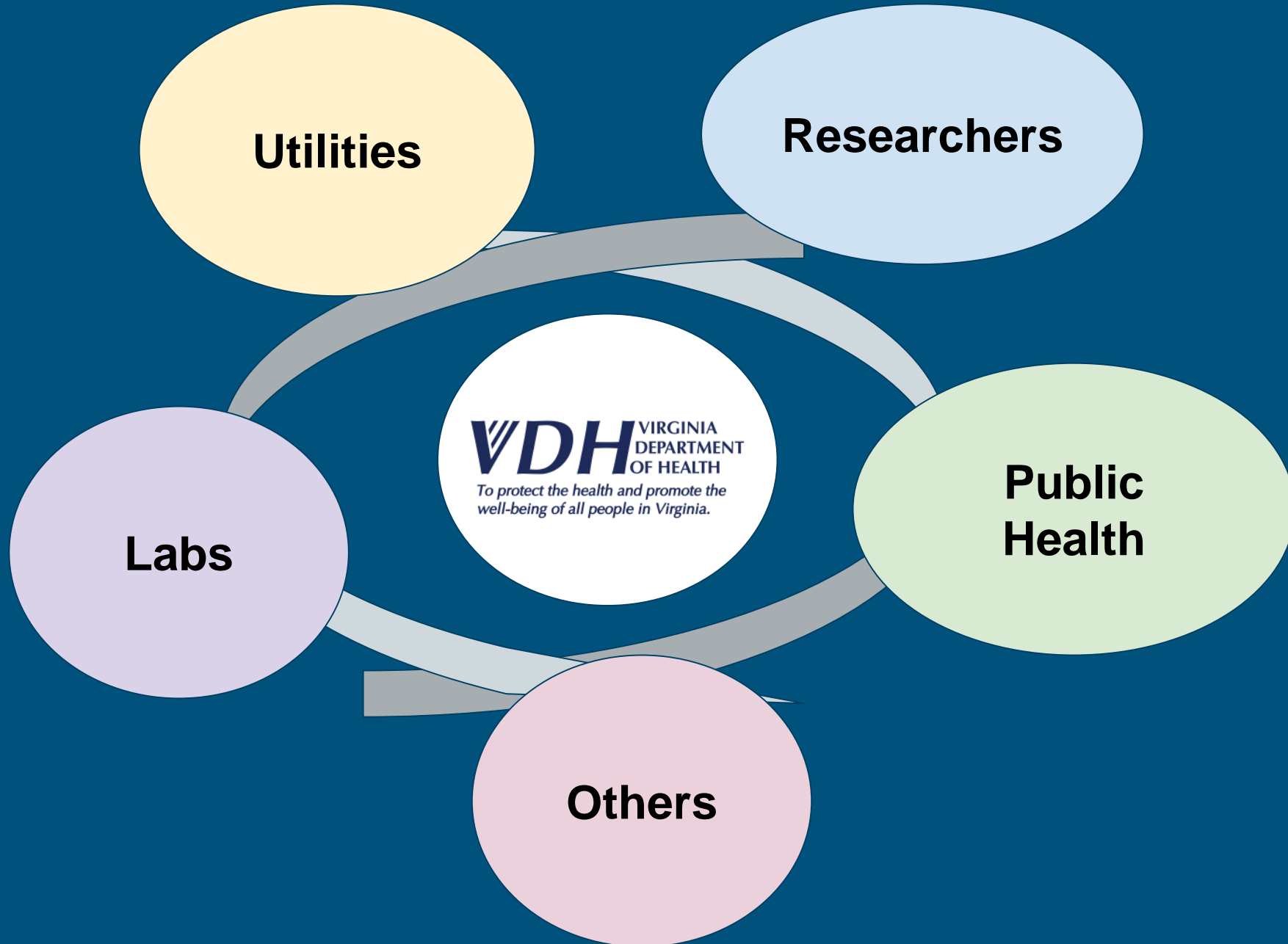
1. Updates & Funding Opportunities (5 mins)

2. Topic(s) of Interest (15 mins):

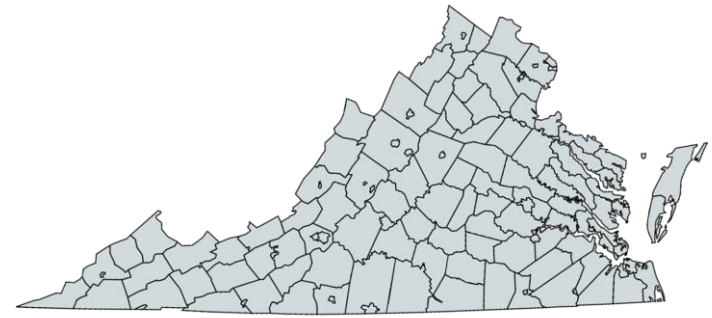
WWS Data Visualization: Behind the Scenes

1. Open Discussion (5-10 mins)





Sentinel Monitoring Updates



SARS-CoV-2 monitoring at influent to 25 wastewater treatment plants statewide

- ❖ **Received applications**
- ❖ **Working with the Office of Epidemiology to select representative sampling sites for a good geographic coverage throughout the state**
- ❖ **Tentative sampling start date: August/September**
- ❖ **Kick-Off Meeting: TBD**



July's Topic of Interest

WWS Data Visualization:
Behind the Scenes



Field Methods: Sample Collection

GRAB



COMPOSITE

Treatment Facility

Field

Field Methods: Sample Location

UPSTREAM
(i.e., within sewershed)



INFLUENT



PRIMARY SLUDGE



**UNTREATED
WASTEWATER**



Laboratory Methods: Concentration Methods

CDC Wastewater Surveillance Recommendations:



Electronegative membrane
filtration



Ultrafiltration



PEG Precipitation



Centrifugation

Laboratory Methods: Controls

CDC Wastewater Surveillance Recommendations:

- Matrix recovery control
 - Assesses amount of virus lost during processing using biologically-similar virus by spiking a known amount at the start

Recovery Efficiency Targets:

- murine coronavirus (murine hepatitis virus)
- bovine coronavirus
- bovine respiratory syncytial virus

Laboratory Methods: Controls

CDC Wastewater Surveillance Recommendations:

- Human fecal normalization
 - Accounts for changes which may impact concentration (e.g., dilution of wastewater, fluxes in population)

Fecal Indicator Targets:

- pepper mild mottle virus (viral)
- crAssphage (viral)
- *Bacteroides* HF183 (bacterial)

Laboratory Methods: Controls

CDC Wastewater Surveillance Recommendations:

- Quantitative measurement controls
 - Performed using calibration curve or sample of known concentration
- Negative controls
 - Assesses contamination during sample processing and molecular analysis

Laboratory Methods: Controls

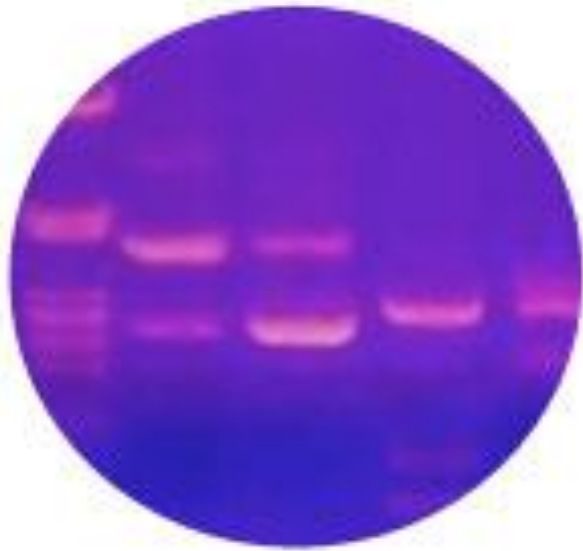
CDC Wastewater Surveillance Recommendations:

- Inhibition assessment
 - Used to optimize accurate measurement of RNA target in a complex matrix such as wastewater

Inhibition Assessment Approaches:

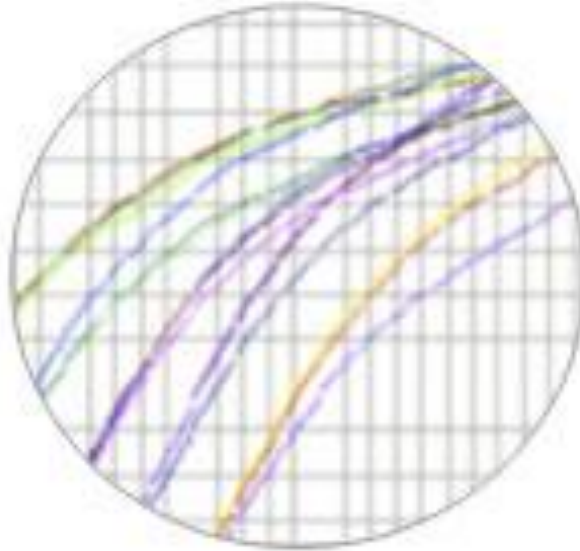
- dilution curve using sample of interest
- dilution curve of spiked virus in sample or no template control

Laboratory Methods: PCR Technologies



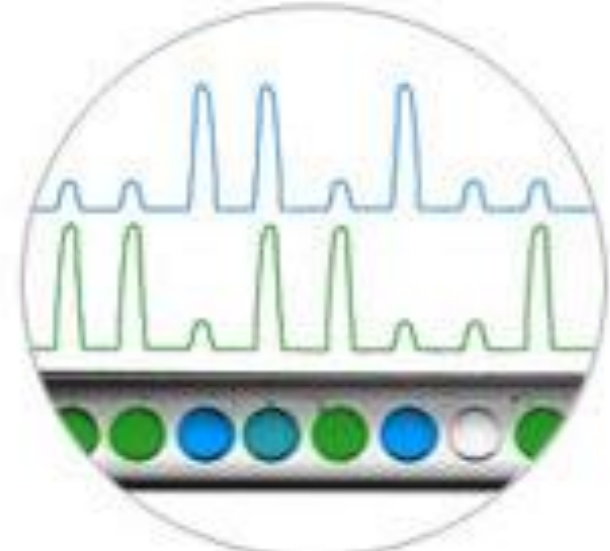
PCR
Qualitative

[PCR]



Real-Time PCR
Relative Quantitation

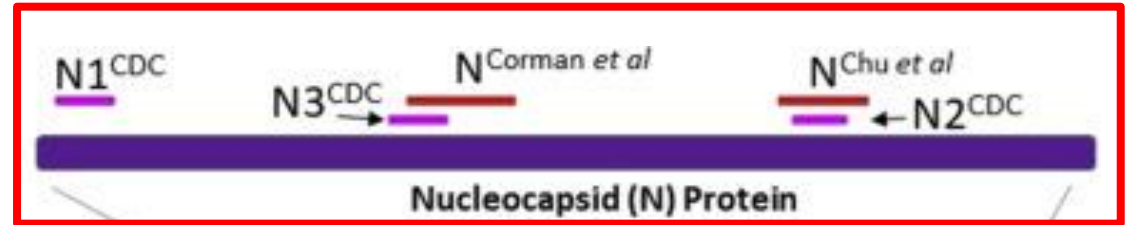
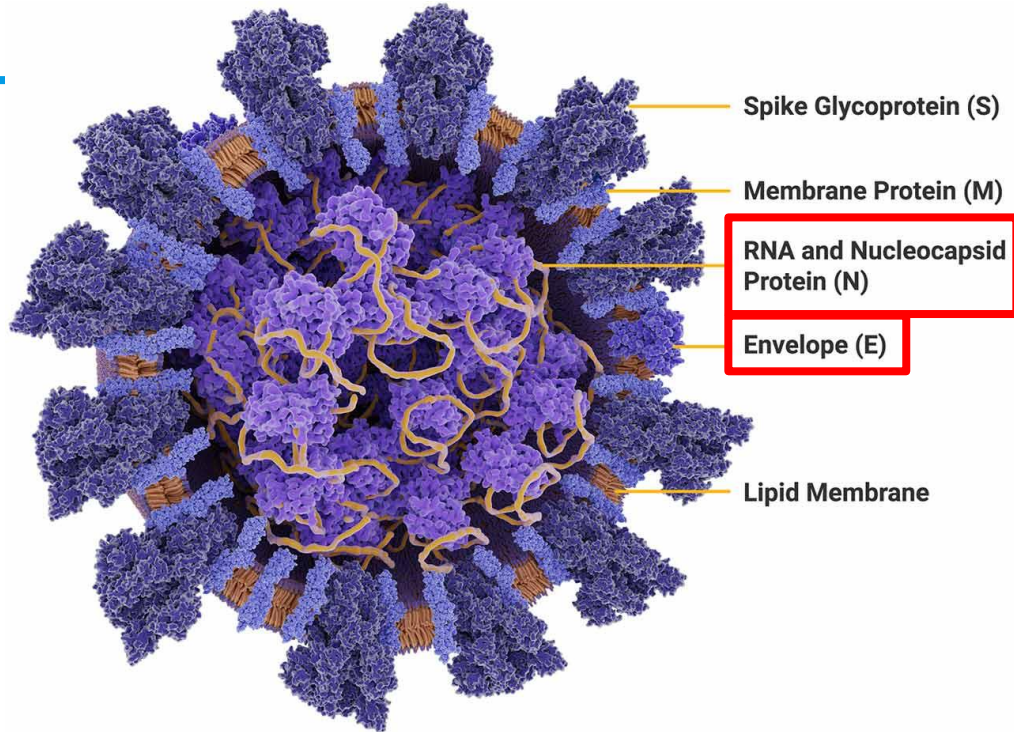
[qPCR]



Droplet Digital PCR
Absolute Quantitation

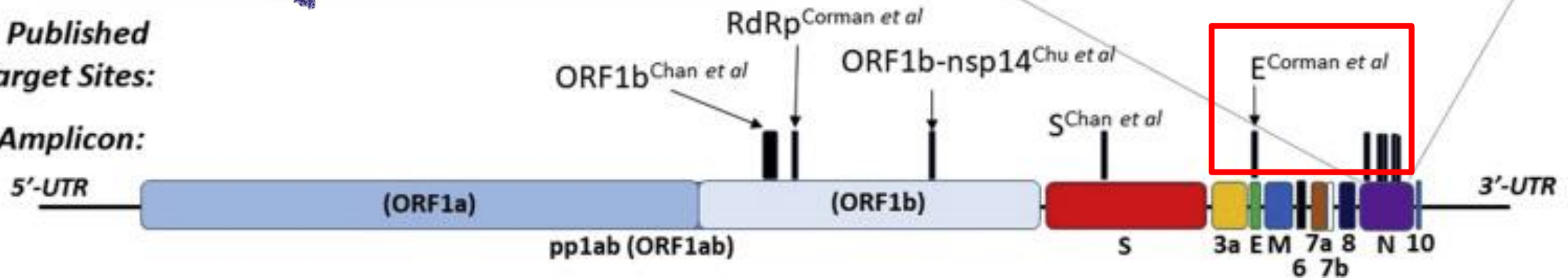
[ddPCR]

Laboratory Methods: Gene Targets

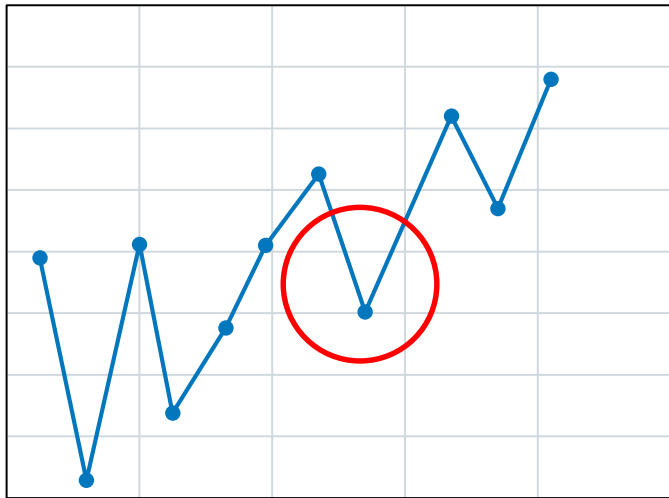


Published Target Sites:

Amplicon:



Analysis Methods: Reporting Units



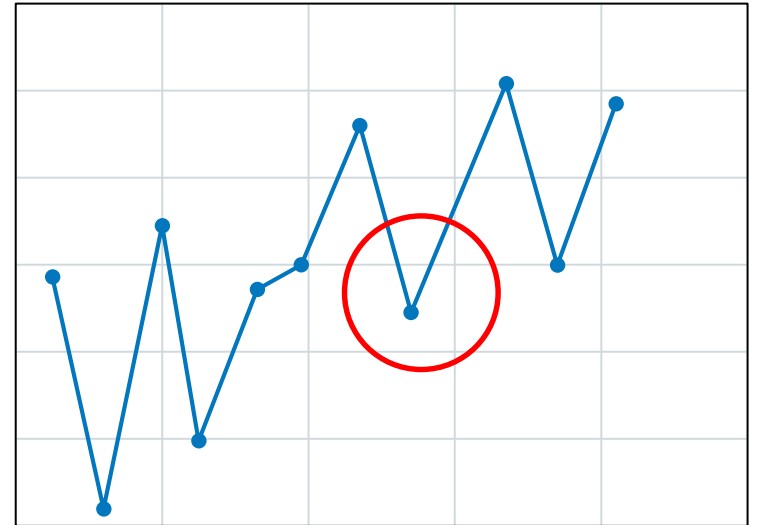
Viral Concentration

[copies/volume]

FLOW



[volume/time]



Viral Load

[copies/time]

Analysis Methods: Epi Limitations

Suppression: removing certain data when anonymity standards are at risk (e.g. extremely low rate areas)

Data Anonymization: removing identifiable information (e.g. names, addresses)

Reasons:

- *Reliability* - low rates may not be reliable/meaningful
- *Confidentiality* - health information is sensitive/protected
- *Unintended consequences*



Analysis Methods: Epi limitations

Current CDC-DCIPHER Rules For Public Sharing

Suppression:

- data from sewersheds < 3,000 persons (or missing population numbers)
- data from specific institutions
- daily COVID-19 case counts between 1-4
- recent COVID-19 case counts (last 14 days)
- free-text fields (either removed or anonymized) -- not including county names

Data Anonymization:

- WWTPs
- Sampling location names
- Laboratory IDs



How to Interpret?

Important Caveats...

SARS-CoV-2 Concentration

- comparisons across samples are variable
 - location factors
 - laboratory factors
- normalization options by WW flow/fecal controls
 - may improve comparability over time
 - **different labs still vary substantially!**

Provisional Data

- jurisdictional updates/control -- data can be modified at any time!

Clinical Correlates

- Support only! -- viral loads are not clinical cases; mutations are not variants



Key Takeaways

- Be mindful of “behind-the-scenes” for varying analyses
 - Sampling Type
 - Sampling Matrix
 - Concentration Method
 - PCR Platform
 - Gene Target
 - Reporting Units
- Important to assess relative trends as opposed to absolute values
- Data has limits which should be carefully considered prior to analysis and visualization to protect the target community

August's Topic of Interest: Data Visuals and Dashboards

Open Q&A/Discussion



Questions?
Comments?

See you next month!

Send inquiries / topics to:
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