SF-RAD:

Development and Proof-of-Concept Implementation of the South Florida Miami RADx-rad SARS-CoV-2 Wastewater-Based Surveillance Infrastructure

Funded by
NIH RADx-rad Grant
1U01DA053941-01

UNIVERSITY OF MIAMI

Weill Cornell Medicine

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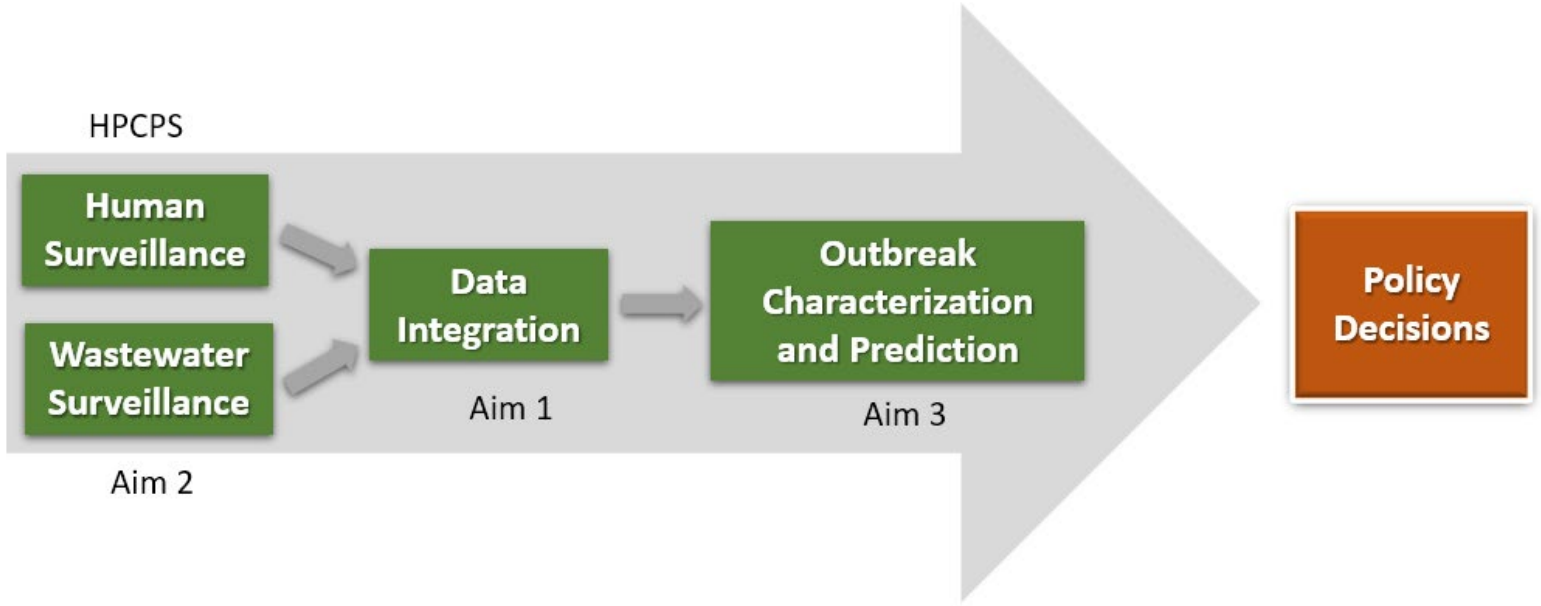
Aims

1. Data standardization and informatics infrastructure
2. Wastewater characterization
3. Integration with human health surveillance



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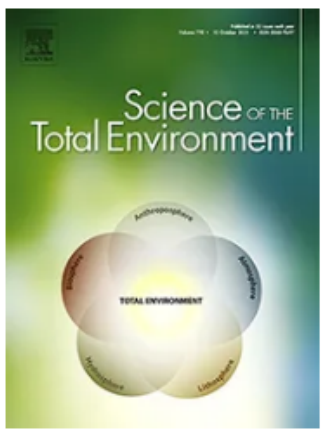
Publications

Presentations

Peer-Reviewed Journal Publications



Lessons learned from SARS-CoV-2 measurements in wastewater

Sharkey ME, Kumar N, Mantero AMA, Babler KM, Boone MM, Cardentey Y, Cortizas EM, Grills GS, Herrin J, Kemper JM, Kenney R, Kobetz E, Laine J, Lamar WE, Mader CC, Mason CE, Quintero AZ, Reding BD, Roca MA, Ryon K, Solle NS, Schürer SC, Shukla B, Stevenson M, Stone T, Tallon JJ Jr, Venkatapuram SS, Vidovic D, Williams SL, Young B, Solo-Gabriele HM. **Lessons learned from SARS-CoV-2 measurements in wastewater.** *Sci Total Environ.* 2021 Dec 1;798:149177.doi.org/10.1016/j.scitotenv.2021.149177 PMID: 34375259; PMC8294117.

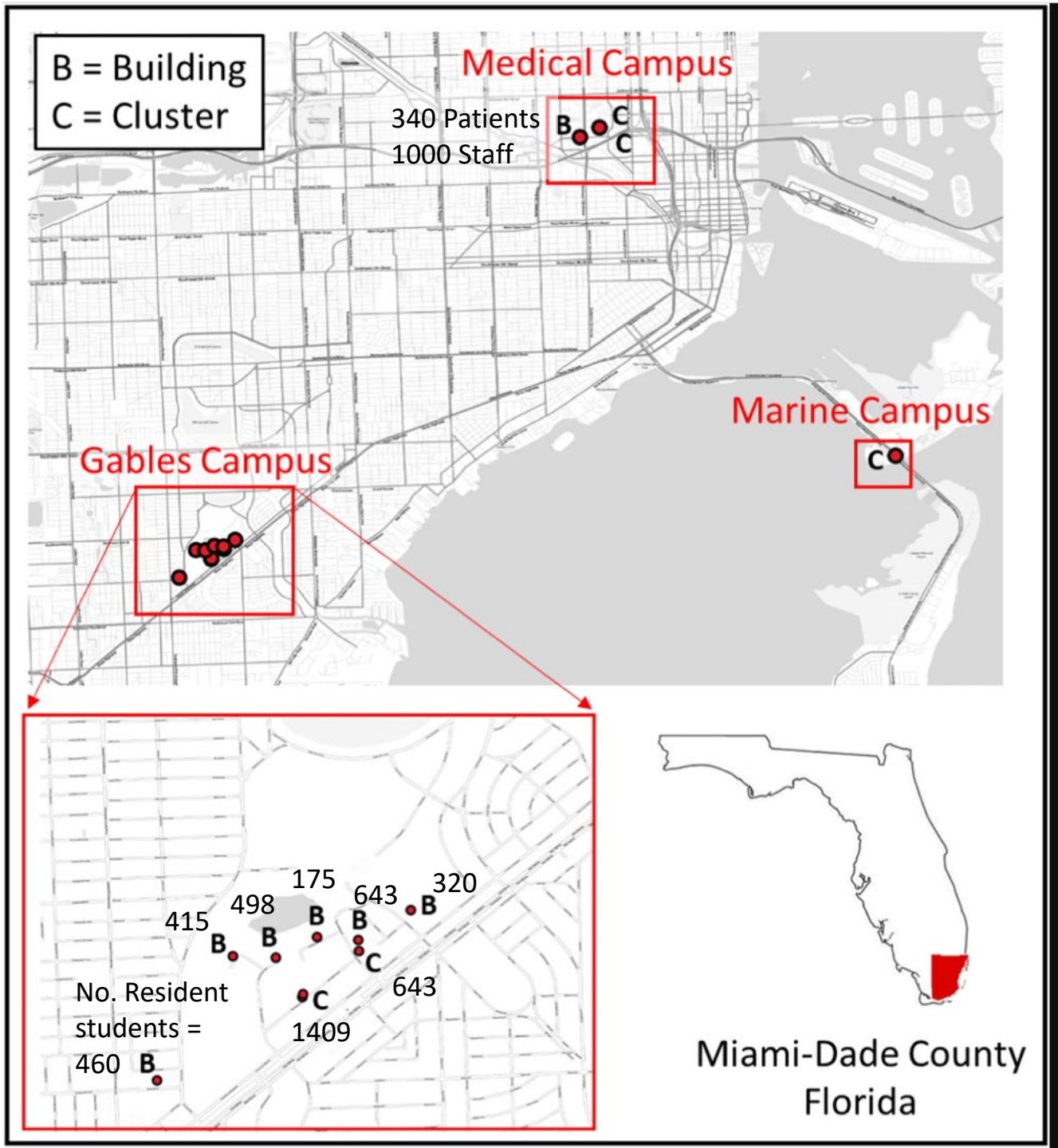
(Download .pdf)

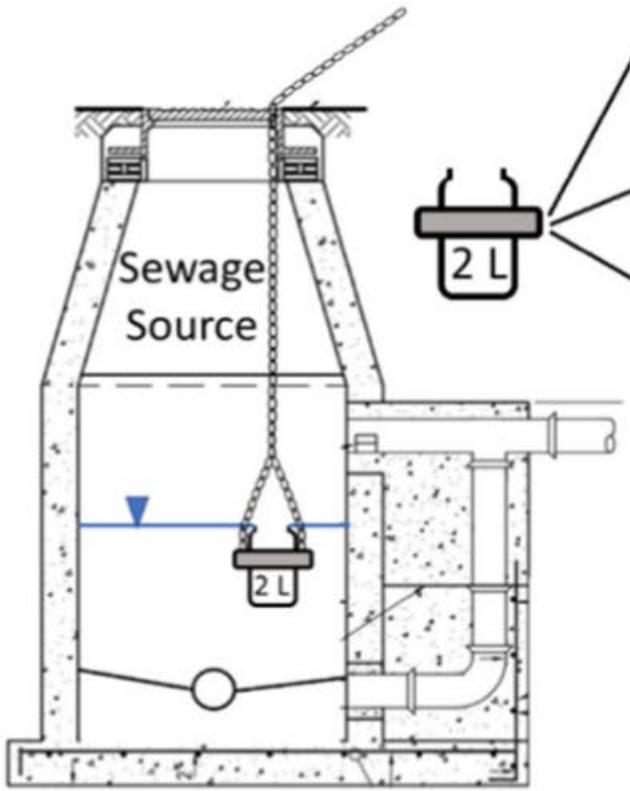
Human Health

- UMiami student/faculty/staff = 34,000 (2000 residents)
- Testing, Tracing, Tracking
- 55,186 tests, students Fall ‘20
- mid-nasal swab and PCR

Wastewater Sampling

- Weekly grab in morning





In field 0.5L T, pH, Salinity/SPC, DO, turbidity

No significant correlations with SARS-CoV-2

Electronegative Filtration

Spike with OC43
Acidify

SM Laboratory → 0.5L → 0.25L → RT-qPCR, N1 & N2 at SM Lab (Mouse Lung Cell Extraction Control)

0.25L → V2G-qPCR, N3 at UM CFAR Lab (HIV-1 Extraction Control)

UM SCCC Lab → 1 L → 0.5L → RT-qPCR, N1 at WCM Lab (Twist Synthetic SARS-CoV-2 RNA Control)

0.5L → Stored for Sequencing

Spike with OC43
Add $MgCl_2$
Acidify

Filters

Filters in DNA/RNA Shield

Centricon

0.5L → Centrifuge → RT-qPCR, N1 at WCM Lab (Twist Synthetic SARS-CoV-2 RNA Control)

Concentrate in DNA/RNA Shield

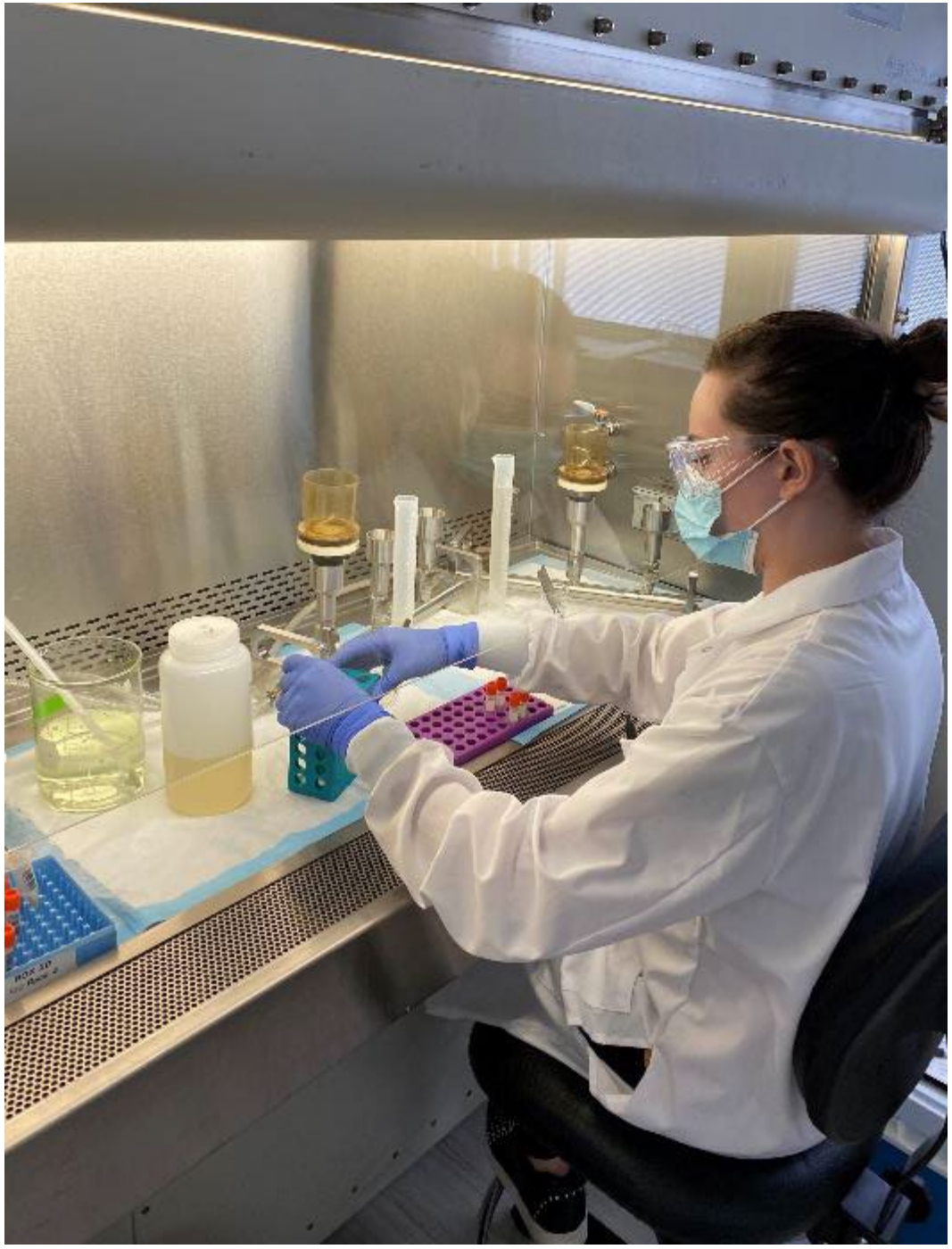
Not different from EN filtration

Ultracentrifugation

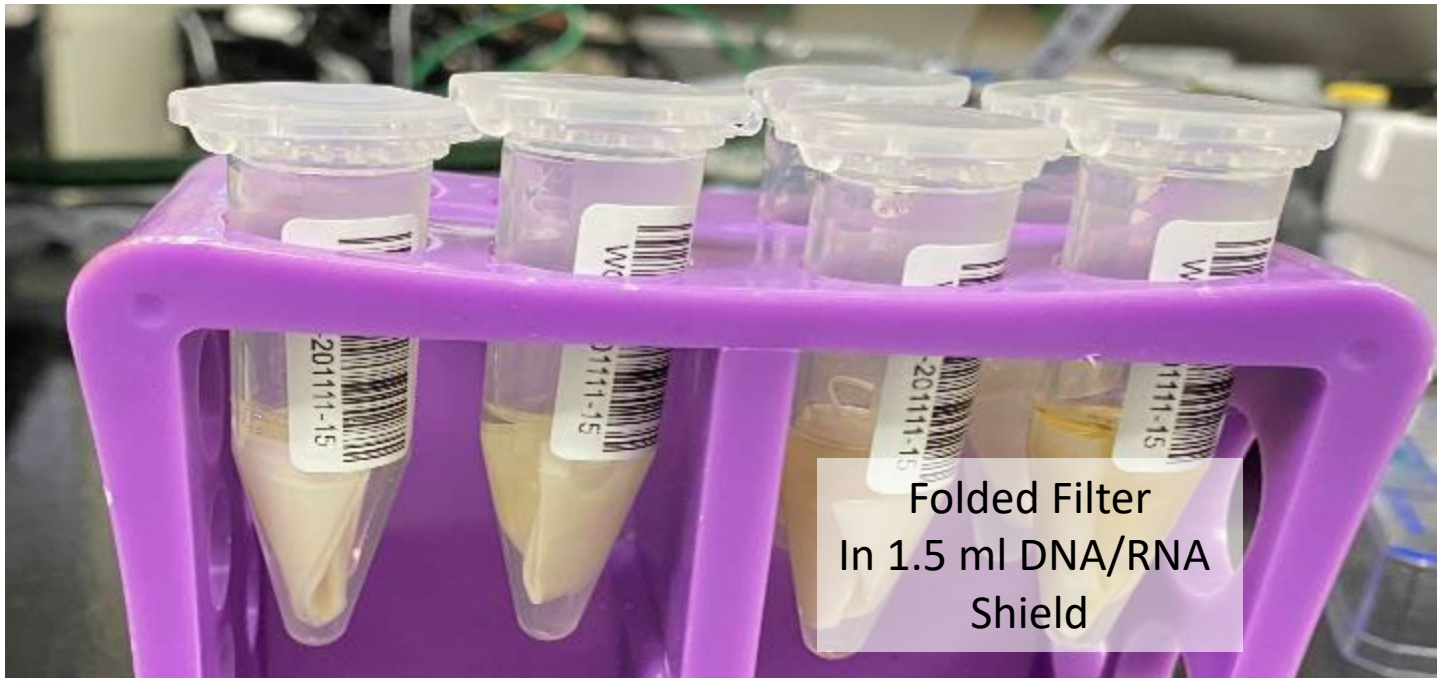
10 mL *Fecal Indicator Bacteria*

Add reductant to reduce Cl_2 residual


1. Add OC43 spike, recovery control
2. Add $MgCl_2$ (50 mM)
3. Acidify to pH 3.5-4.5
pH meter
Continuous Stirring
1
2
U
S
H
E
L
P




Electronegative
Filter


Folded Filter
In 1.5 ml DNA/RNA
Shield

UMiami Center For AIDS Research (Dr. Mark Sharkey, V2G-qPCR)

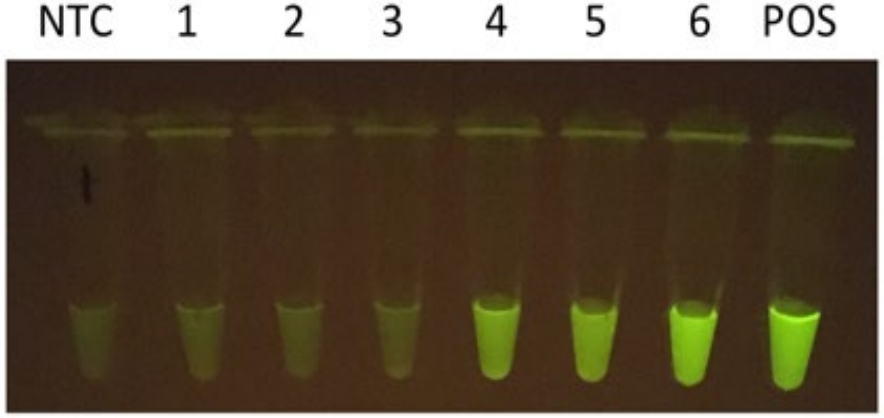
Volcano Second Generation (V2G):

a) Novel polymerase, uses both RNA and DNA as templates

   Avoids cDNA synthesis step

b) Sequence-specific fluorescent hydrolysis probes

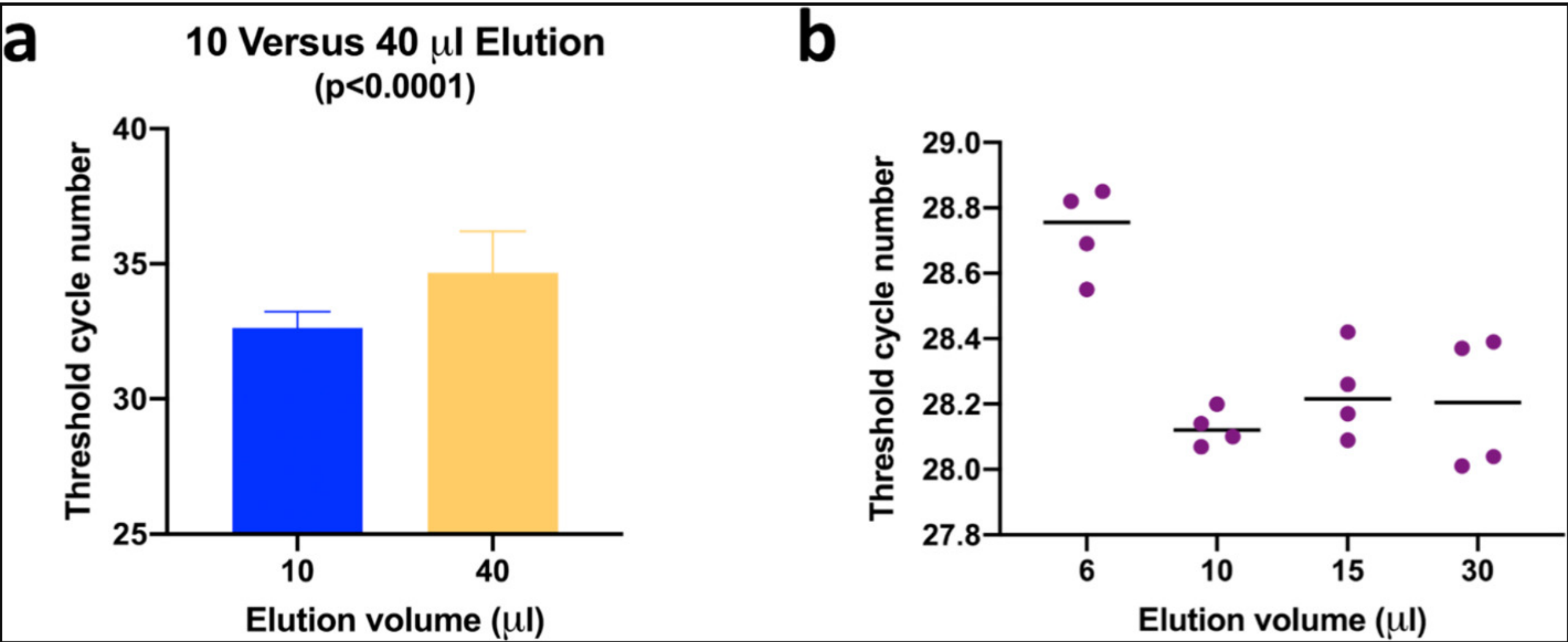
c) 2.5 hours turn-around time

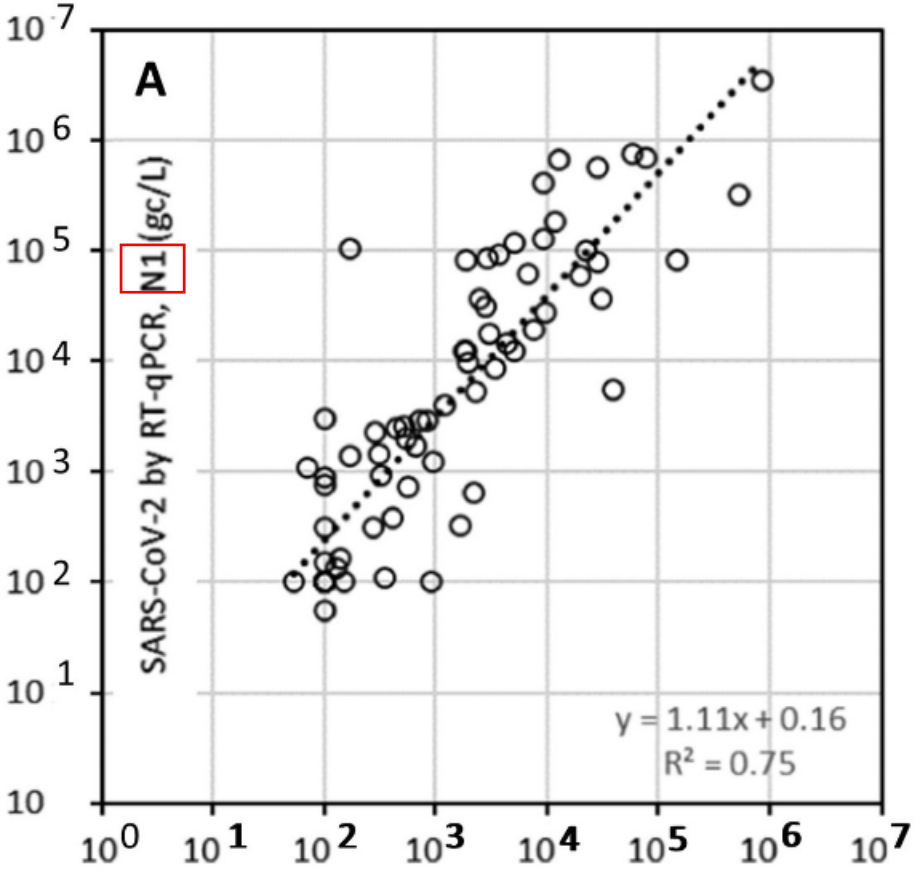


NTC, no template control POS, positive control

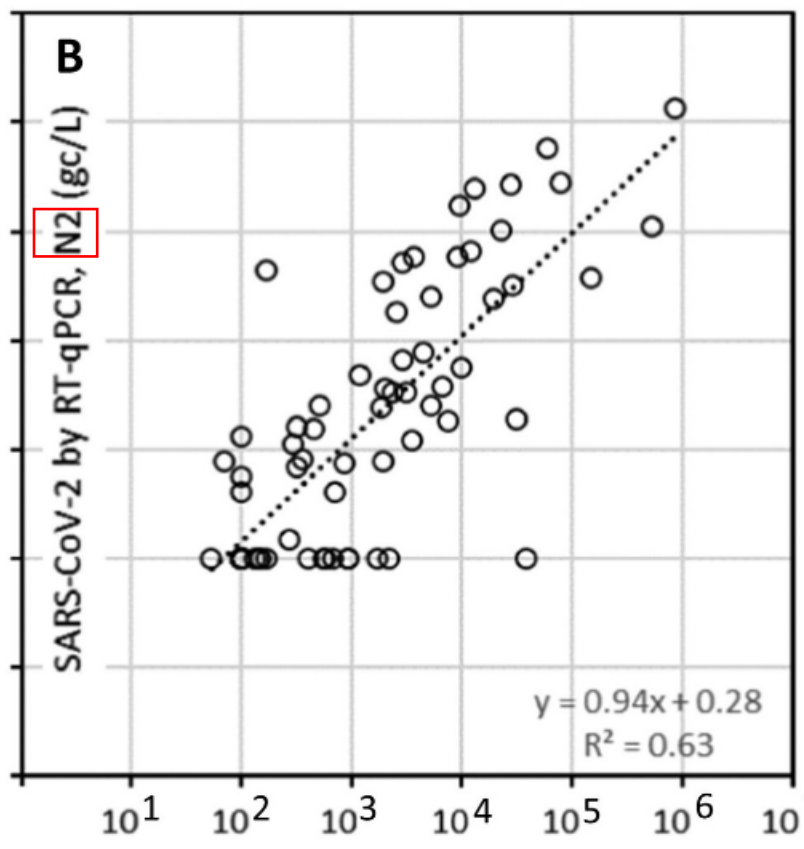
Direct PCR detection of SARS-CoV-2 RNA. Detection of viral RNA using previously tested negative (1-3) and positive (4-6) saliva samples.

Faster and less expensive the RT-qPCR ★


a
10 Versus 40 µl Elution
(p<0.0001)
Threshold cycle number
40
35
30
25
10
40
Elution volume (µl)
b
Threshold cycle number
29.0
28.8
28.6
28.4
28.2
28.0
27.8
6
10
15
30
Elution volume (µl)



SARS-CoV-2 by V2G-qPCR (gc/L)



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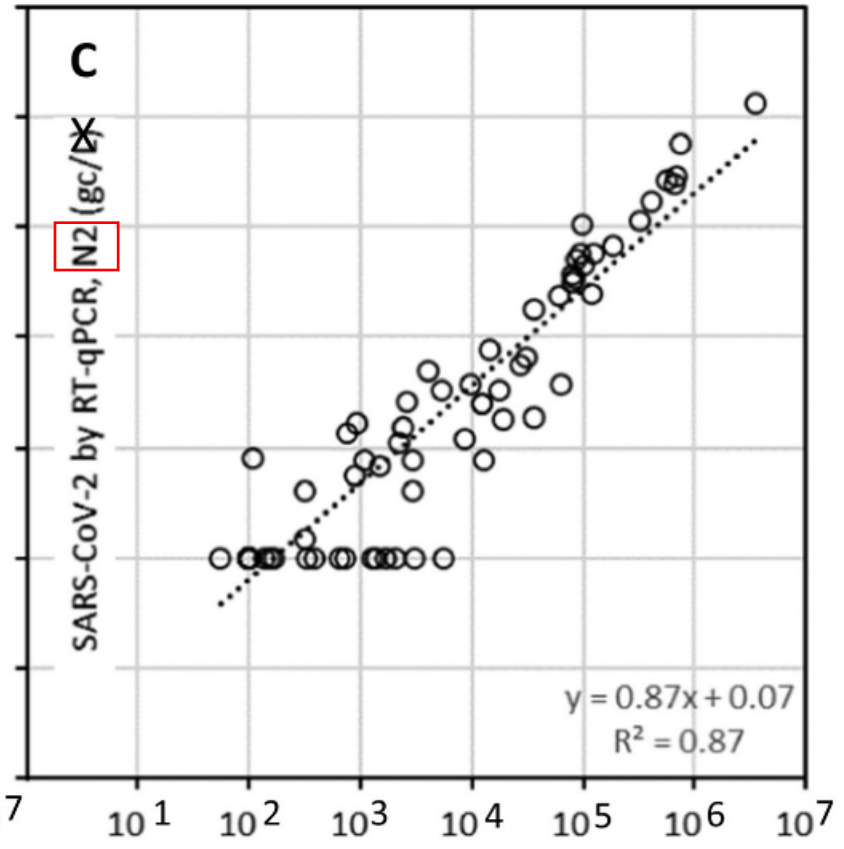


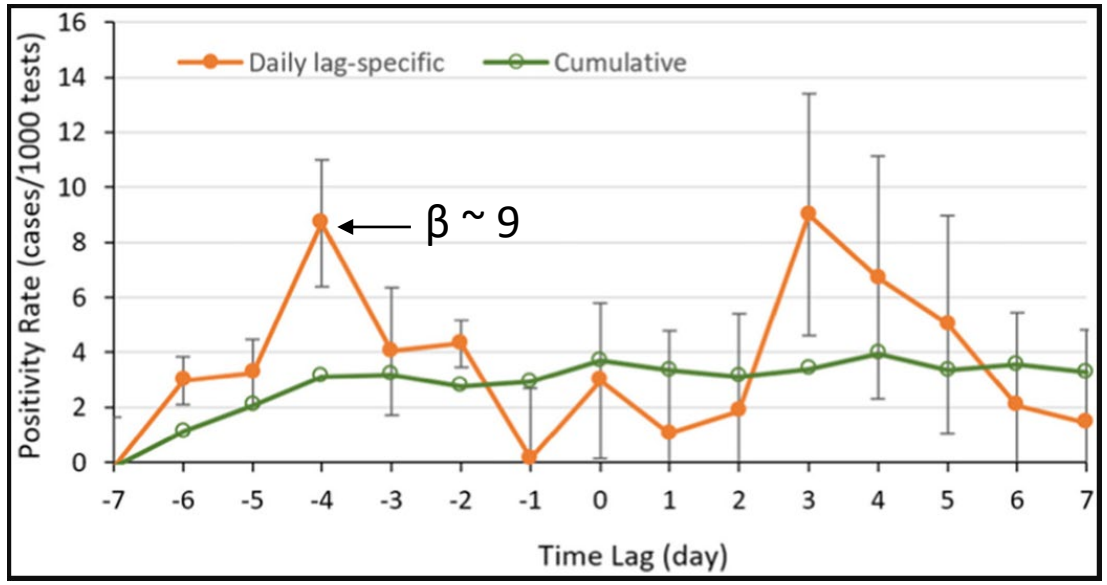
SARS-CoV-2 by RT-qPCR N1 (gc/L)

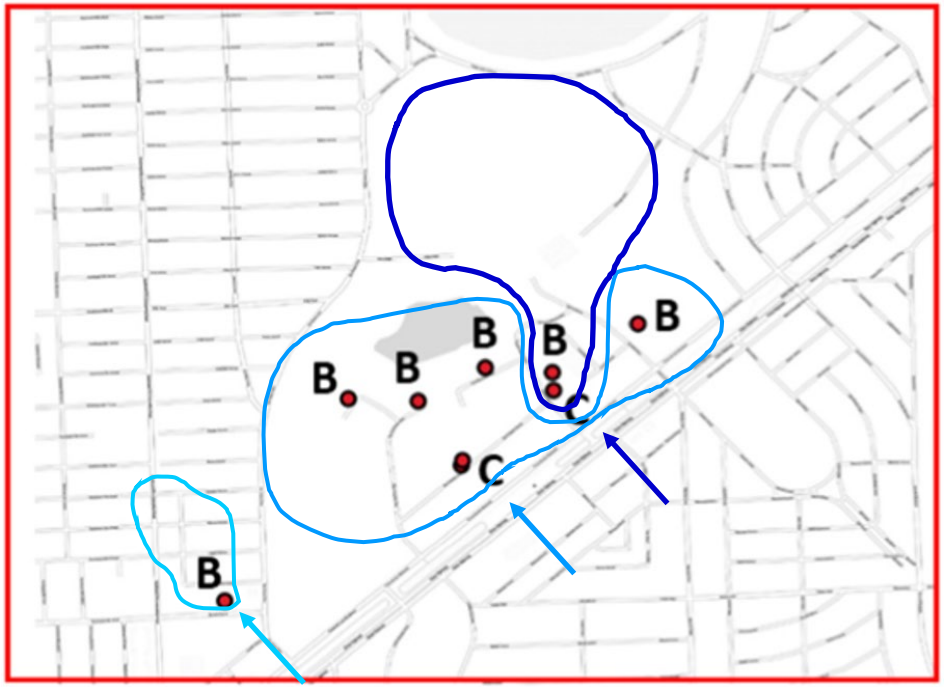
Relationships with Human Health (Dr. Naresh Kumar)

$$\text{Positivity (\%)} = \beta \frac{\ln(C)}{10}$$

C = SARS-CoV-2 RNA in wastewater (gc/L)
e.g., 100 gc/L → 4% positivity

- Evaluated lag-specific COVID-19 positivity (#positive/#tested)
- -7 to 15 days after diagnosis
- Cumulative. For example, -4 (cumulative from -7 to -4)
- Wastewater data log transformed





Lessons Learned

- Buildings more variable than clusters
- Water quality of sewage influenced by water source (know your water source)
  - Neutralize for chlorine residual
  - Lime softened groundwater subject to pH ranges
- Physical-chemical parameters lack significant correlations
  - T, pH, Spec Cond, Turbidity, DO
- Consider normalizing data by a measure of fecal inputs
- Results possible within 12 hours

Summary

A new innovative detection method, V2G-qPCR, was evaluated

100 gc/L of SARS-CoV-2 in wastewater associate with a 4% positivity rate

SARS-CoV-2 in wastewater was a 4-day lead indicator

More frequent sampling is recommended for model development

Publications

Lessons learned from SARS-CoV-2 measurements in wastewater

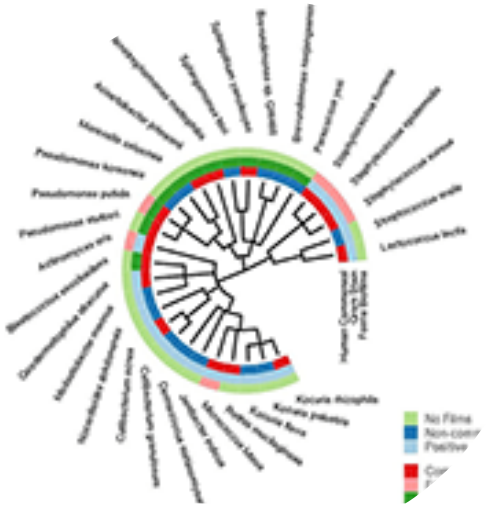
Sharkey ME, Kumar N, Mantero AMA, Babler KM, Boone MM, Cardentey Y, Cortizas EM, Grills GS, Herrin J, Kemper JM, Kenney R, Kobetz E, Laine J, Lamar WE, Mader CC, Mason CE, Quintero AZ, Reding BD, Roca MA, Ryon K, Solle NS, Schürer SC, Shukla B, Stevenson M, Stone T, Tallon JJ Jr, Venkatapuram SS, Vidovic D, Williams SL, Young B, Solo-Gabriele HM. **Lessons learned from SARS-CoV-2 measurements in wastewater**. *Sci Total Environ.* 2021 Dec 1;798:149177.doi.org/10.1016/j.scitotenv.2021.149177 PMID: 34375259; PMC8294117.

A rapid, isothermal, and point-of-care system for COVID-19 diagnostics

Christopher Mozsary, Duncan McCloskey, Kristina M. Babler, Juan Boza, Daniel Butler, Benjamin Currall, Sion Williams, Anne Wiley, George S. Grills, Mark E. Sharkey, Prem Premsrirut, Helena Solo-Gabriele, Yoslayma Cardentey, David Erickson, Christopher E. Mason. **A Rapid, Isothermal, and Point-of-Care System for COVID-19 Diagnostics**. *J Biomol Tech.* 2021 Sep;32(3):221-227. doi: 10.7171/jbt.21-3203-019. PMID: 35136383, PMCID: PMC8802758.

A global metagenomic map of urban microbiomes and antimicrobial resistance

David Danko, Daniela Bezdan, Evan E. Afshin, Sibo Zhu, Christopher E. Mason, et al. **A global metagenomic map of urban microbiomes and antimicrobial resistance,** *Cell* Vol. 184, Issue 13, pp. 3376-3393, June 24, 2021. Published by Elsevier Inc. https://doi.org/10.1016/j.cell.2021.05.002



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Thank you
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