Automated PCR For Wastewater Disease Surveillance in Communities and Public Schools

Be Right™

NAACHO Presentation
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Topics Covered

- Wastewater surveillance and its public health benefits
- GeneXpert Technology
  - Validation Studies
  - Community Case Study
- Current/Future Targets
- Rice/Houston DOH Public School Case Study

Wastewater Disease Surveillance: Multi-Platform Collaboration within Danaher

There is a Strong Case for Wastewater Surveillance

Wastewater surveillance for disease monitoring involves analyzing sewage to detect and track the presence of pathogens or biomarkers associated with diseases within a community.

Complimentary to most public health initiatives:

- Can enable more proactive and targeted actions

Leading Indicator over Clinical Testing:

- Evidence suggests it is effective for monitoring COVID-19 (Leading indicator vs. Clinical methods) in sewers with results up to 5-7 days before clinical detection

Representative of Macro Community:

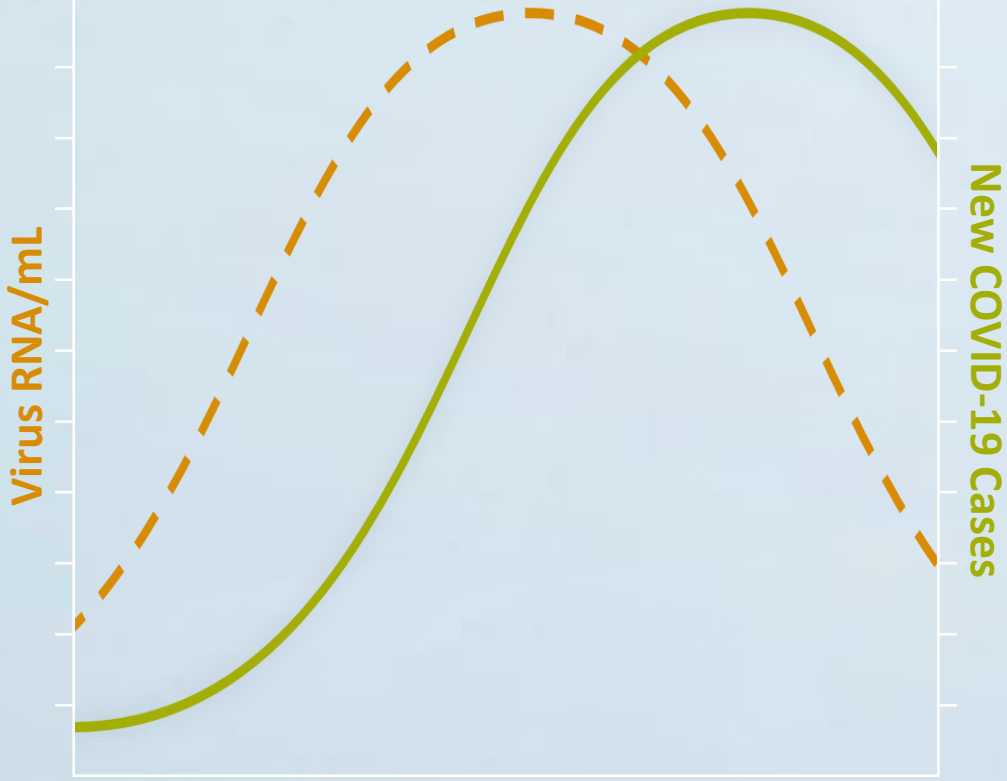
- Reflects asymptomatic and symptomatic population
- Independent of healthcare-seeking behaviors and access to clinical testing

Comprehensive Coverage:

- 75-80% of US households are served by municipal sewage systems

Economically Monitor Spread, Decline, and Re-emergence in the community:

- Fewer tests per capita required



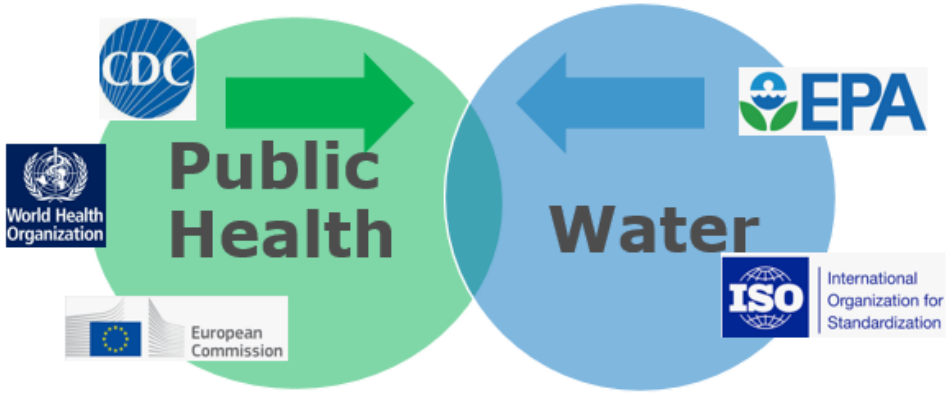
Wastewater Surveillance: A New"ish" Intersection between Public Health and Water

Public Health and Water

- **Historically Focused on *Consumption/Contact* based diseases**
  - Bacteria, viruses, heavy metals, organics, PFAS
- **Solutions involved Treatment and Testing Mitigations**

A new intersection of Public Health and Water: Wastewater Surveillance

- Using wastewater to monitor trends in community public health
  - In theory 1 wastewater sample can represent an entire community
- **The pandemic accelerated the maturity of wastewater surveillance**
  - Used as a leading indicator or trends in the spread of COVID
  - Wastewater Surveillance has evolved into a relatively mature science since the start of the pandemic
  - Over the last couple years, several other targets of interest have also shown public health value



Public Health and Wastewater Surveillance 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 regions/communities or smaller scale WWTPs

Impact

- **Almost no in-house testing:** PCR tests 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
- **Underserved communities/regions have little access:** Low-to-no access to central labs and/or skilled lab technicians

GeneXpert Simplicity (1-step testing, <1 minute hands-on)

Traditional Viral Extraction

Sample Prep System/Cartridge

300 µL

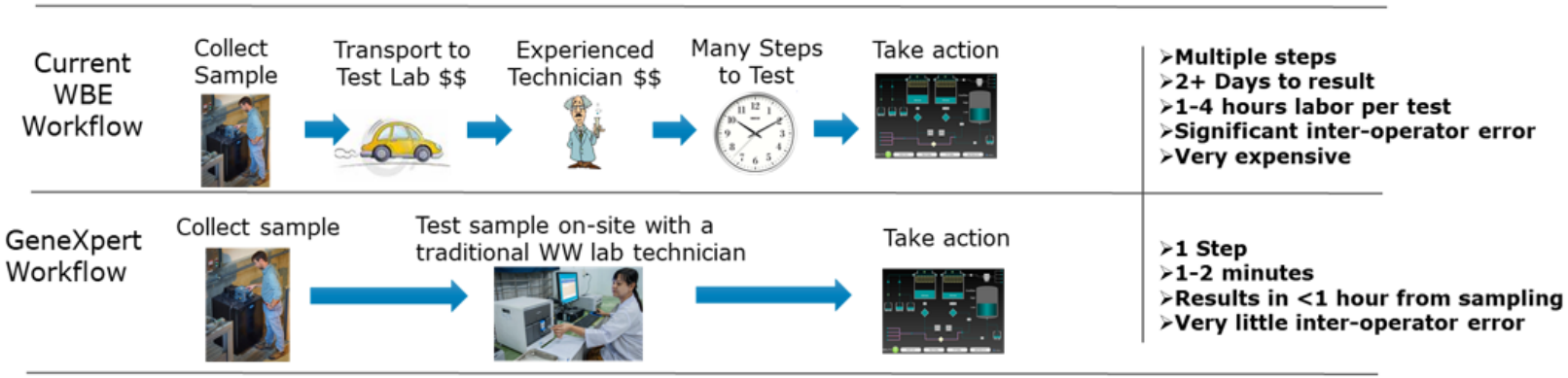
PCR System (~1' x 1' x1')

Time Consuming & Complex

Multiple pipetting steps, sample transfers, filtration, etc.; 1-4 hours per test, in many cases requires very skilled lab technicians

Simple, Faster, Cheaper & Portable

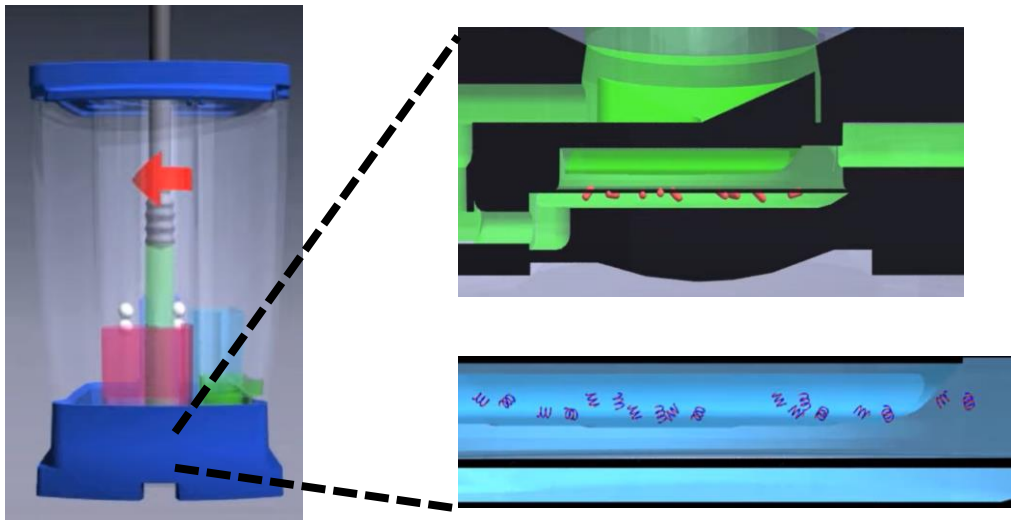
Cepheid Cartridge/System automates all pipetting, filtering and sample preparation
(1 minute of hands-on time per test, <45 minutes to result)



Jim Huang (CSU): **"He's able to take just a small amount of wastewater (~300uL) and put it into a Cepheid pod, which goes into GeneXpert for processing, thermocycling, and reading! This set-up can do my job, which normally takes ~7 hours and does it in ~40 minutes!"**

Sample Preparation/Analysis Automation: GeneXpert Cartridge

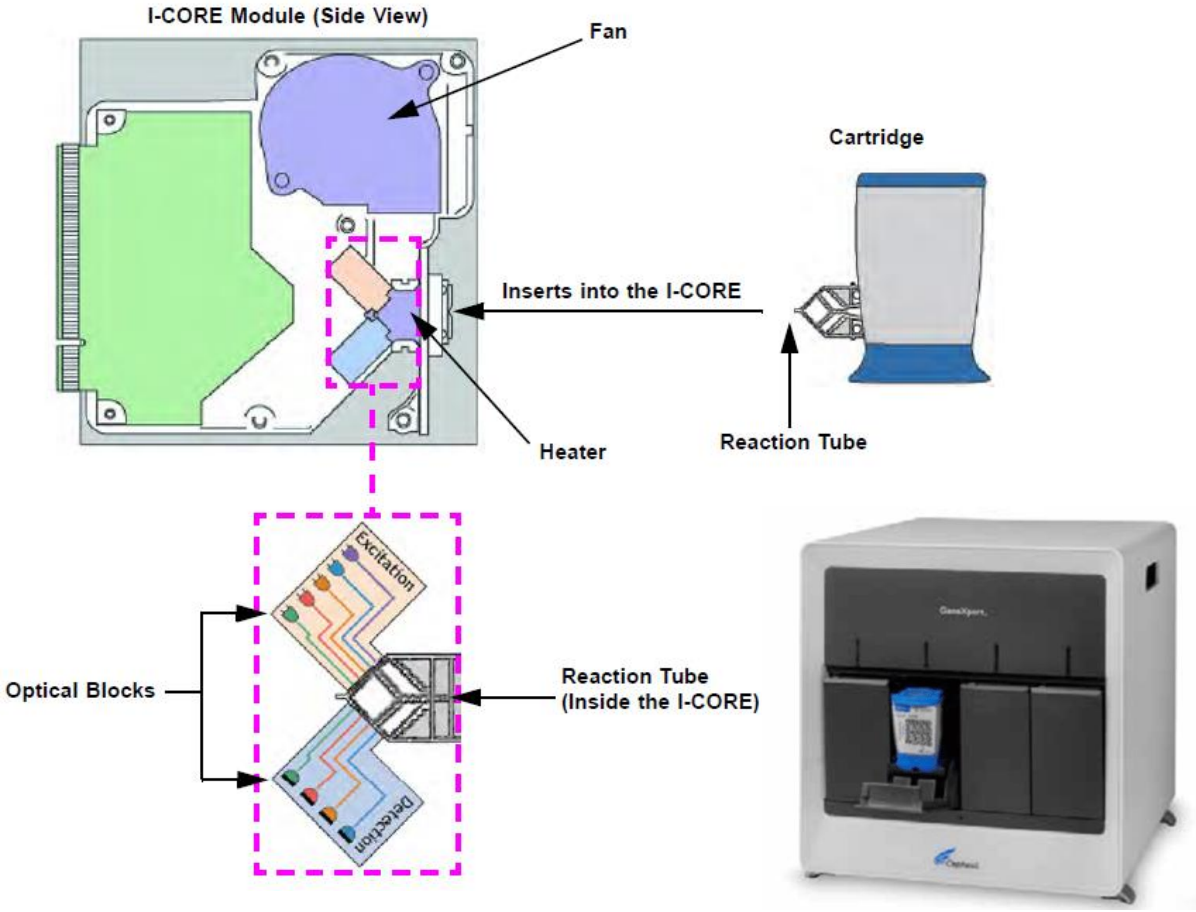
Sample Preparation



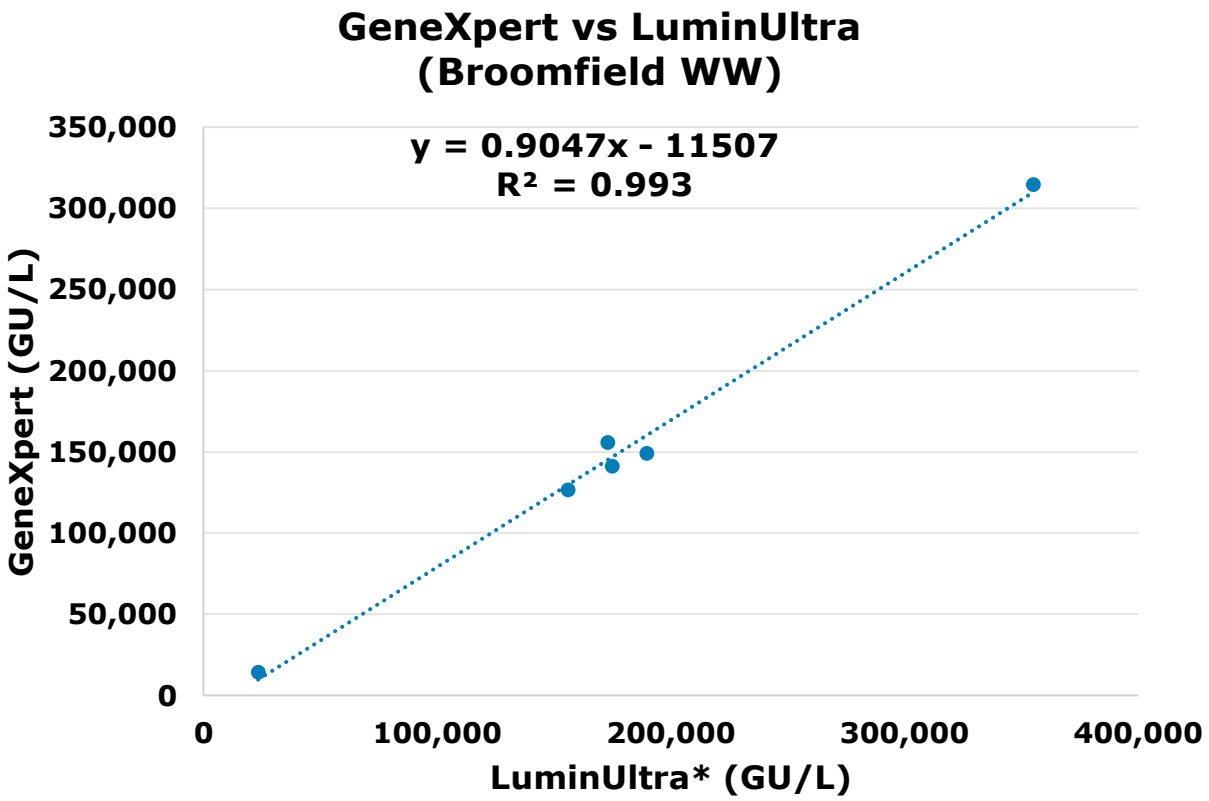
Chambers contain reagents, primers, probes, buffers and extracted sample. Syringe and valving system moves the sample and reagents

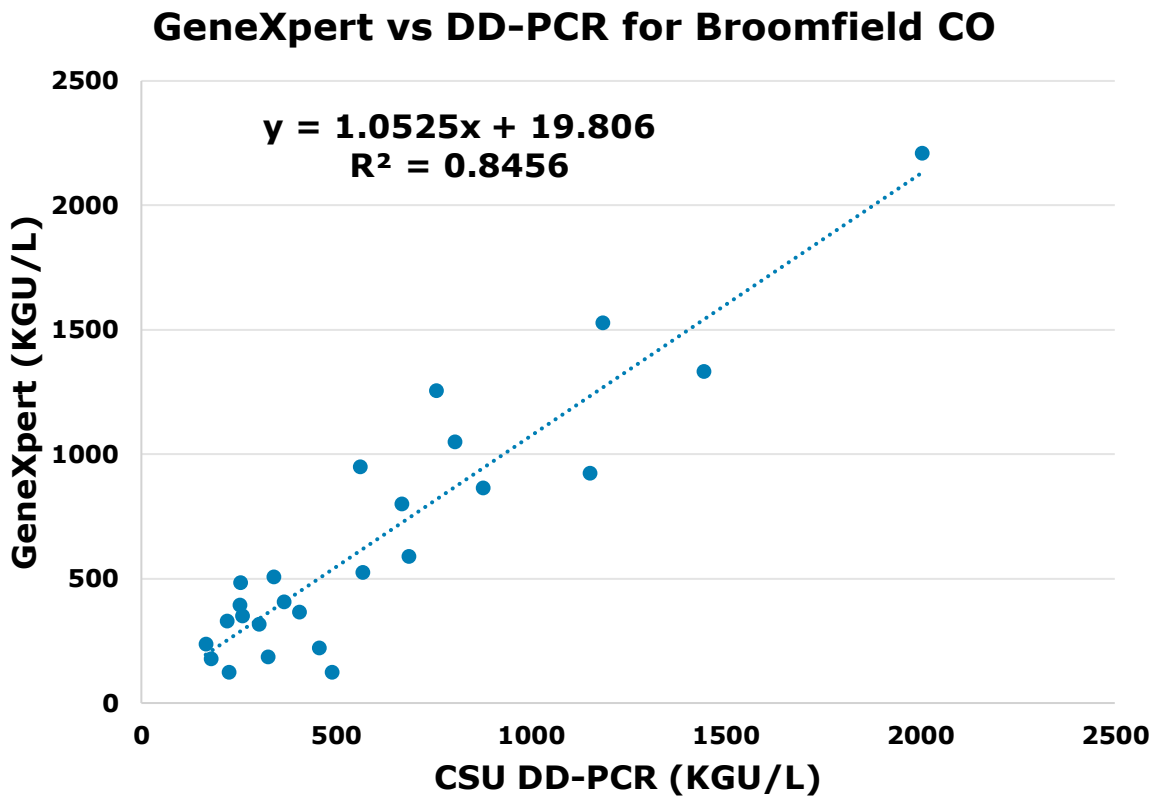
An integrated membrane isolates the virus prior to lysing and the releasing of the targeted RNA

Measurement



Comparison to other methods

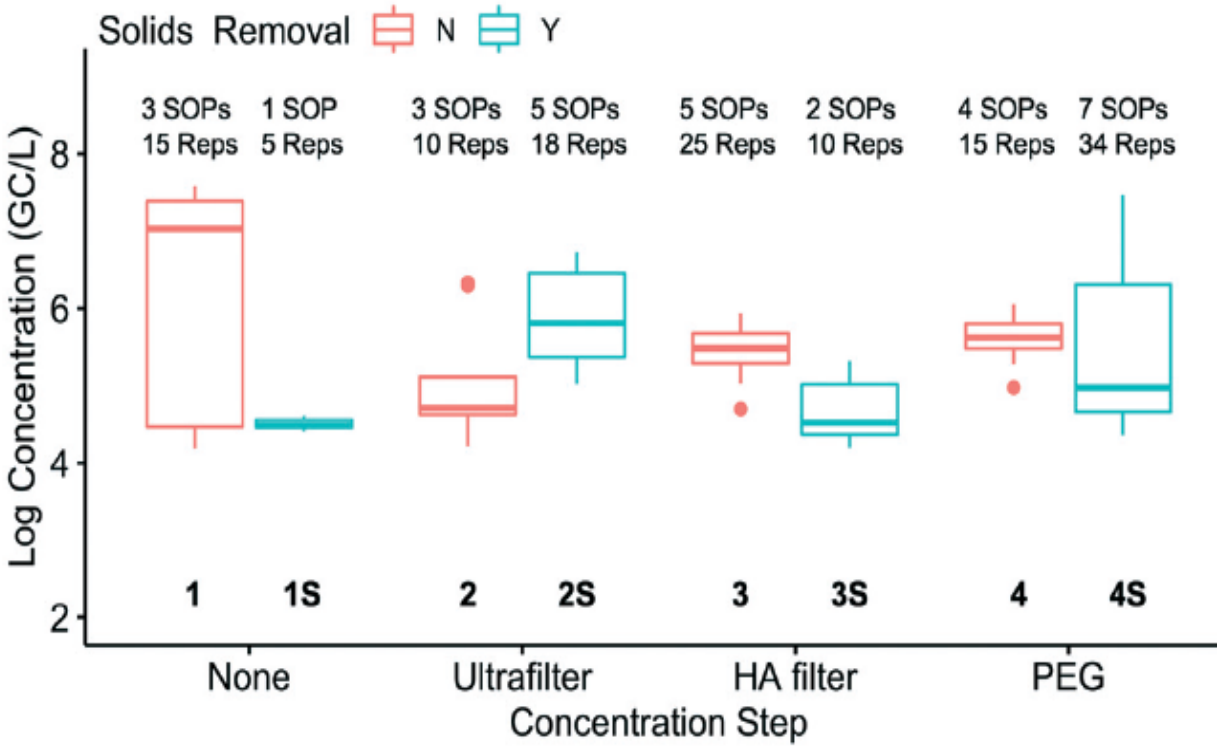


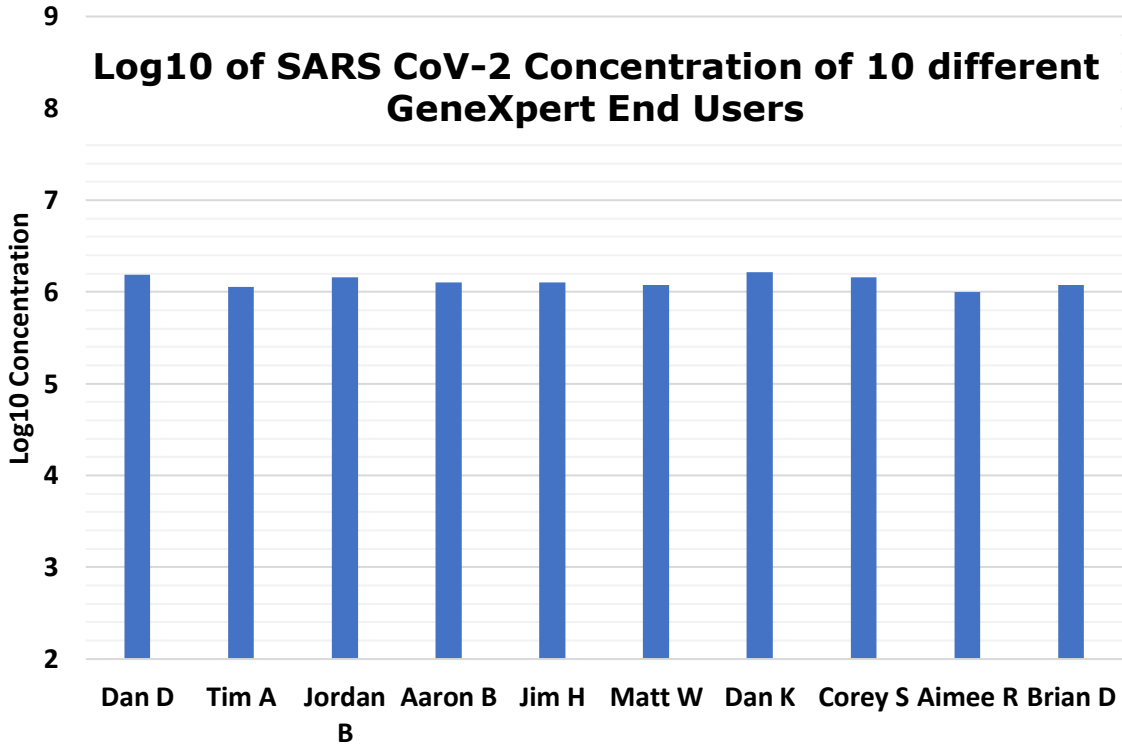


*Magnetic bead extraction

Note that the LuminUltra comparison was done on the same sample in the same lab. The comparison to the digital droplet PCR results were a weekly average comparison done on different samples at different sites (Hach and Colorado State University)

Automation Standardizes Results Between Operators and Sites





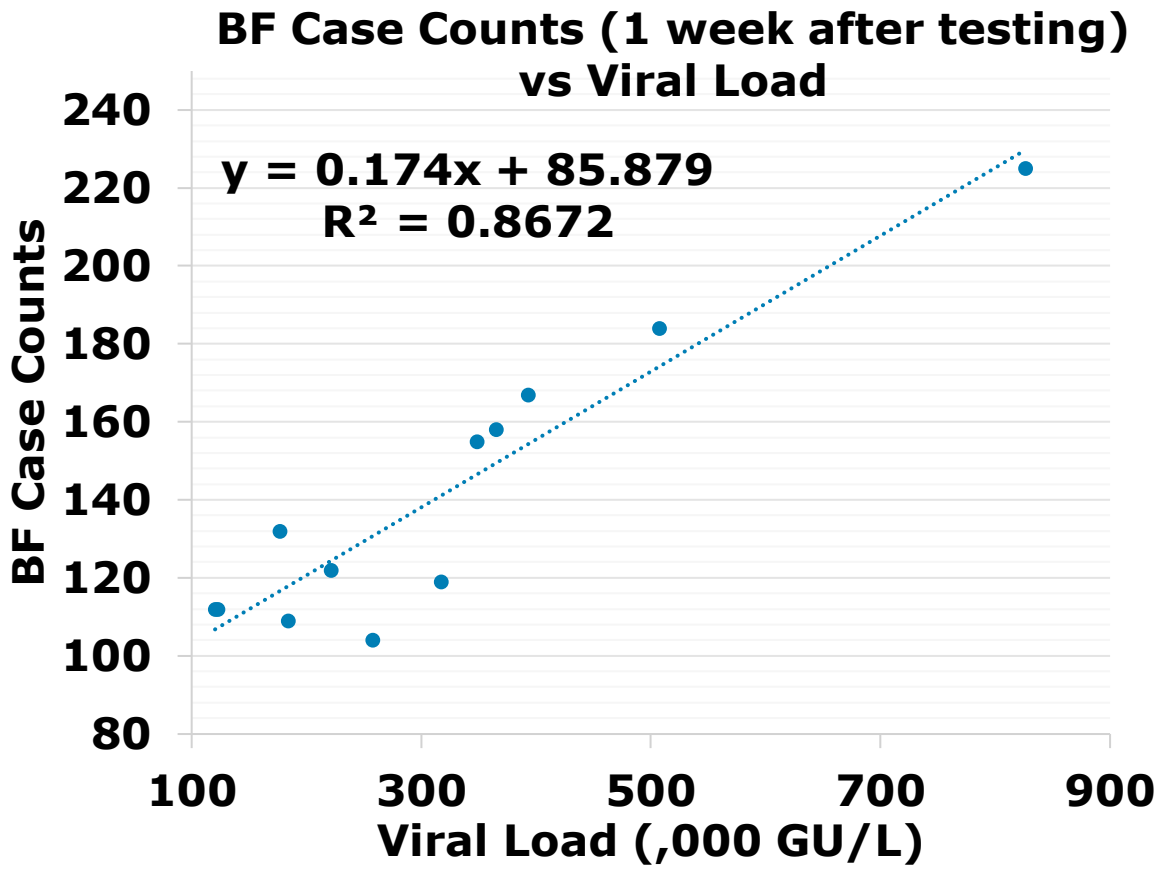
Environmental Science: Water Research & Technology, 2021, 7, 504

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.†

Brian M. Pecson, ‡*[a] Emily Darby,‡*[a] Charles N. Haas, [b] Yamrot M. Amha,[c] Mitchel Bartolo,[d] Richard Danielson,[e] Yeggie Dearborn,[e] George Di Giovanni,[f] Christobel Ferguson,[g] Stephanie Fevig, [g] Erica Gaddis,[h] Donald Gray,[i] George Lukasik,[j] Bonnie Mull,[j] Liana Olivas,[c] Adam Olivieri,[k] Yan Qu[c] and SARS-CoV-2 Interlaboratory Consortium§

10 different users ran a SARS CoV-2 wastewater test on the GeneXpert with 15 minutes of training Backgrounds ranged from Distinguished Scientist to Plant Maintenance

Can GeneXpert Data be Used to Model Community Spread?

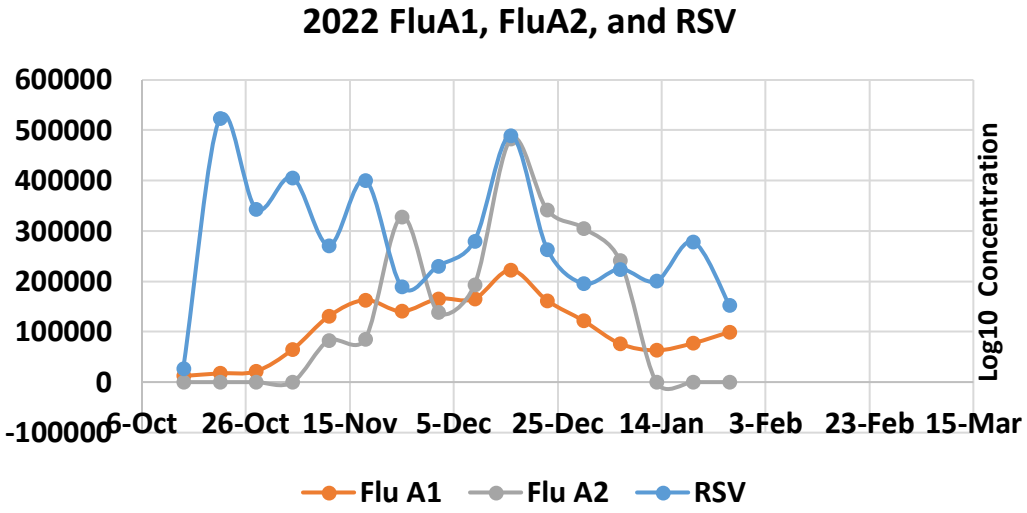


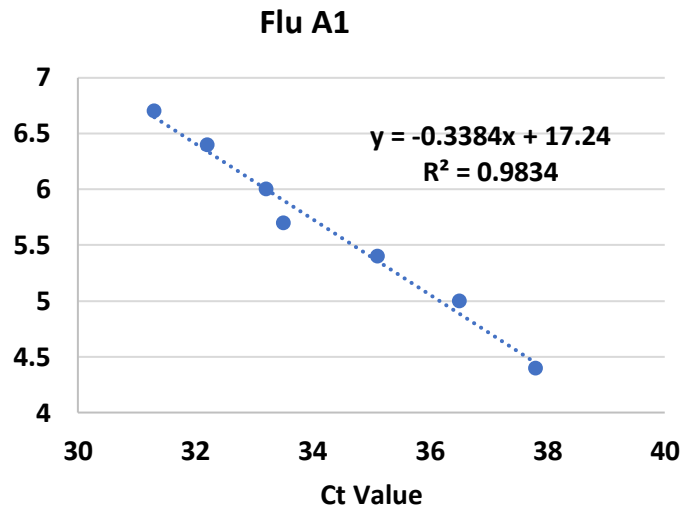
Prediction for Week....	Viral Load (K Gu/L)	Case Counts	Predicted (1 week in advance)	Error	Abs Error
8/22/2021	120	112	109	-3%	3%
8/29/2021	123	112	109	-2%	2%
9/5/2021	221	122	125	2%	2%
9/12/2021	317	119	140	18%	18%
9/19/2021	365	158	147	-7%	7%
9/26/2021	184	109	119	9%	9%
10/3/2021	177	132	118	-11%	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		

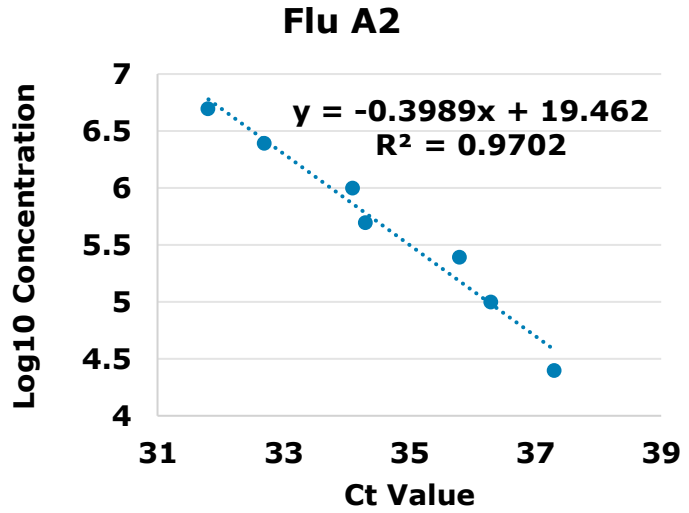
Test Frequency	Hands-On Time (Per Week)	Predictive Capability (Average Error)
Daily	10 Minutes	+/- 8%
2X per Week	4 Minutes	+/- 21%
Once a week	2 Minutes	+/- 47%

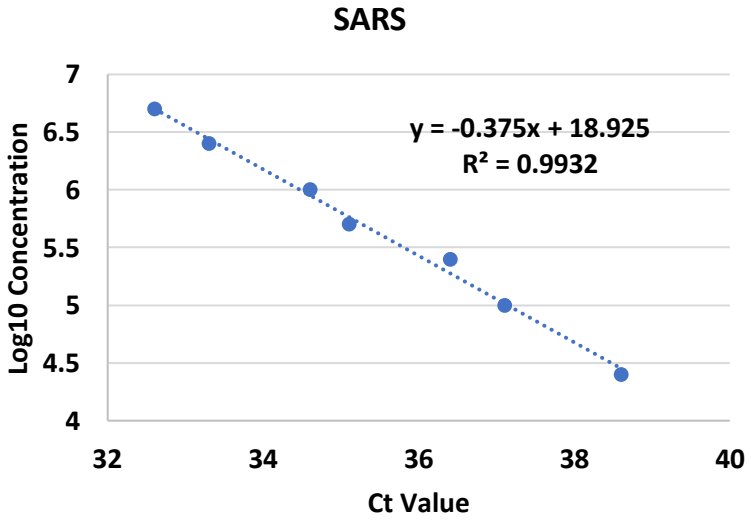
What Else Can/Could the GeneXpert Do?

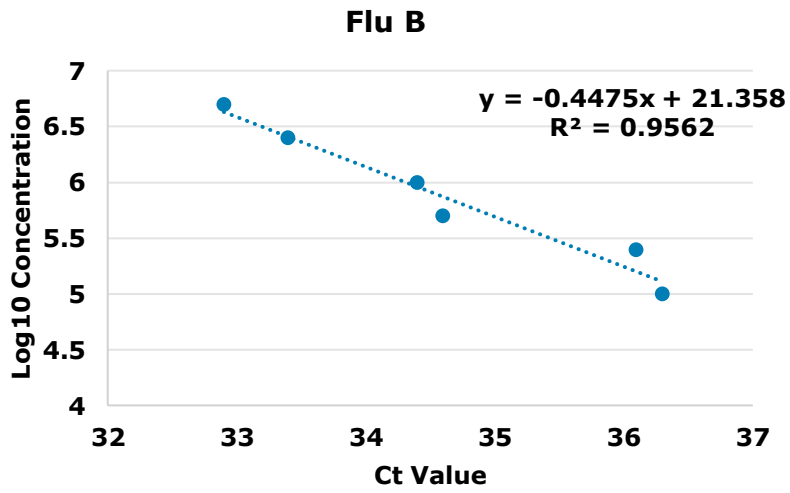
Targets that can be measured in parallel (Flu A1, Flu A2, Flu B, RSV, and SARS Cov-2 in one cartridge)

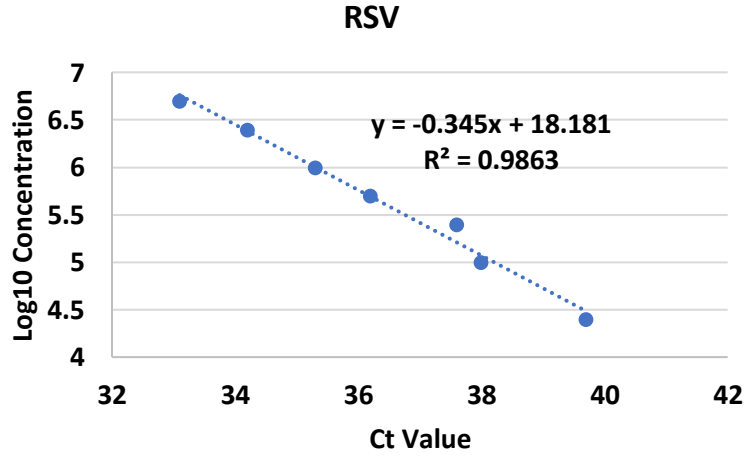






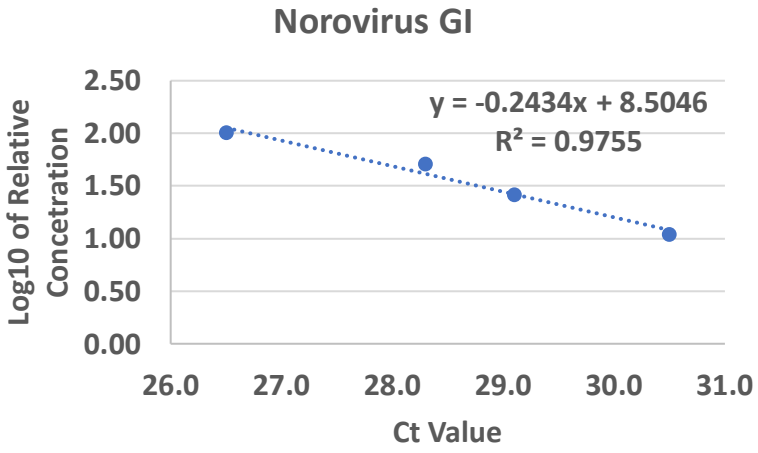




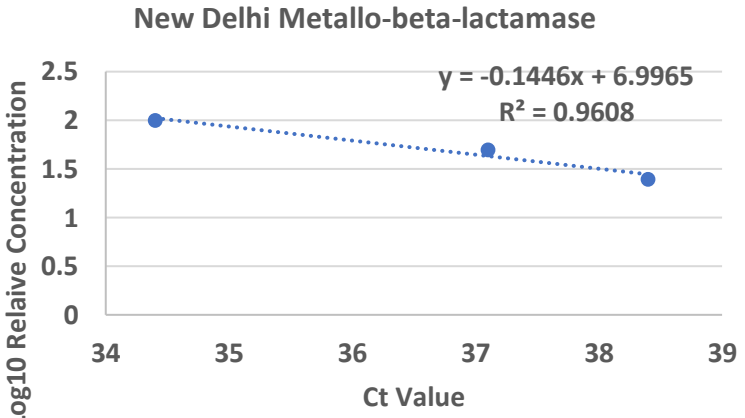


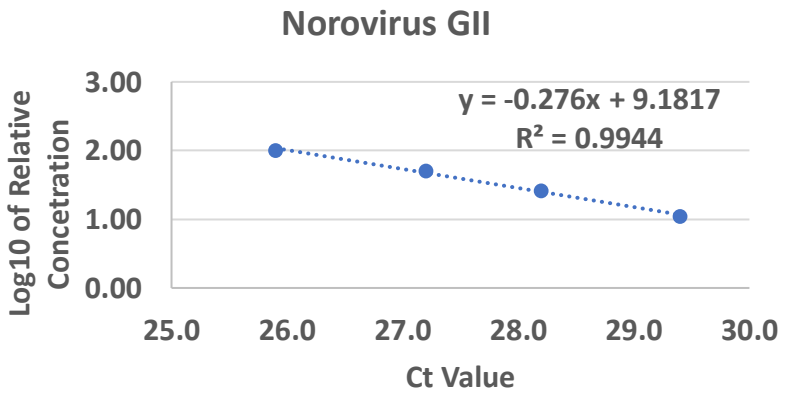
Approach: A Multi-target standard was spiked into raw sewage at different levels that were 2X different (equivalent to 1 Ct value)

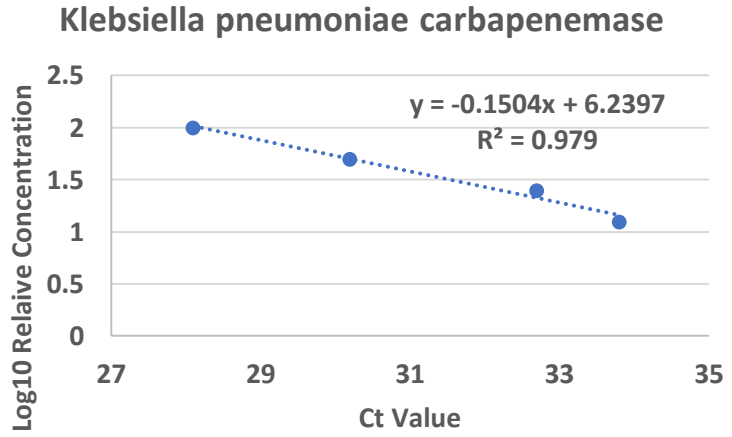
Norovirus and Antibiotic Resistant Bacteria Data

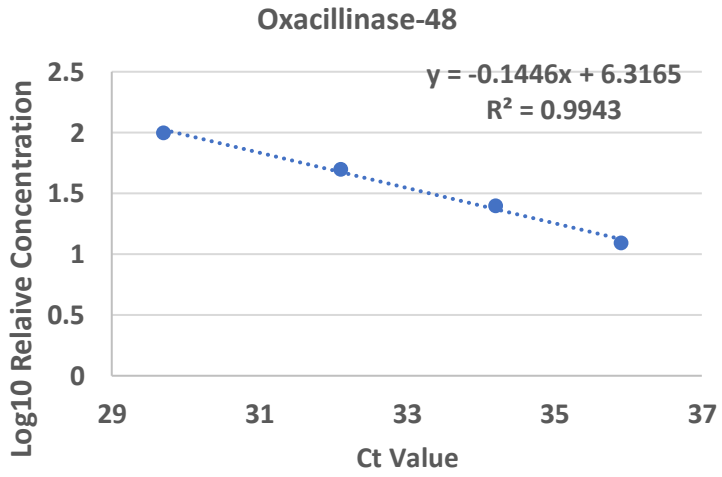


	Spike receoveries in WW
Imipenemase	84%
Verona Integron-Encoded Metallo-beta-lactamase	87%
New Delhi Metallo-beta-lactamase	103%
Klebsiella pneumoniae carbapenemase	139%
Oxacillinase-48	74%









2 strains of Norovirus and 3 different ARBs were found in Broomfield WW and were diluted to determine that the GeneXpert response was linear.

Current Offering and Long Term Vision

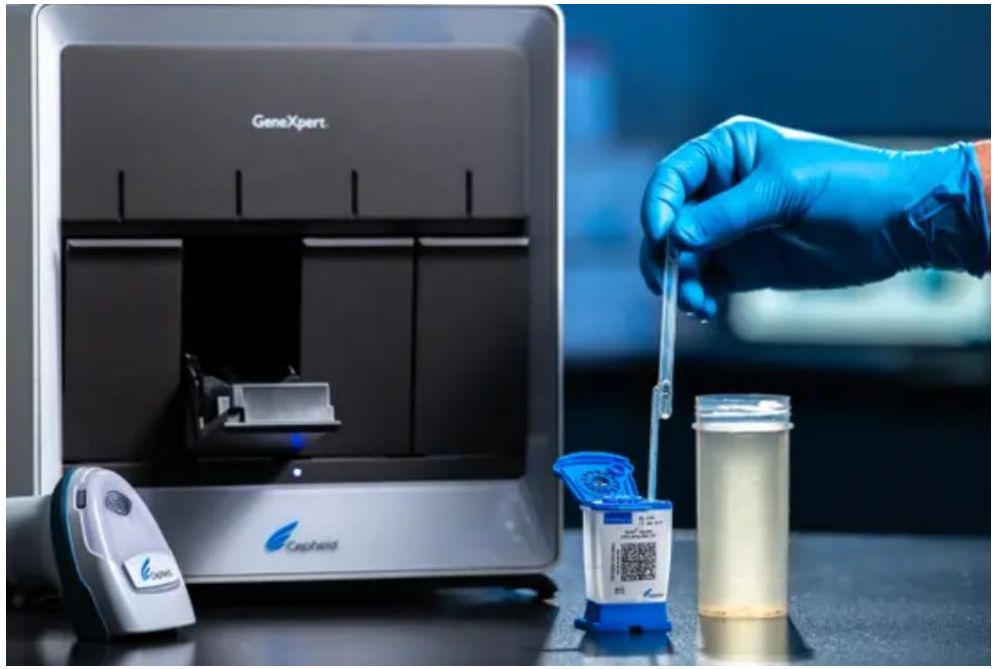
Current Targets

SARS-CoV-2

- N2, E, and RDRP (RNA dependent RNA polymerase)

Mixed Target Cartridge

- Flu A, Flu A1, Flu B, Respiratory Syncytial Virus (RSV), and SARS-CoV-2



Vision

A comprehensive wastewater public health cartridge(s)

Possible Targets:

- Carbapenem/Antibiotic Resistant Bacteria
- Norovirus MTB/RIF Ultra
- Hepatitis C
- Hepatitis B, chlamydia, gonorrhea
- Extensively drug resistant TB
- Mpox
- Polioviris
- Adenovirus
- Cholera

March 20, 2024
NACCHO Webinar

Assessment and application of GeneXpert rapid testing for respiratory viruses in school wastewater

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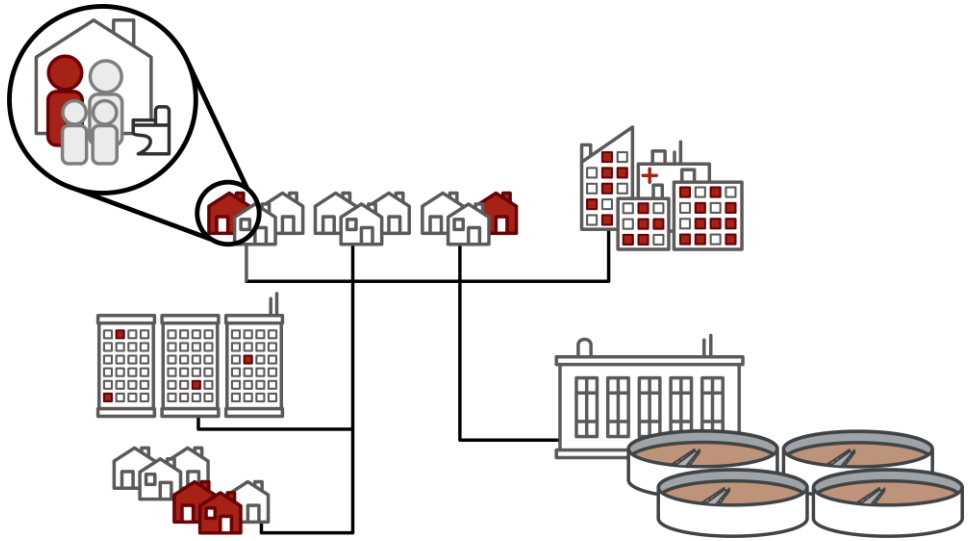

Our Team

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Wastewater

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Dr. Loren Hopkins

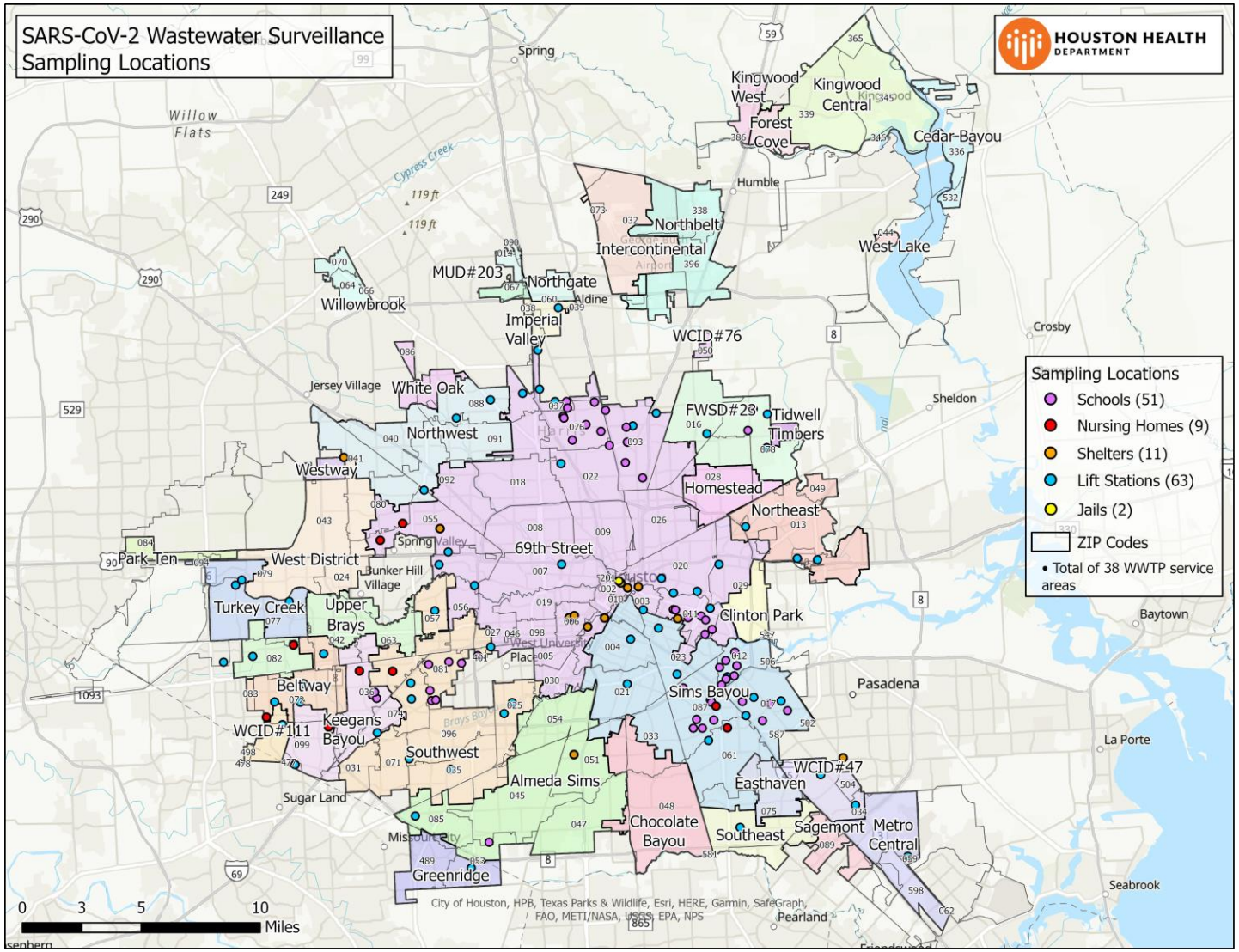
Implementation and Leadership

Kaavya Domakonda, Rebecca Schneider, Anthony Mulenga, Kelsey Caton, Komal Sheth

Houston's wastewater monitoring system

Current sampling sites:

- **39 wastewater treatment plants**
  - serve ~600 to 550,000 people
- **14 lift stations**
  - serve ~950 to 374,000 people
- **69 manholes**
  - 48 public schools
  - 11 shelters
  - 8 nursing homes
  - 1 jail (2 sites)



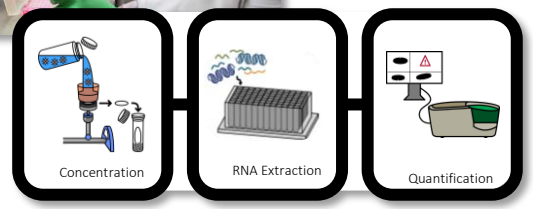
Website: https://hou-wastewater-epi.org/

Process Overview

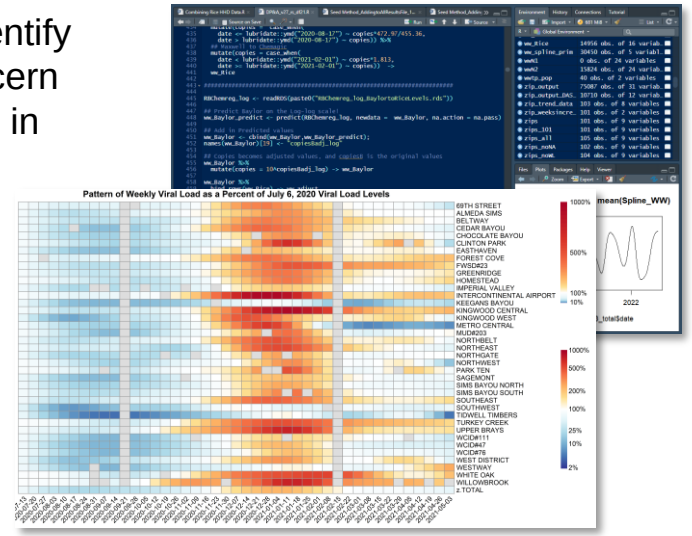
24-hour composite influent/wastewater samples are collected from wastewater treatment plants (serving 2.3M+), schools, congregate living facilities, and lift stations each week

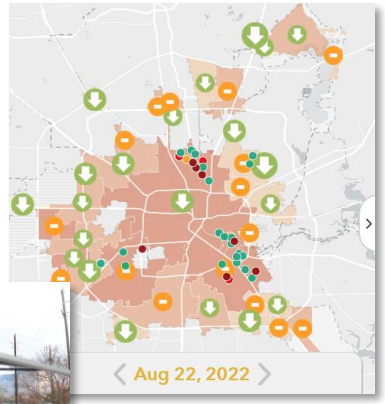
Viruses quantified in replicate samples in two independent laboratories

Variant screening performed using SARS-CoV-2 genome sequencing.



Raw data is input into statistical models that identify geographic areas of concern with significant increases in wastewater virus concentration.

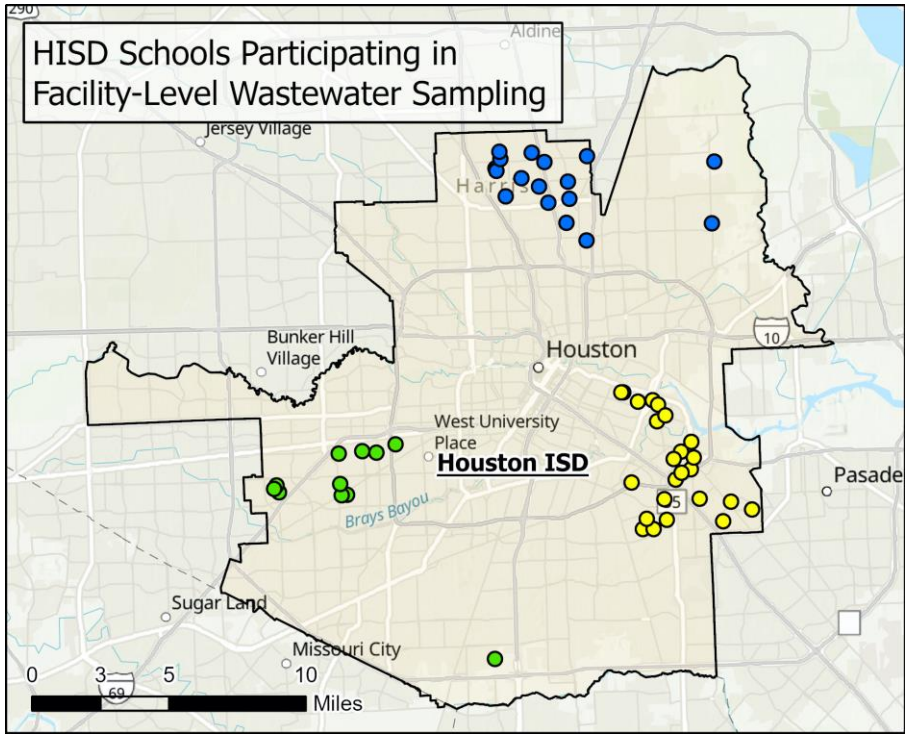




Health department uses wastewater data in public health interventions at zip-code and facility-level

PreK-12 school wastewater monitoring of respiratory viruses to support public health response

Why? Respiratory viruses are a leading cause of hospitalizations in children and schools are sources of respiratory viral outbreaks.



We have an established and successful preK-12 school monitoring program

- **48** preK-12 schools participating
- **Weekly** samples collected from each school
- Results reported back to schools and parents

33 months, 3 school years

24 months, 2.5 school years

12 months, 1.5 school years

March 2020:
Began citywide wastewater monitoring for SARS-CoV-2 in Houston

December 2020:
Added school sampling and analysis for SARS-CoV-2

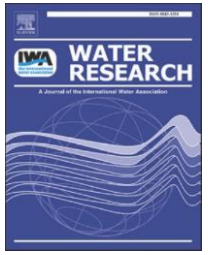
September 2021:
Added influenza analysis to school wastewater samples

September 2022:
Added RSV analysis to school wastewater samples

Today!
Measles, mumps, rubella
Enterovirus d68

School wastewater surveillance is reflective of local infections at several population levels

- Wastewater concentrations of SARS-CoV-2 were strongly associated with:
  - COVID-19 cases in schools, and
  - Community positivity rates
- Houston Health Department uses **schools as a springboard into the community** to encourage vaccinations



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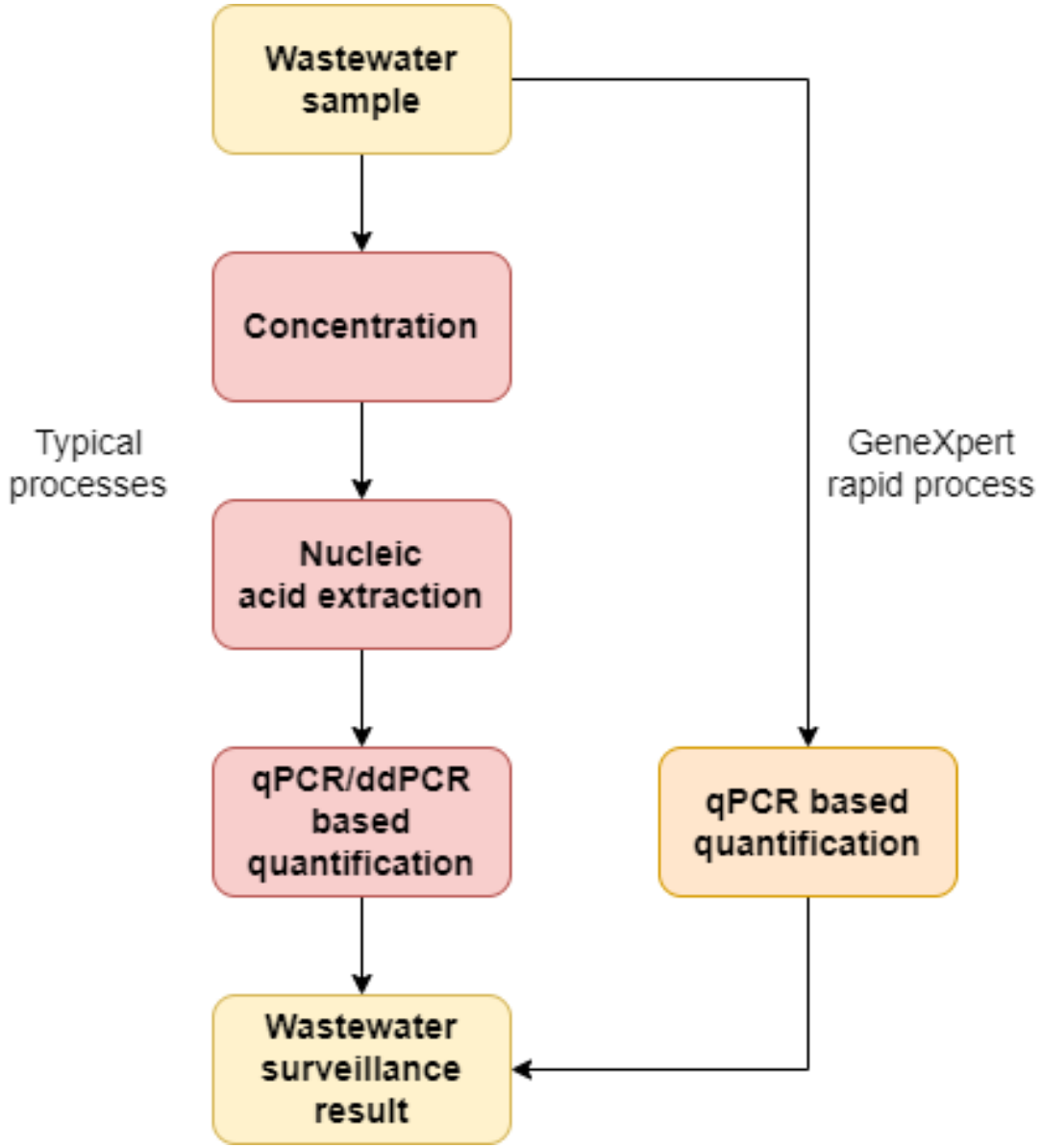
GeneXpert rapid test for wastewater monitoring



GeneXpert rapid test:

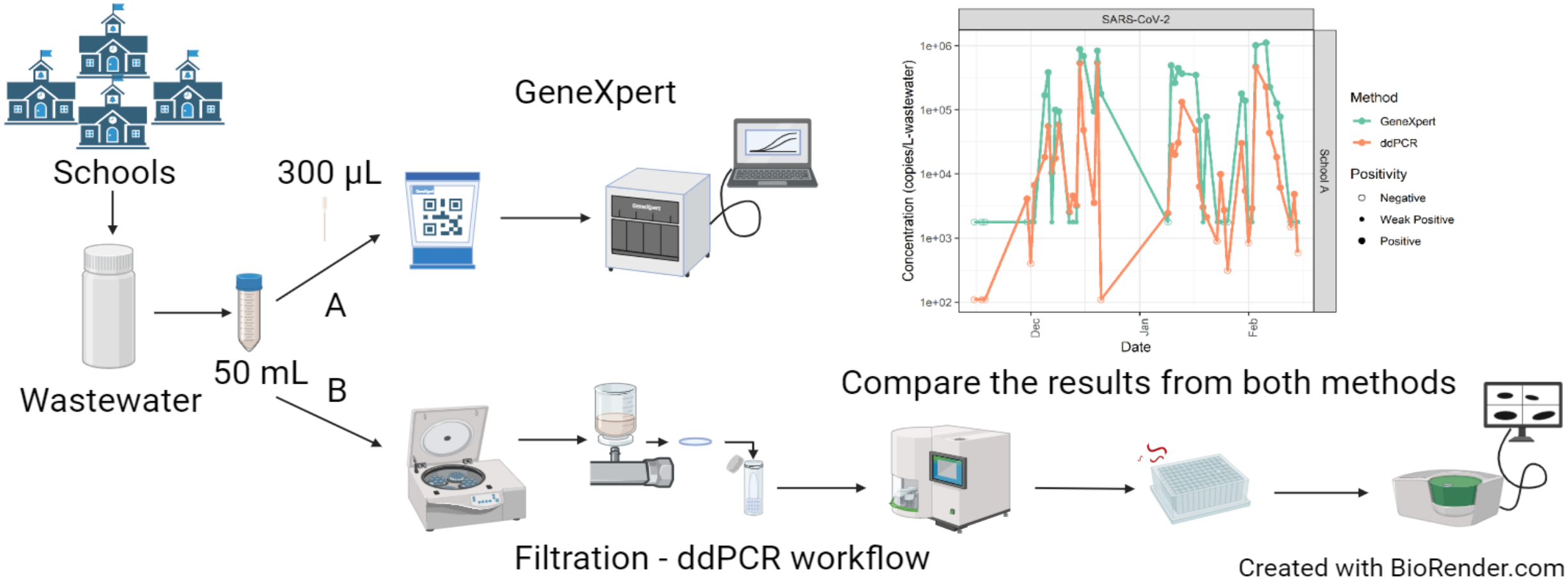
Pros:

- Fewer steps involved
- Less laborious
- Less equipment required
- Can be processed on site
- Fast (~1 hour)

Cons:

- Less accurate (?)
- Less sensitive (?)
  - No concentration step

Project overview



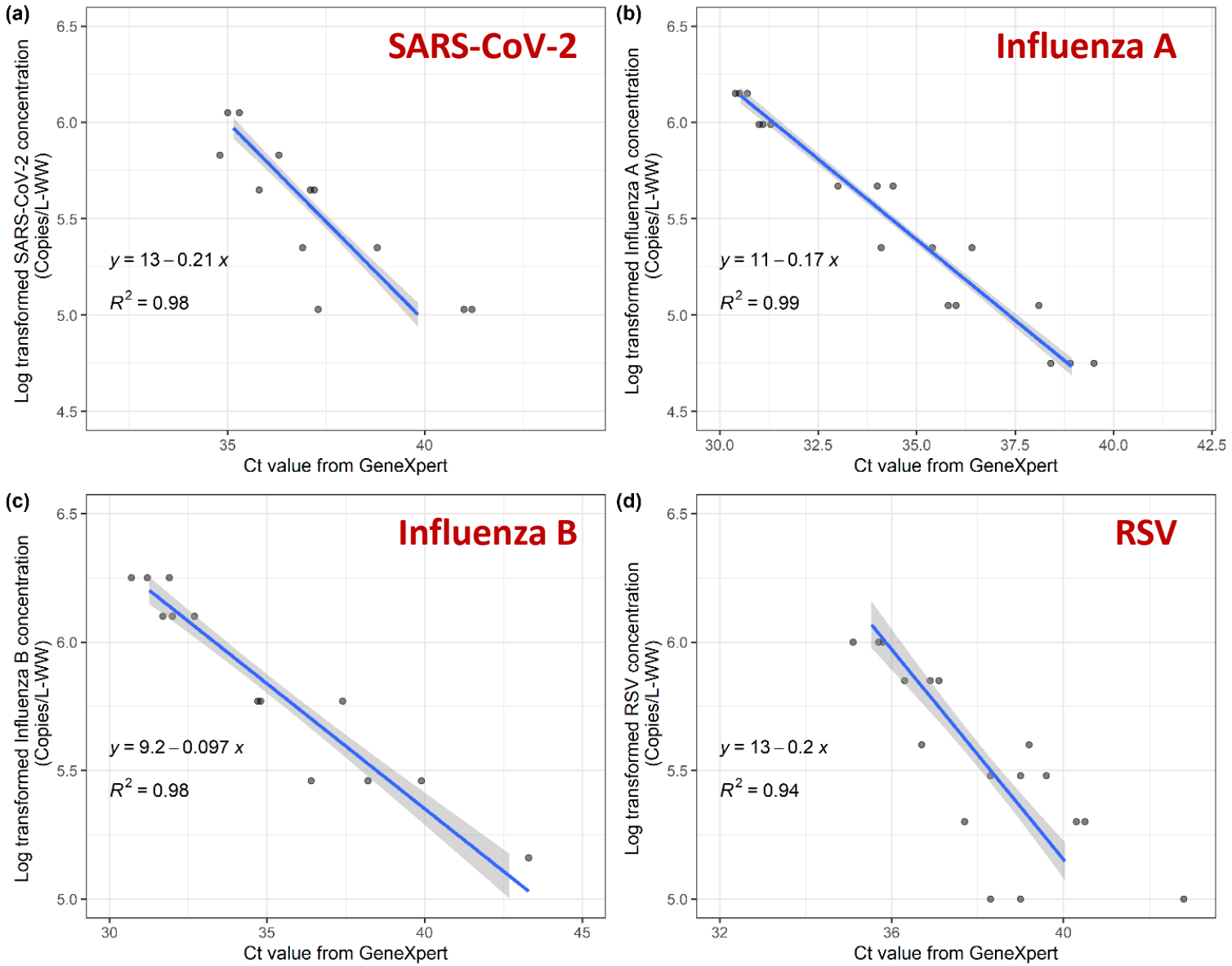
Project objectives

Evaluation of the GeneXpert system	GeneXpert application for school wastewater surveillance	Comparison of GeneXpert and filtration-ddPCR workflow results	Cost analysis of GeneXpert versus filtration–ddPCR workflow
• Development of a standard curve in wastewater to convert Ct to viral concentration for each target virus • Reproducibility test	• 3-month daily surveillance using wastewater samples from 4 schools	• Compared sensitivity • Compared quantitative trends generated by each method • Performed a linear regression to relate GeneXpert and filtration - ddPCR workflow quantitative results	• Compared annual total cost of each method • Analyzed as a function of total number of samples per week

GeneXpert targets (Xpress-SARS-CoV-2/Flu/RSV cartridge): SARS-CoV-2, Influenza A, Influenza B, RSV

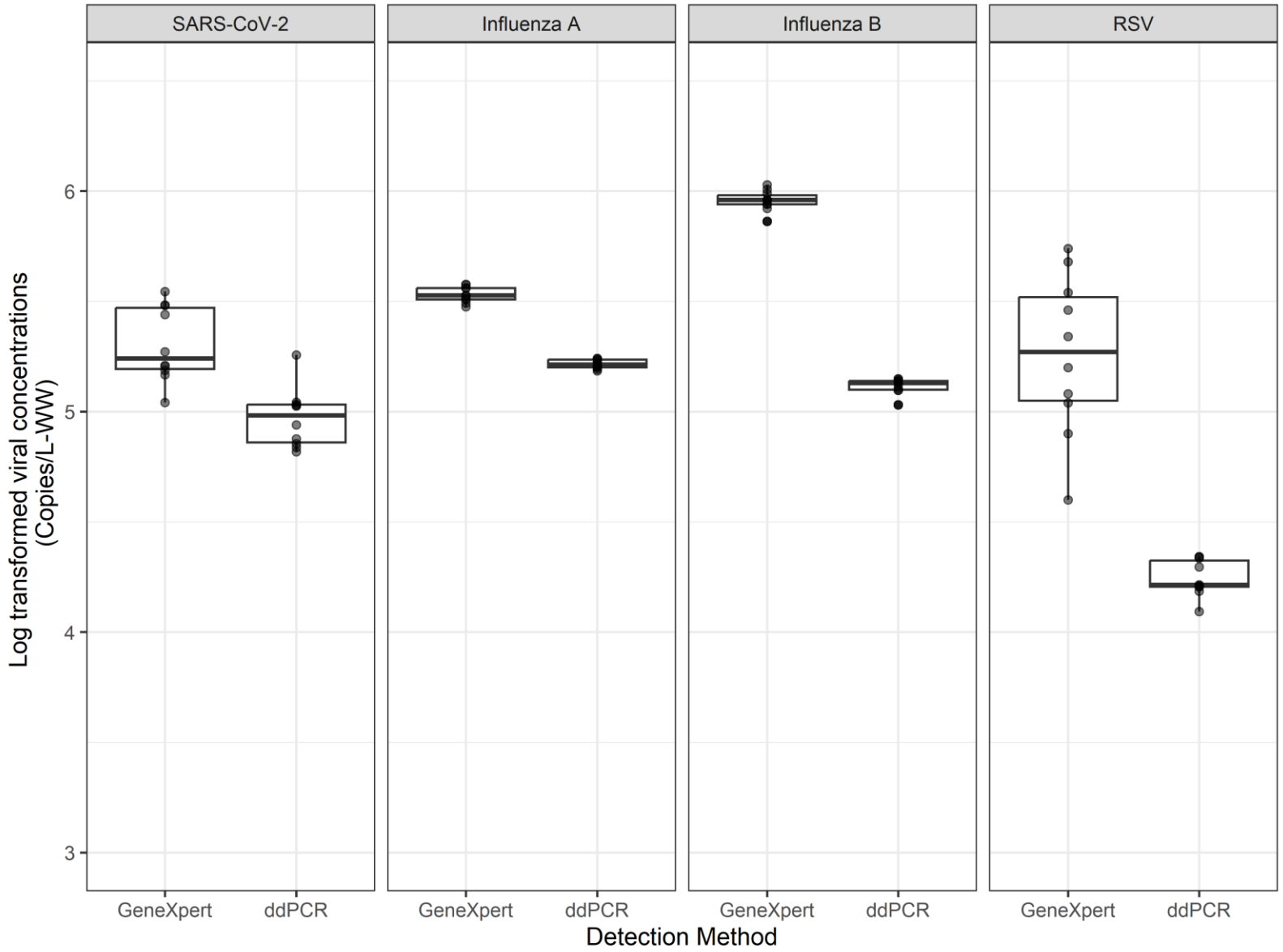
Filtration-ddPCR targets: SARS-CoV-2-N1, SARS-CoV-2-N2, Influenza A, Influenza B (only detected for reproducibility test), RSV

Standard curves were developed to convert GeneXpert Ct values to viral wastewater concentrations



- Well-fit linear relationships were observed between the Ct values and the amount of target virus spiked into the school wastewater samples for all targets.
- R^2 values were greater than 0.94.

Quantification results from the GeneXpert system were generally reproducible

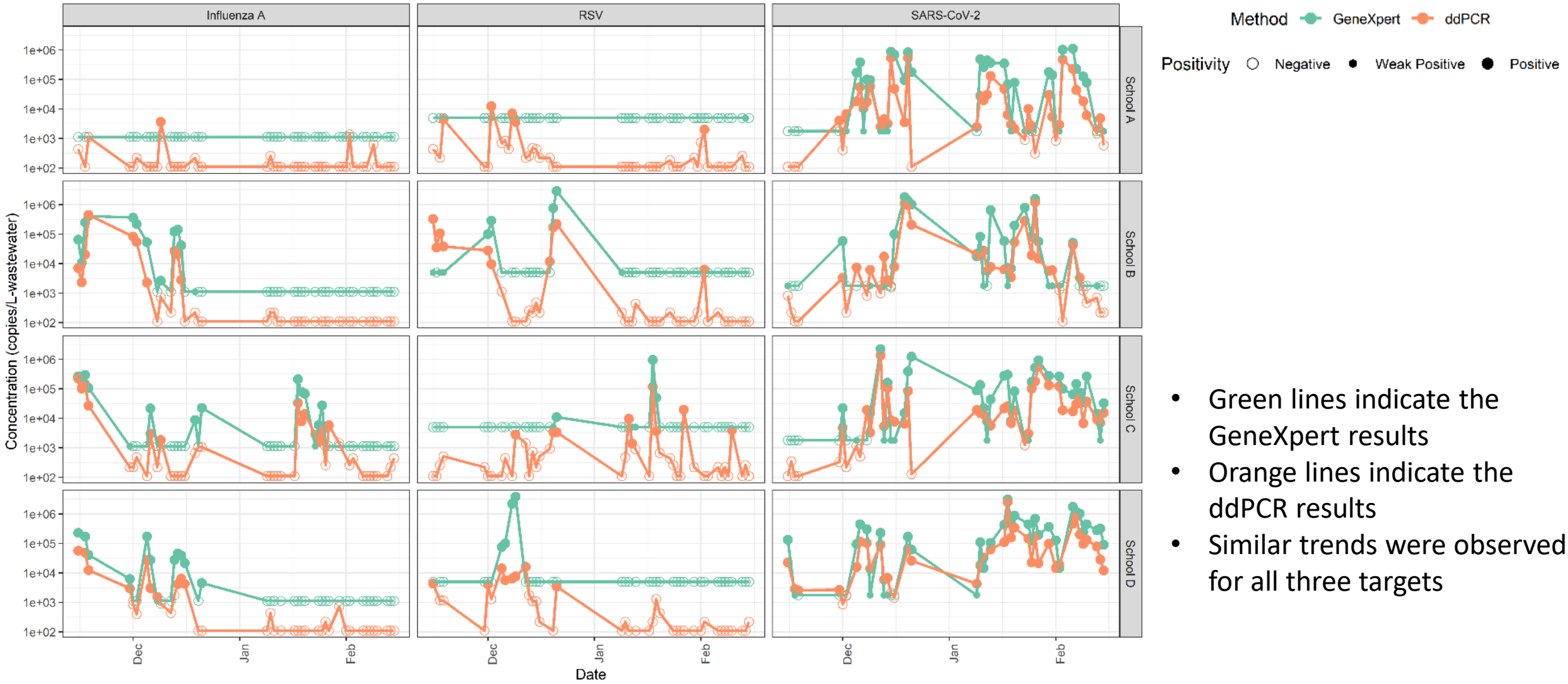


- Variance of the RSV concentration was significantly greater for GeneXpert than filtration-ddPCR results (p-value = 0.002).

3 month daily school sampling results:
GeneXpert and filtration-ddPCR results (positive vs. negative) were consistent

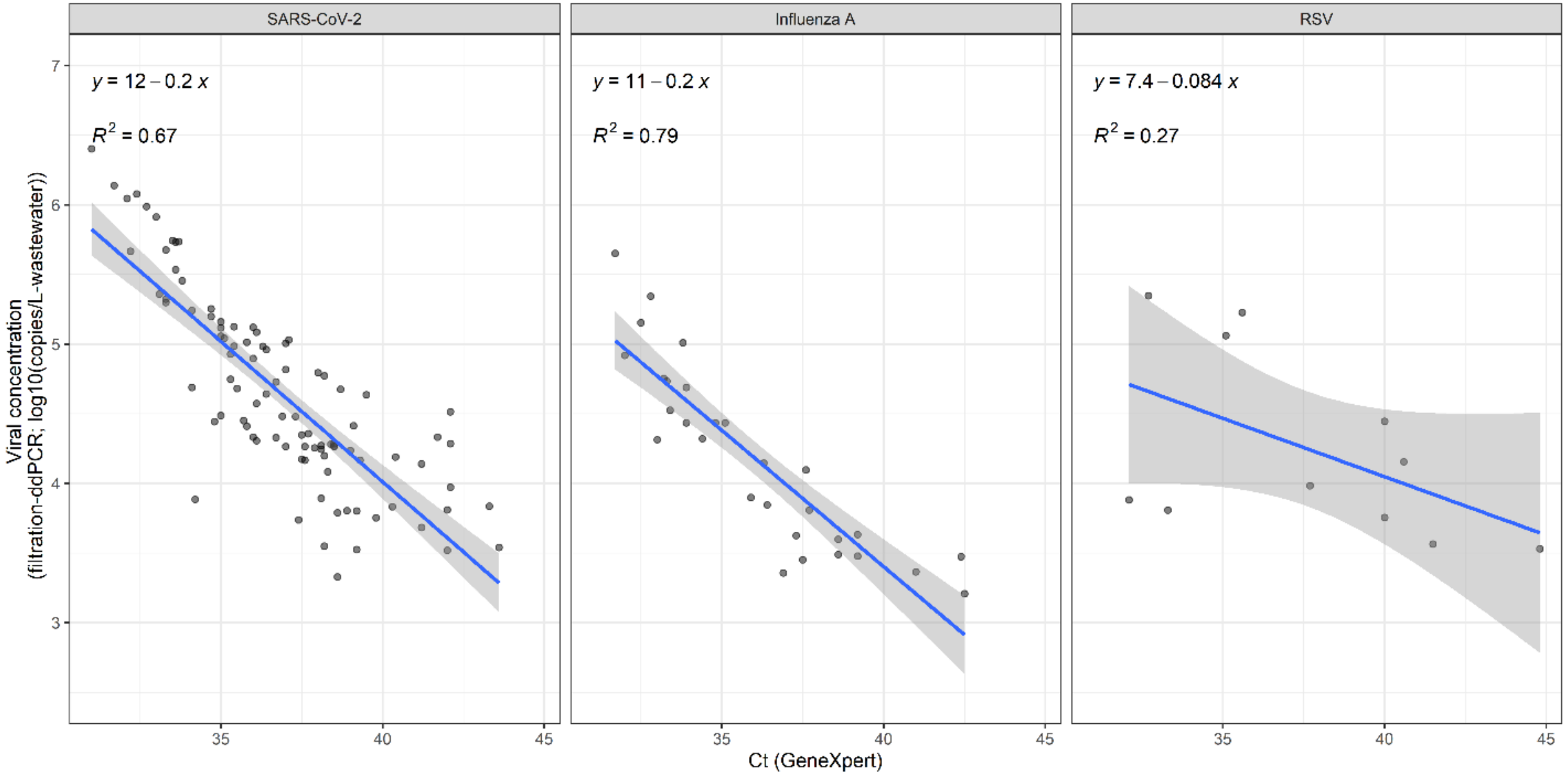
		GeneXpert system					
		SARS-CoV-2		Influenza-A		RSV	
	Positivity	Positive	Negative	Positive	Negative	Positive	Negative
Filtration-ddPCR workflow	Positive	119	11	31	3	19	13
	Negative	11	28	7	128	3	134
		87.0% consistent		94.1% consistent		90.5% consistent	

3 month daily school sampling results:
GeneXpert and filtration-ddPCR results showed similar quantitative trends



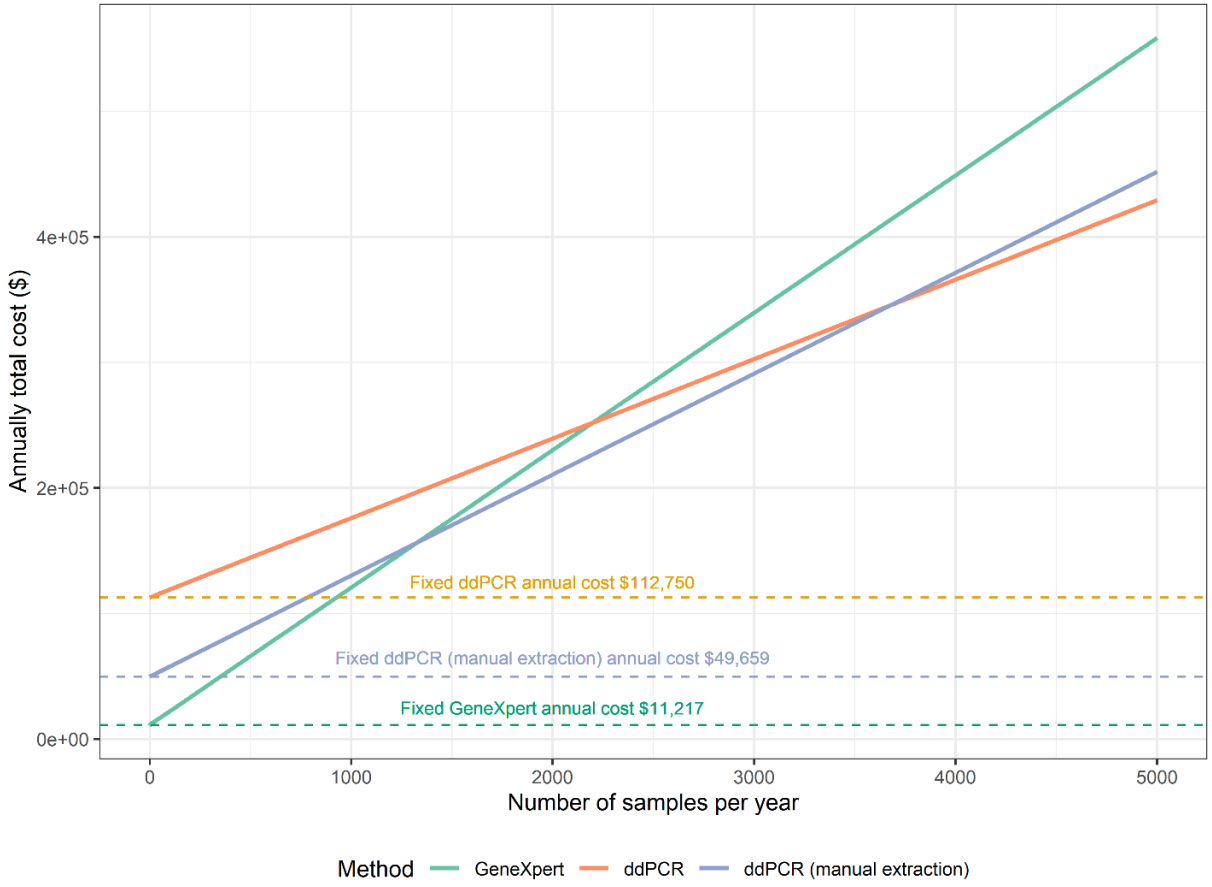
- Green lines indicate the GeneXpert results
- Orange lines indicate the ddPCR results
- Similar trends were observed for all three targets

Relationship between GeneXpert Ct value and filtration-ddPCR viral concentrations



GeneXpert is cost-effective for <42 samples/week

		GeneXpert system	Filtration-ddPCR workflow	Filtration- ddPCR workflow (manual extraction)
Capital cost ($)	Concentration	50,000.00	19,849.00	19,849.00
	Extraction and PCR preparation		290,449.69	5,165.50
	Quantification		192,794.25	192,794.25
	Total	**50,000.00**	**503,092.94**	**217,808.75**
Annual maintenance cost ($)		**7,050.00**	**70,826.25**	**31,507.88**
Consumable cost ($)	Concentration	100.76	6.02	6.02
	Extraction and PCR preparation		10.90	26.98
	Quantification		23.05	23.05
	Total	**100.76**	**39.97**	**56.05**
Labor cost ($)	Concentration	8.67	9.75	9.75
	Extraction and PCR preparation		10.83	11.92
	Quantification		2.71	2.71
	Total	**8.67**	**23.29**	**24.38**



- GeneXpert system requires less start-up costs but more processing costs than the filtration-ddPCR methods.
- GeneXpert is more cost-effective for testing lower quantities of samples.

Summary

- The GeneXpert system is effective for monitoring the dynamics of respiratory viruses in school wastewater with a limited testing volume, providing (1) qualitative results on positivity, and (2) quantitative viral concentrations.
- GeneXpert results were reproducible and comparably sensitive to filtration-ddPCR
- Significant positive relationships were observed between the quantitative results using the two methods
- GeneXpert is cost-effective for systems that analyze low numbers of samples (<42/week)

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