



Wastewater Surveillance: Monitoring Community Transmission of Diseases Using Automated PCR



James Harbridge, Aaron Anson and Scott Tucker
Hach, Loveland, CO

Abstract

Wastewater surveillance data provides valuable insights for public health actions and decision-making. Key areas where wastewater data has proven its worth include:

- Confirming trends by comparing it with other health data.
- Informing public health policies like masking and social distancing.
- Allocating resources effectively in public health.
- Guiding allocation of clinical resources.

However, the widespread adoption of wastewater surveillance has been hindered by challenges associated with SARS-CoV-2 extraction from wastewater samples. Complex, costly, and variable extraction processes have limited in-house testing at wastewater treatment plants, resulted in high outsourcing costs, delayed data, and reduced confidence in result usability.

To address these challenges, Cepheid's GeneXpert technology offers an efficient solution for SARS-CoV-2 analysis in wastewater. This technology automates the entire process, including capturing and lysing viral RNA, and employs real-time reverse transcriptase PCR for measurement. The GeneXpert cartridge contains all necessary components, including internal controls, ensuring reliable and quality results.

With a successful track record of over 15 years in clinical applications, the GeneXpert technology has proven effective in wastewater analysis. The PCR instrumentation enables simultaneous testing of multiple samples, with each analysis taking less than 40 minutes and requiring minimal hands-on time. Additionally, the system can measure other pathogens and indicators, such as Flu, RSV, antibiotic-resistant bacteria, and norovirus, through a streamlined preparation process.

This paper will explore the working principles of GeneXpert technology, compare its performance with traditional viral extraction methods in wastewater, and present a two-year case study on monitoring SARS-CoV-2 in a Colorado community. By showcasing its effectiveness, this research aims to promote widespread adoption of GeneXpert technology and its contribution to enhancing wastewater surveillance for public health purposes.

PCR Automation Eliminates Complexity and Minimizes Costs

Traditional Viral Extraction Options

Sample Prep System/Cartridge

300 µL

PCR System (~1' x 1' x 1')

Time Consuming & Complex
Multiple pipetting steps, sample transfers, filtration, etc. (1-4 hours per test, in many cases requires very skilled lab technicians)

Simple & More Affordable
Cepheid Cartridge/System automates all pipetting, filtering and sample preparation (1 minute of hands-on time per test, <45 minutes to result)

Challenges with Wastewater Surveillance

Public Health and WBE Challenges

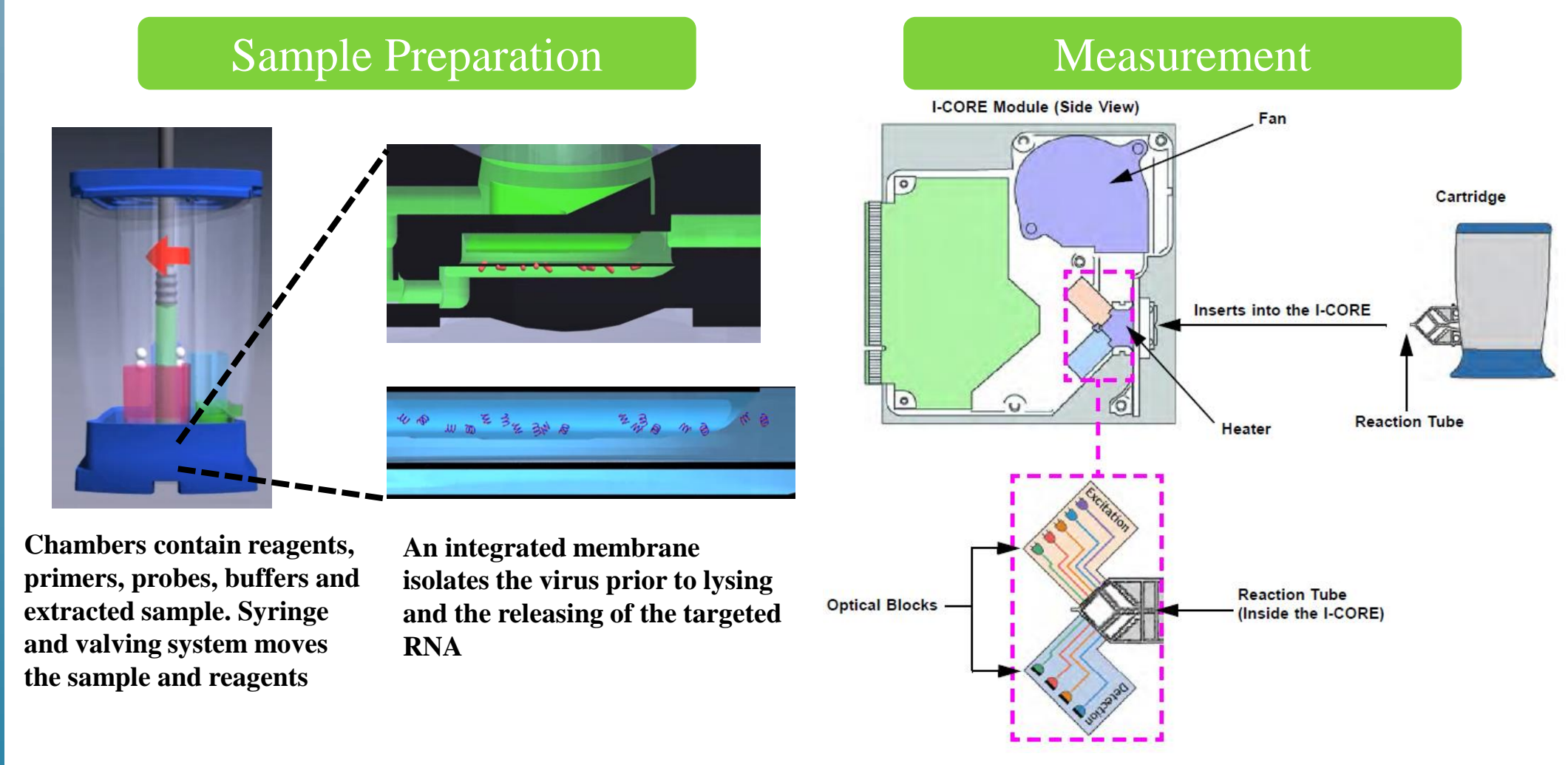
- **Test complexity and time:** extraction of virus from raw sewage is complicated and time consuming
- **Very Expensive to start a program:** Centrifuges, concentrators, PCR instruments/reagents, etc.
- **Multiple Methods/Consistent Results:** Different methods can lead to different results; inter-operator errors create additional variability/uncertainty (e.g. sample prep)
- Technically and practically **not possible to reach** some remote or smaller scale WWTPS

Impact

- **Almost no in-house testing:** PCR test are usually only performed by highly trained specialists or outsourced
- **Outsource costs are high:** test prices can exceed \$500 per test making surveillance programs hard to justify
- **Actionability:** delayed data from outsourcing testing leads to less impactful decision making
- **Reliability/Performance:** test complexity and method differences generally lead to variable results, leading to less confidence in WBE dependent public health decisions

Methodology/Technology

- 1) Collect a representative sample (Composite sampling or Passive Sampling)
- 2) Mix sample and pipette 300 µL into the GeneXpert cartridge
- 3) Place the cartridge in an open bay and start the analysis (~37 minutes)



Analytical Performance

Linearity & Correlation Coefficients

E Gene: 0.9908, N2 Gene: 0.9886, RdRp: 0.9431
• Slopes and correlation coefficients are similar in calibration curves created in both DI water and wastewater effluent

Precision

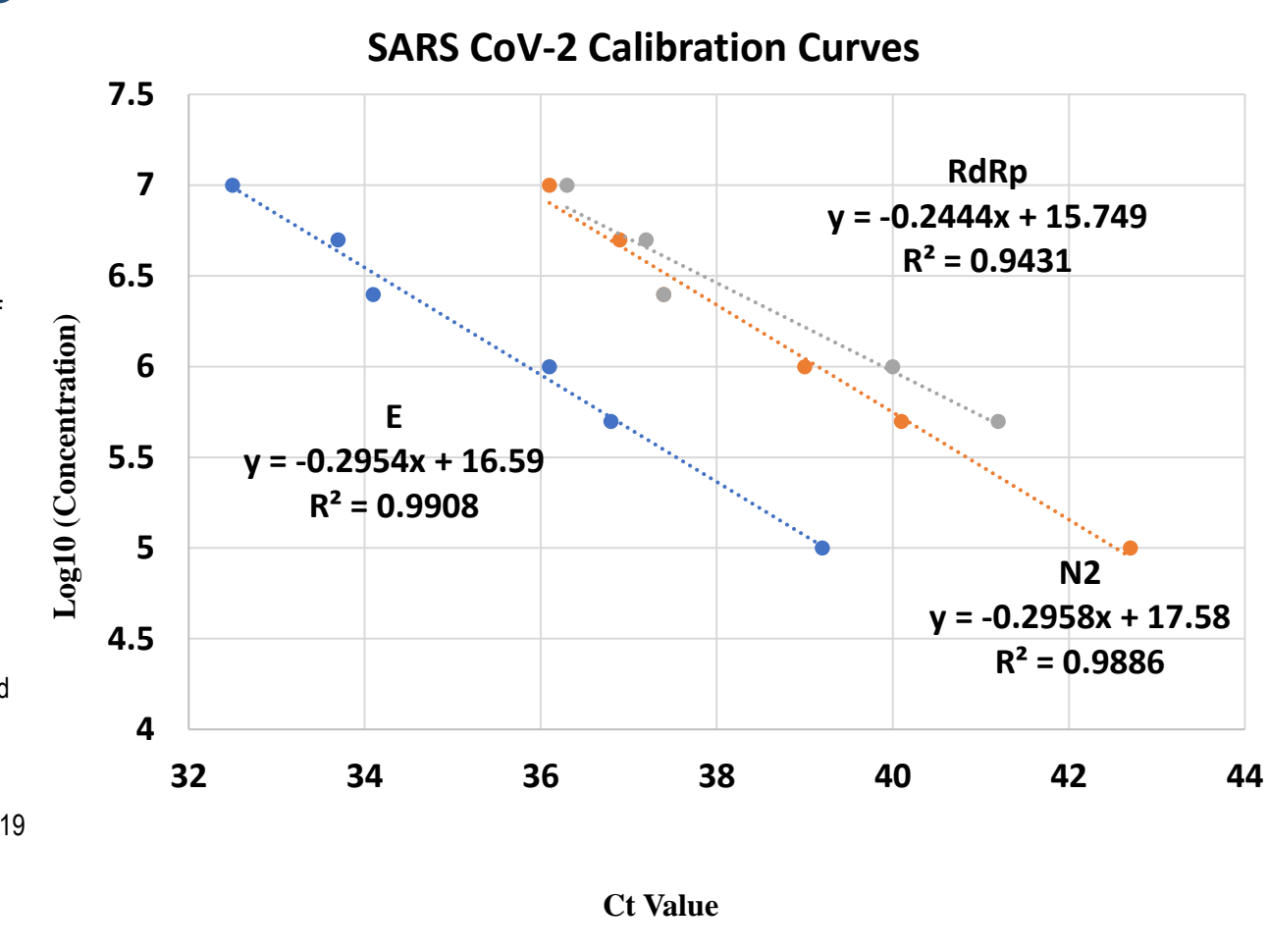
12% Relative Standard Deviation (RSD) on 4 spikes of 1M copies per liter (E gene 3%, N2 Gene 22%; overall 12%)
15% RSD on 7 reps of a raw wastewater sample @ ~200K copies/L
DD-PCR with InnovaPrep Concentration on the same sample: 17% RSD

Accuracy (Spike Recoveries and Correlation to existing methods)

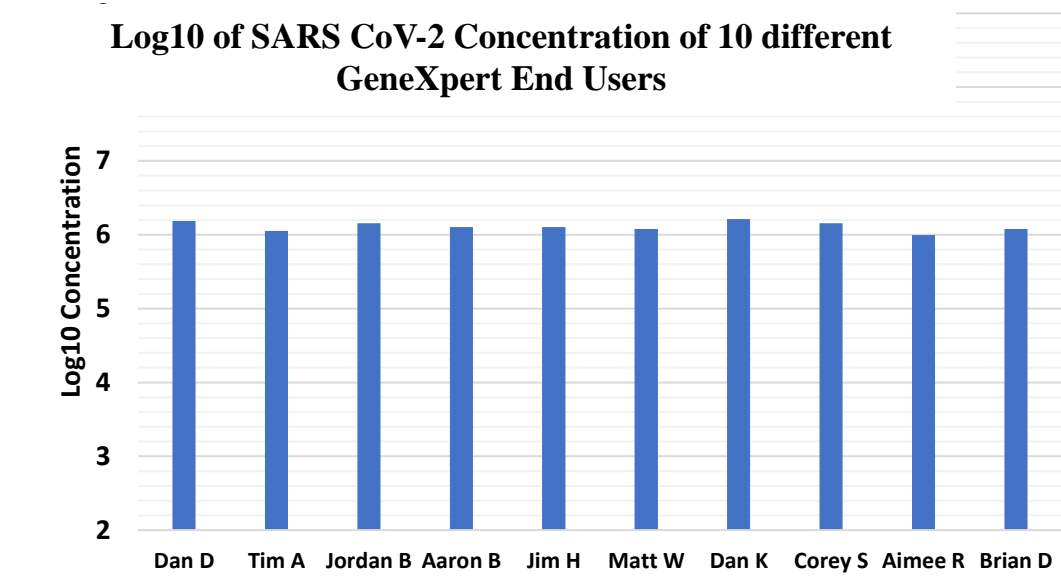
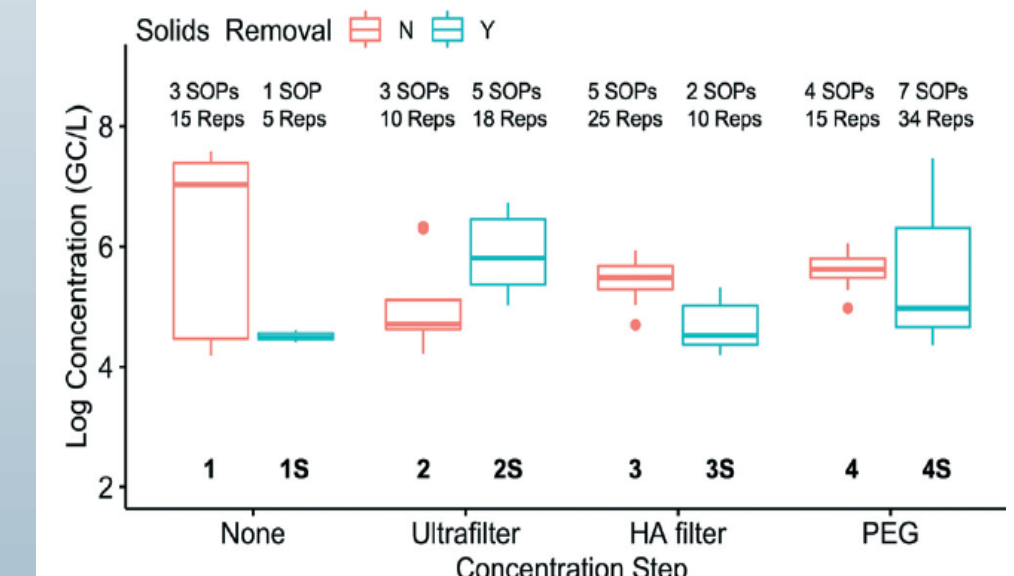
• Average of 97% recovery for 4 influent WW samples spiked with 1M Copies per liter (Averaged over E & N2 genes)
• Correlation with other methods:
LuminUltra (0.9931 r², slope 1.09, 6 tests)
DD-PCR at Colorado State (0.7296 r², slope 0.88, 19 tests)

Detection Limits

• Theoretical detection limit of the system is 11,000 copies/L. In practice, ~32,000 copies/L.

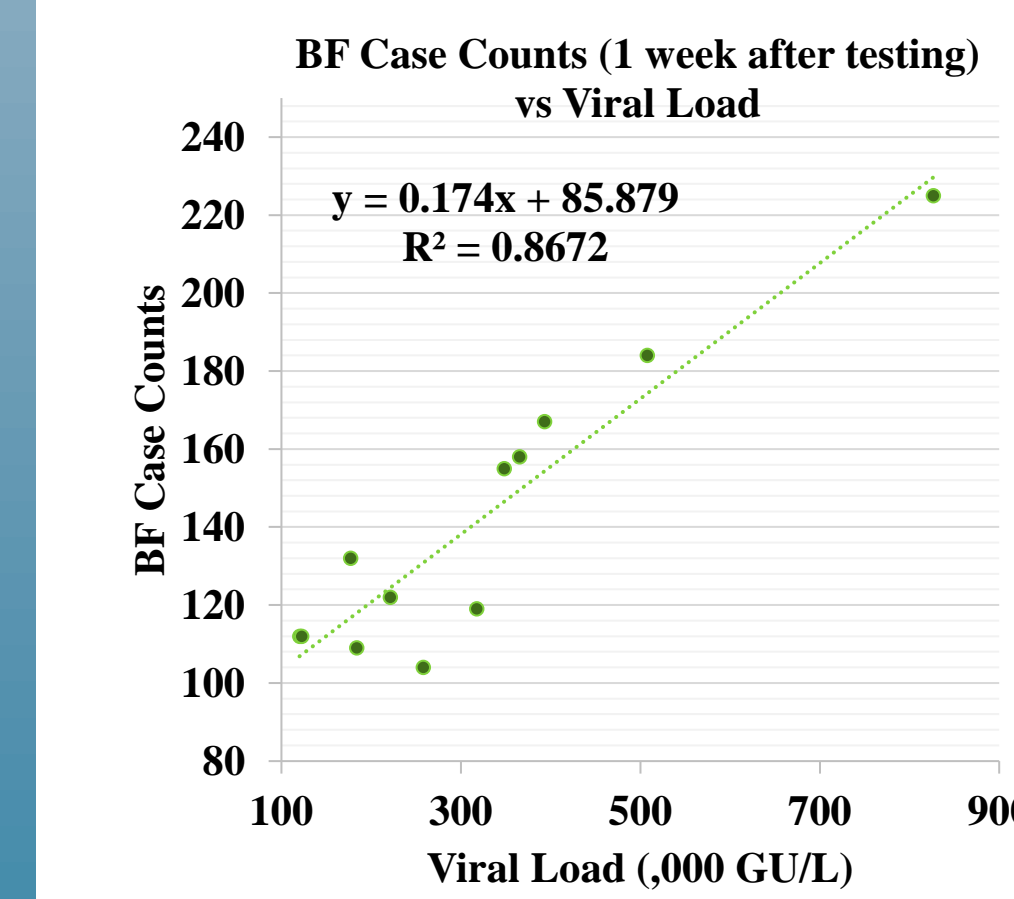


Elimination of Inter-Operator/Method Error



Environ. Sci.: Water Res. Technol., 2021, 7, 504
Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the U.S.¹
Brian M. Peccoon, Emily Darby, Charles N. Haas, Yamin M. Amha, Mitchell Bartolo, Richard Danielson, Yegor Deebom, George Di Giovanni, Christoffer Ferguson, Stephanie Feig, Erica Gaidis, Donald Gray, George Lukash, Bonnie Mull, Liara Olivas, Adam Oliver, Yan Gu, and SARS-CoV-2 Interlaboratory Consortium

Predictive Capability of Wastewater Surveillance

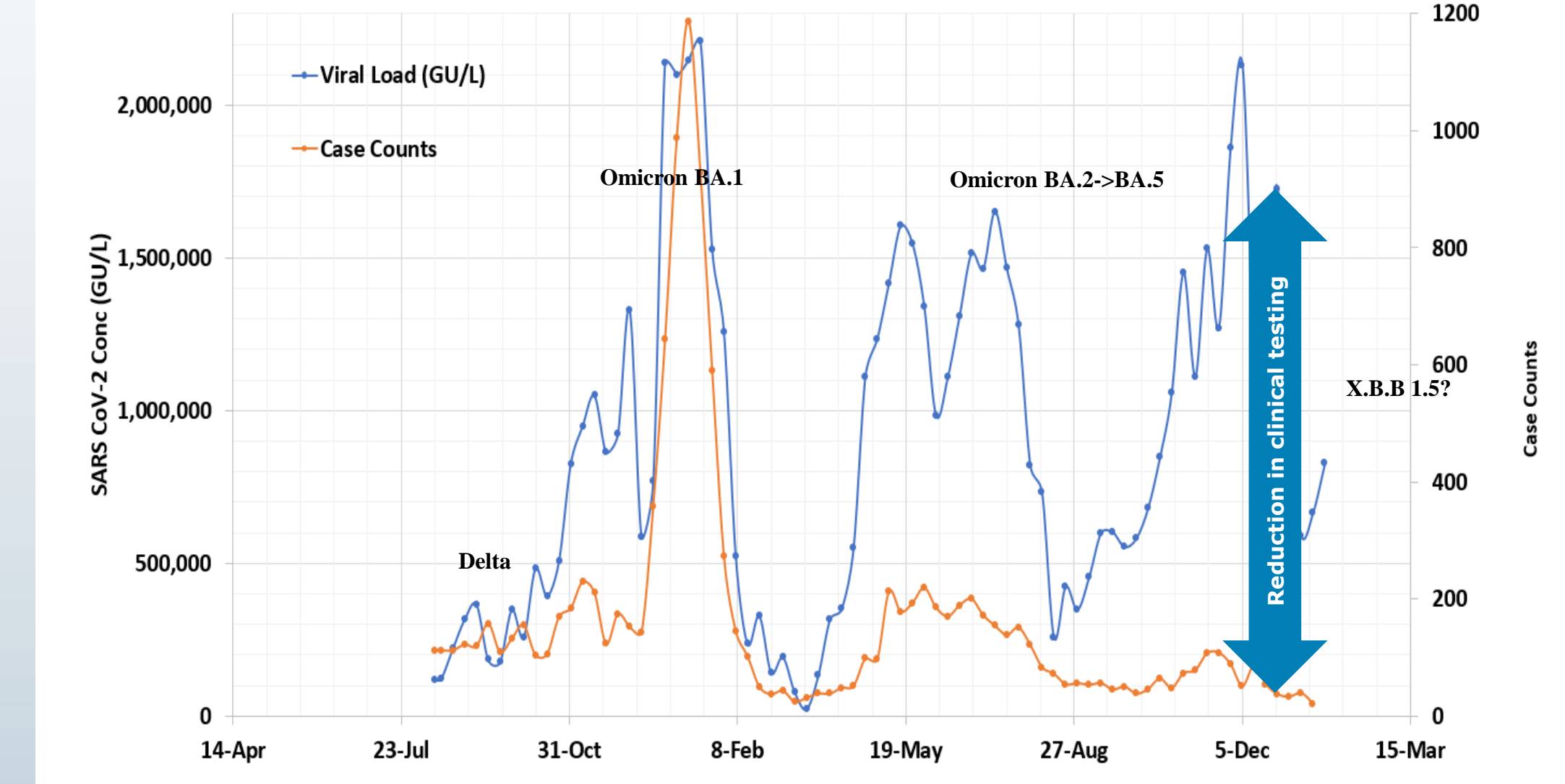


Prediction for Week...	Viral Load (K Gu/L)	Case Counts	Predicted (1 week in advance)	Error	Abs Error
8/22/2021	120	112	109	-3%	3%
8/29/2021	123	112	109	-2%	2%
9/5/2021	221	122	125	2%	2%
9/12/2021	317	119	140	18%	18%
9/19/2021	365	158	147	-7%	7%
9/26/2021	184	109	119	9%	9%
10/3/2021	177	132	118	-11%	11%
10/10/2021	348	155	145	-7%	7%
10/17/2021	258	104	131	26%	26%
10/24/2021	393	167	152	-9%	9%
10/31/2021	508	184	170	-8%	8%
11/7/2021	827	225	220	-2%	2%
11/14/2021	949	239			
11/21/2021	1,050	255			

Test Frequency	Cost (Per Week)	Hands-On Time (Per Week)	Predictive Capability (Average Error)
Daily	\$250	10 Minutes	+/- 8%
2X per Week	\$100	4 Minutes	+/- 21%
Once a week	\$50	2 Minutes	+/- 47%

Case Study Results

2021-2023 GeneXpert SARS CoV-2 Case Study (Broomfield, CO)

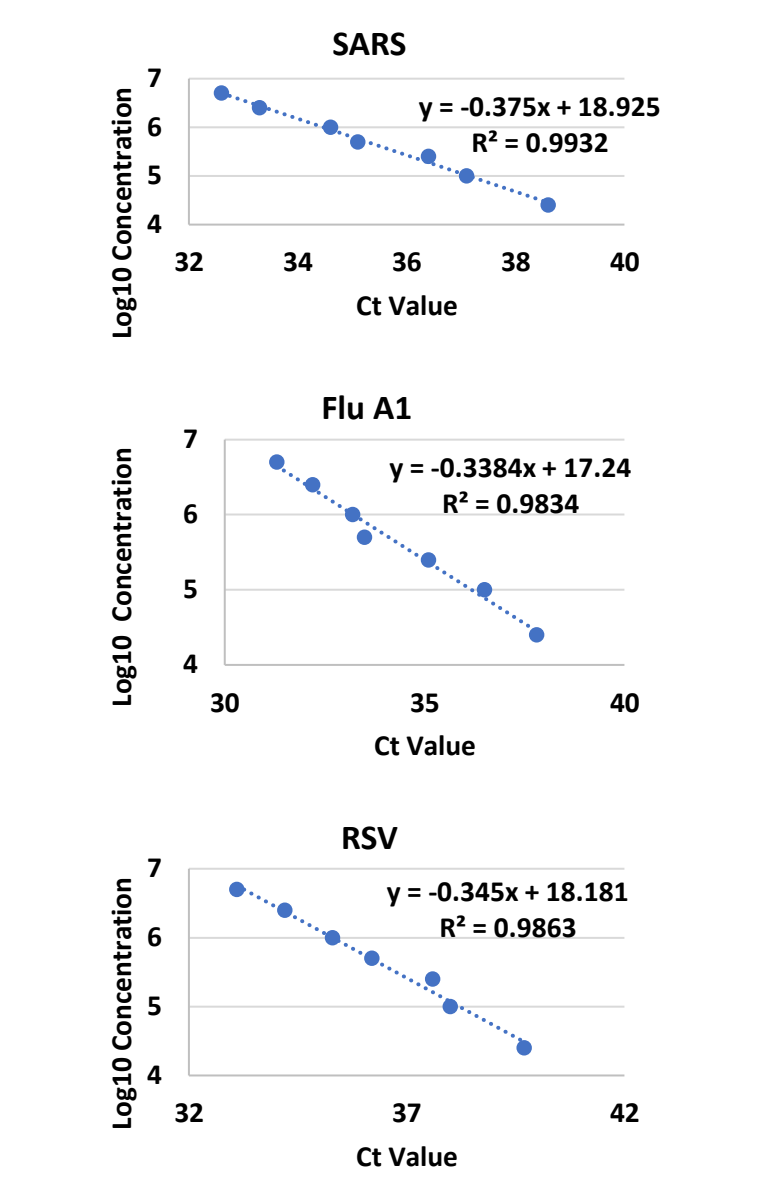


As clinical testing declines WBE shows community spread is still a big problem

Houston Public School Pilot SAR CoV-2, Flu and RSV

Comparison of the GeneXpert Against Digital Droplet PCR Results ((4 schools, 107 samples) GeneXpert Multiplex Cartridge (SARS CoV-2, Flu, and RSV in one)

- SARS CoV-2**
96 out of 107 were aligned (90%)
75 for 81 correlation with DD-PCR for DD Positives
21 for 26 correlation with DD-PCR on DD Negatives
- RSV**
98 out of 107 were aligned (92%)
20 for 28 correlation with DD-PCR for DD Positives
78 for 79 correlation with DD-PCR for DD Negatives
- Flu A**
100 out of 107 were aligned (93%)
28 for 30 correlation with DD-PCR for DD Positives
72 for 77 correlation with DD-PCR for DD Negatives
28 for 30 correlation



Acknowledgements

- Broomfield CO DOH and WWTP for providing wastewater samples for almost 2 years
- Rice University and Houston DOH for performing the side-by-side analyses during their public-school wastewater testing