Virginia Wastewater Surveillance Program: Community of Practice Meeting

WWS Team
VDH | Office of Environmental Health Services
April 26, 2023

VDH VIRGINIA DEPARTMENT OF HEALTH

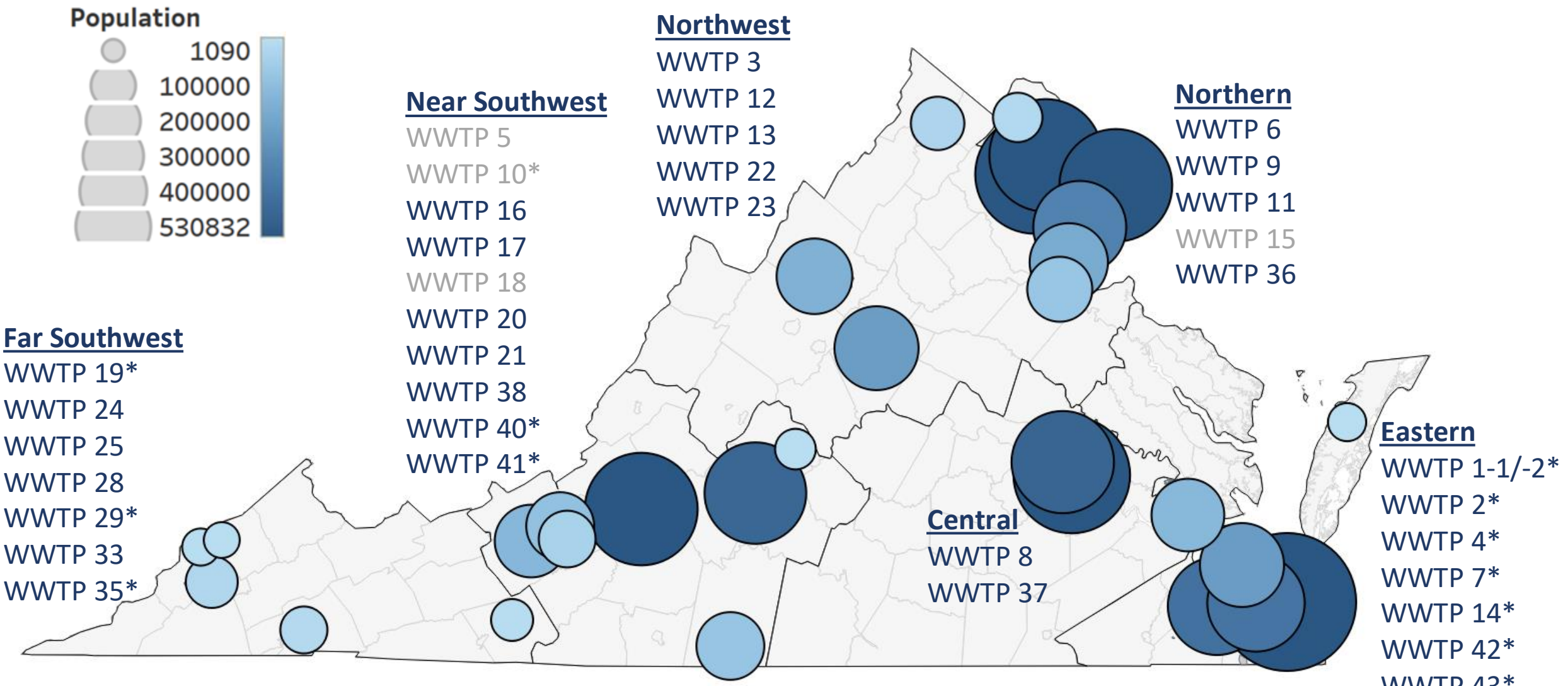
Agenda

- ❑ **Updates & Funding Opportunities**
- ❑ **Topic(s) of Interest:**

  An Evaluation of a New Rapid qPCR Test for the Detection of 2019 Novel Coronavirus Nucleocapsid (N1) Gene in Wastewater in Roanoke and Salem VA Sewersheds

- ❑ **Open Discussion**

Sentinel Monitoring Facilities



Northwest
WWTP 3
WWTP 12
WWTP 13
WWTP 22
WWTP 23

Near Southwest
WWTP 5
WWTP 10*
WWTP 16
WWTP 17
WWTP 18
WWTP 20
WWTP 21
WWTP 38
WWTP 40*
WWTP 41*

Northern
WWTP 6
WWTP 9
WWTP 11
WWTP 15
WWTP 36

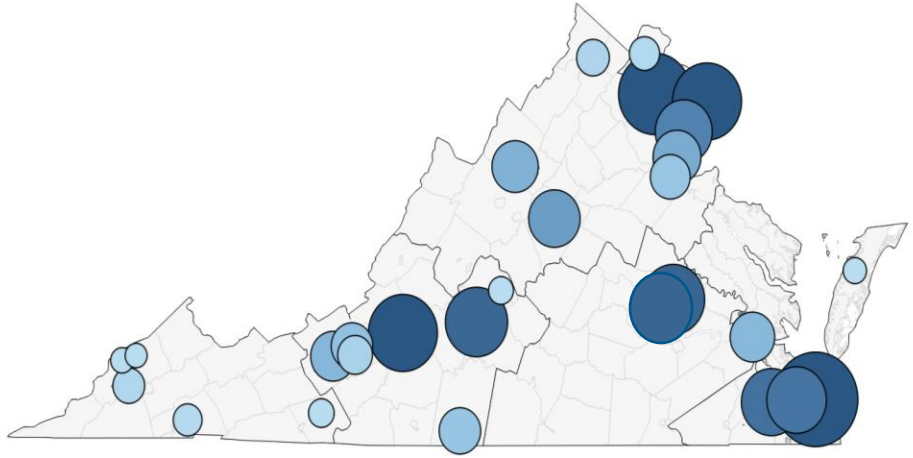
Far Southwest
WWTP 19*
WWTP 24
WWTP 25
WWTP 28
WWTP 29*
WWTP 33
WWTP 35*

Central
WWTP 8
WWTP 37

Eastern
WWTP 1-1/-2*
WWTP 2*
WWTP 4*
WWTP 7*
WWTP 14*
WWTP 42*
WWTP 43*
WWTP 44*
WWPT 45*

*sample type: grab

Program Updates



- **SARS-COV-2 MONITORING**
  - Total **36** wastewater treatment plants
    - 13 sampling *twice* weekly
    - 23 sampling *once* weekly
  - *Still **enrolling** (up to 50 sites)*
  - **Internal dashboard updated weekly**
  - **Tentative go-live date for public facing dashboard: *5/9!***
- **VARIANT SEQUENCING**
  - 20-23 sites weekly (rotating some sites)
  - **Working on internal sequencing dashboard!**

Sampling and Funding Opportunities

- Initiated statewide analysis of Monkeypox, Influenza A&B
- **Localized Projects and Pilot Projects** *($200,000)*

  Roanoke Project: Sampling

  Chesapeake Project: Final developmental phase will start sampling in May

  Alexandria Renew Project: Initial developmental phase

  SW VA -Virginia Tech Project: Application submitted
- **CDC-Biobot Commercial Sampling**
  - 15 sites were enrolled from Virginia; 7 sites transitioned to VDH program!
  - Generating data for Monkeypox
  - Sampling: twice/week

An Evaluation of a New Rapid qPCR Test for the Detection of 2019-Novel Coronavirus Nucleocapsid (N1) Gene in Wastewater in Roanoke and Salem VA Sewersheds

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Radford University Carilion, VA

Susan Tolliver

Basic Science Research Lab Manager,

Carilion Clinic, VA

An Evaluation of a New Rapid qPCR Test for the Detection of 2019-Novel Coronavirus Nucleocapsid (N1) Gene in Wastewater in Roanoke and Salem VA Sewersheds

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Acknowledgement

Anna Lewis and Willow Lehrer, Biomedical Sciences Program students at Radford University Carilion, presented this research at Carilion Clinic Research Day.

This presentation uses their slides as foundational material.

We are grateful.

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Today

- Beginnings
- Overview
- Background
- Comparisons
- Our Conclusions
- Limitations

Brought to you by:

Sara Houser
Assistant Professor
Radford University Carilion

Susan Tolliver
Basic Science Research Lab Manager
Carilion Clinic

How it began

Hach and LuminUltra partner to deliver first rapid test for SARS-CoV-2 in wastewater

FEBRUARY 25, 2021 FEATURED NEWS CORPORATE UPDATES COVID-19 | BY LUMINULTRA TEAM

NEWS » CORPORATE UPDATES

Loveland, Colo. – Hach® (www.hach.com), a global leader in water analysis and LuminUltra (luminultra.com), a global leader in biological diagnostic testing, have partnered to offer the world's first rapid, on-site testing solution for the detection of SARS-CoV-2 in wastewater. SARS-CoV-2 is the virus that causes COVID-19. The partnership is a direct response to consumer and community need for same-day test results and accessible, cost-efficient solutions.

"Hach's mission is to ensure water quality for people around the world. When the pandemic hit, Hach immediately looked to see if there was a way that we could help fight the spread. Wastewater-based epidemiology (WBE) was the logical area for us to contribute," says James Harbridge, Business Development Manager for Hach. "This partnership with LuminUltra enabled us to quickly bring a product to market with the potential to help slow virus spread. We are excited to see how communities, businesses and schools use this solution to make a positive impact on public health."

https://www.luminultra.com/blog/hach-and-luminultra-partner-to-deliver-first-rapid-test-for-sars-cov-2-in-wastewater/

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Hypothesis

The new method using the Hach LuminUltra may be useful for public health applications for detecting the viral loads of SARS-CoV-2 in wastewater.

Overview

- Compared “Established” SARS-CoV-2 wastewater detection methods to the “New” method
- Over a 12-week period ranging from December 2021- April 2022
- Collected weekly from 7 locations in the Roanoke/Salem, VA sewershed (n=84)
- The target gene was the nucleocapsid (N1) gene of the SARS-CoV-2 virus
- Comparisons
  - Cost
  - East of use
  - Time to results
  - Sample preparation parameters
  - Analysis parameters
  - Results

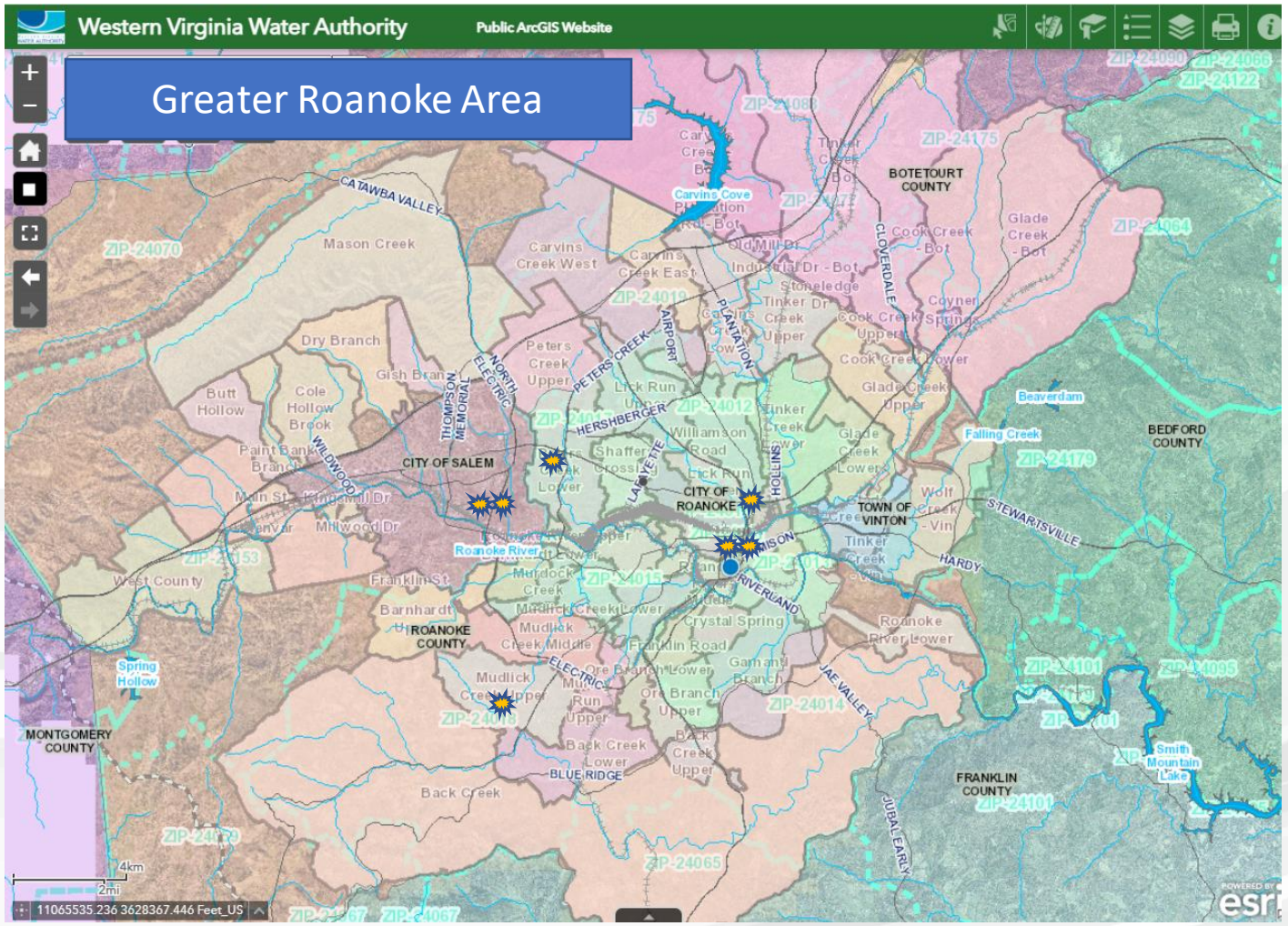
Background: Collaboration

- Virginia Department of Health (VDH)
  - Funding
  - Oversight
  - Study advice
- Western Virginia Water Authority (WVWA)
  - Site selection, training
  - Collection
- Radford University Carilion Biomedical Sciences (RUC)
  - Collection Hub
  - Sample preparation and analysis, novel: concentration, extraction, analysis Hach/LuminUltra
  - Sample preparation, traditional: concentration, extraction for Bio-Rad qPCR
- Carilion Clinic Basic Science Research Lab (BSRL)
  - qPCR analysis using Bio-Rad

Background: Collection from Seven Sites

- Radford University Carilion
  - Grab samples from manholes for selected zip codes
- Western Virginia Water Authority
  - Composite samples
    - Influent at treatment plant
    - Tinker Creek interceptor

Background: Seven Sites



Background: Collection at Manholes

Pop lid

Drop pail

Pour into bottles

Take to RUC lab

Background: RUC Lab

New

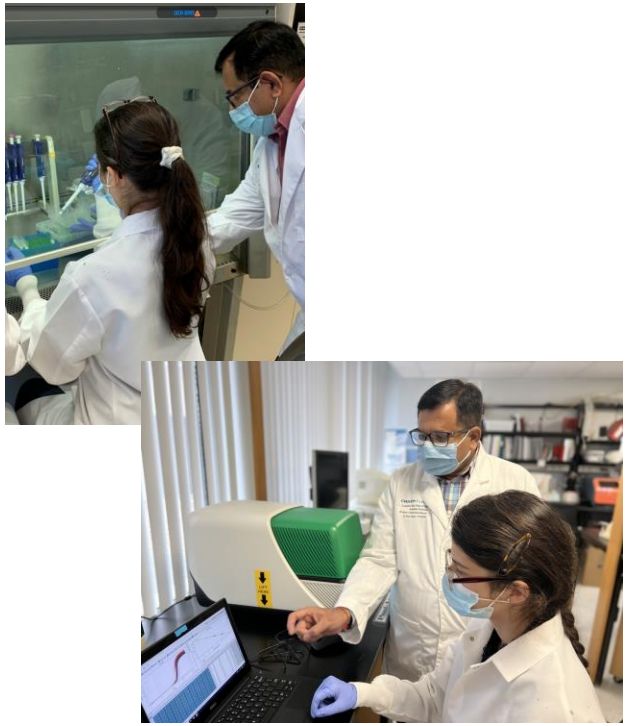
- Hach LuminUltra prepared kit start to finish
  - Concentration
  - RNA Extraction
  - qPCR Analysis
    - GeneCount Q-16
  - Interpret results

Established

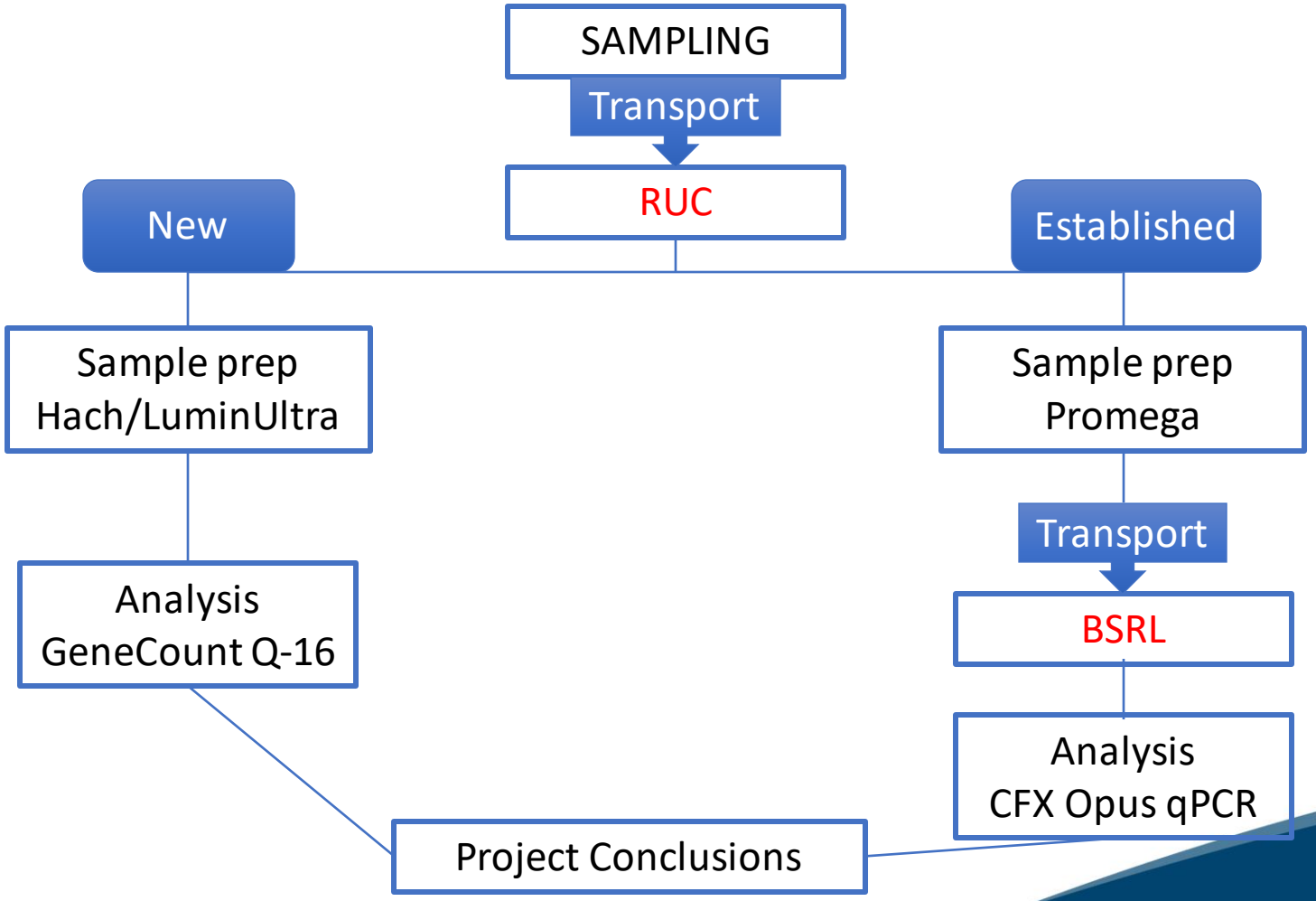
- Promega kit
  - Concentration
  - RNA Extraction
- Take to Basic Science Research Lab for analysis
  - Bio-Rad CFX Opus qPCR

Background: Basic Science Research Lab

- Analyze RNA extracted by RUC
  - Bio-Rad CFX Opus qPCR
- Interpret results



Background: The process



Comparison Considerations

- COST
- EASE OF USE
- TIME TO RESULTS
- SAMPLE PREPARATION PARAMETERS
- ANALYSIS PARAMETERS
- RESULTS

Comparison: Cost

	New	Established
Instrument	LuminUltra GeneCount Q-16	Bio-Rad CFX Opus
Instrument Cost	$15,359.04	$25,994.22
Shipping Cost Instrument	$495.50	$126.30
Start up equipment/supplies	$621.83 Pipets, vials, test tube rack, magnetic rack… Soon ordered additional mag racks to streamline processing	$408.00 PCR plates, film
Total instrument and start up	$16,476.37	$26,528.52
Reagents (does not include "incidental" supplies)	Assay Kit 96 samples $3839.04 Per sample $39.99 Includes sample prep and analysis	Promega Wastewater Kit List: $360/25 tests, $14.40/test qPCR reagents $40/test Includes protocol development and validation

Comparison: Ease of Use

	New	Established
Instrument	LuminUltra GeneCount Q-16	Bio-Rad CFX Opus
Training/Technical Support	Videos Difficult to get technical information, “proprietary”	Videos; on-site, email, and virtual meeting support
Platform Transparency	Not transparent Would not share particulars about test or instrument—proprietary Closed platform—cannot (easily) use outside their kits	Very transparent Know all components Open platform
Ease of use	Designed to be plug and play for non-scientist	Requires technical knowledge, technique expertise
Pre-analysis preparation	None	Many hours designing and modifying protocols

Comparison: Time to Results

	New	Established
Instrument	LuminUltra GeneCount Q-16	Bio-Rad CFX Opus
Sample processing time	2-3 hr	4-6 hr
Analysis time	2 hr	2 hr
Total	4-5 hr	6-8 hr

Comparison: Sample Preparation Parameters

SAMPLE PREPARATION PARAMETERS	Hach/LuminUltra	Promega
Starting wastewater volume	1 ml	40 ml
Extraction volume	50 ul	40 ul
Method	Magnetic beads/membrane	Precipitation/membrane

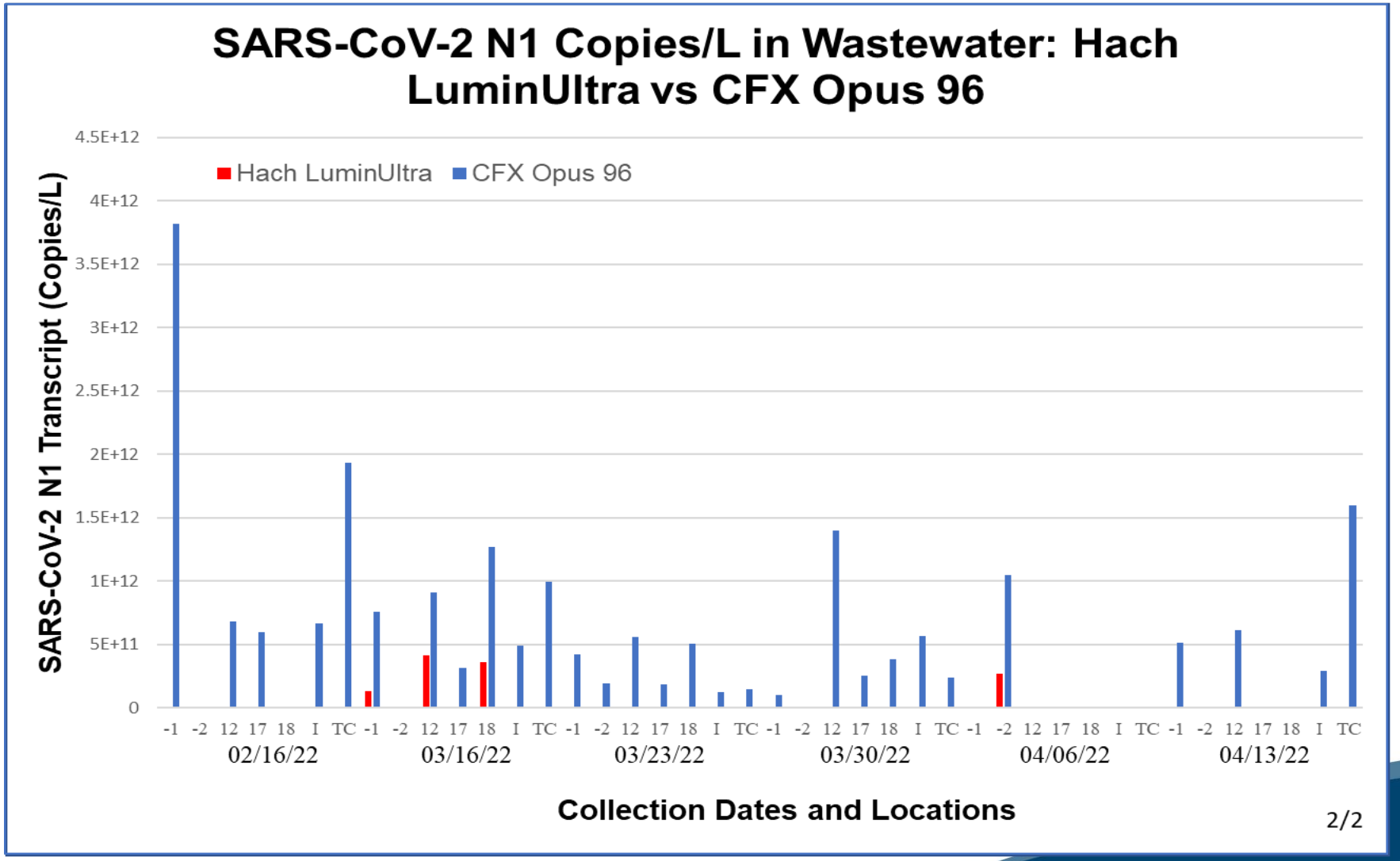
Comparison: Analysis Parameters

	New	Established
Instrument	LuminUltra GeneCount Q-16	Bio-Rad CFX Opus
Pos control	Optional	Yes
Neg control	Yes	Yes
NTC	No	Yes
Standard Curve	No (assume part of software; not analyzed separately)	Yes
Gene target	N1	N1
Number of analyses per sample	Single	Duplicate
Minimum copies detected	4 per instrument/method specs	4 as calculated

Comparison Considerations

- COST
- EASE OF USE
- TIME TO RESULTS
- SAMPLE PREPARATION PARAMETERS
- ANALYSIS PARAMETERS
- RESULTS

Results



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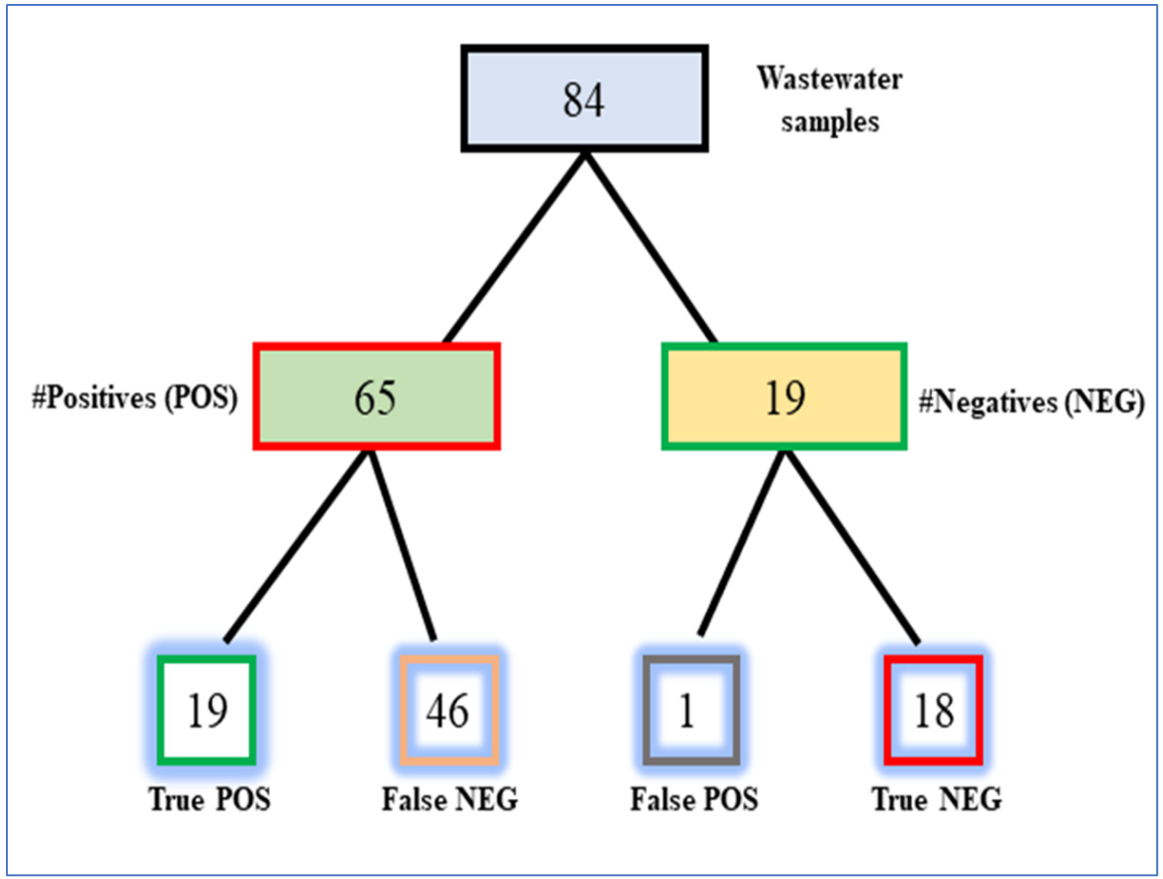
Results

Comparing Hach LuminUltra against Bio-Rad CFX Opus (as “gold standard”)



Hach LuminUltra Statistics Compared to Gold Standard CFX Opus 96	
Specificity	94.74%
Sensitivity	29.23%
Accuracy	44.05%
Positive predictive value	95.00%
Negative predictive value	28.13%
Positive likelihood ratio	5.56
Negative likelihood ratio	0.75

Comparison Summary

	Hach/LuminUltra	Promega/Bio-Rad
Cost	Less	
Ease of Use	Easier to operate Minimal technical expertise required	Better training and support More transparent Need more technical expertise
Time to Results	Shorter	
Sample Preparation	1 ml sample Quicker, easier, less sample	40 ml sample
Analysis	Single Positive control not required No NTC Standard curve?	Duplicate Standard qPCR parameters
Results	High specificity Low sensitivity	Demonstrated more positive results

Discussion/Conclusion

- "New" method had…
  - High specificity
  - Was more affordable
  - Consumed less time
  - Required less technical expertise
  - High confidence in a true positive

therefore…

The Hach LuminUltra methods can be useful for public health applications.

Which is better?

Depends…

- Need for data
- Site doing testing
- Available personnel
- Available time
- Available money

Limitations of This Study

- Processing differences
  - H/L samples usually processed and analyzed day of collection
  - Promega extraction performed day after collection or on frozen samples (1-4 weeks typically)
- CFX analysis performed on frozen RNA
- Further analyses to improve this study became cost and time prohibitive

If we had resources

- Run same day start-to-finish using fresh samples on both methods in parallel.
- Measure extracted nucleic acid.
- Cross test:
  - Perform CFX qPCR on H/L RNA.
  - Perform H/L qPCR on Promega RNA.

The Team

- Jayasimha Rao, Carilion Clinic
- Sara Houser, RUC
- Susan Tolliver, RUC then Carilion Clinic
- Anna Lewis, RUC Student
- Willow Lehrer, RUC Student
- Lacy Burnette, WVWA
- Marcia Degen, VDH
- Rekha Singh, VDH
- Haniyyah Majeed, VDH
- Michelle Yancey, VDH

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- Radford University Carilion (RUC)
- Carilion Roanoke Community Hospital (CRCH) – Carilion Basic Science Research Lab (CBSRL)

References

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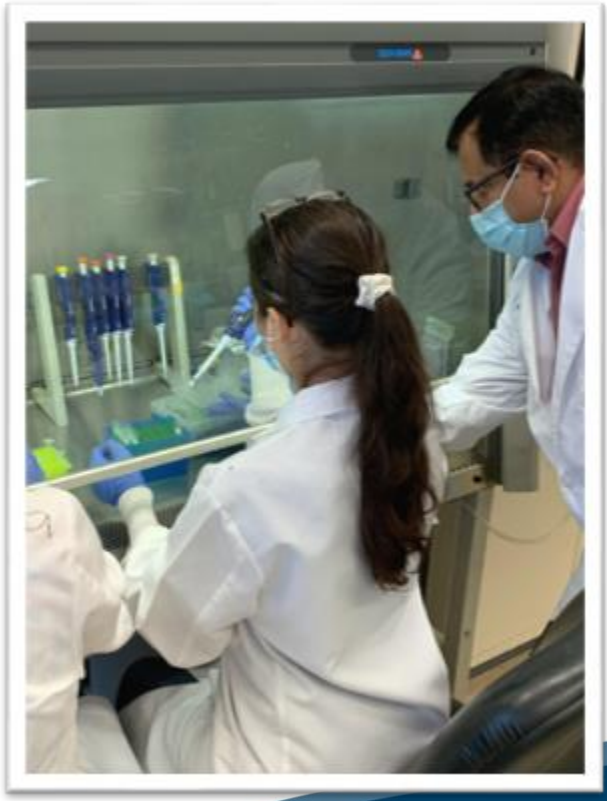
Questions?



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THANK YOU!

See you again!

Send inquiries / topics:

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