South Florida - RAD

Wastewater-Based Monitoring of COVID-19

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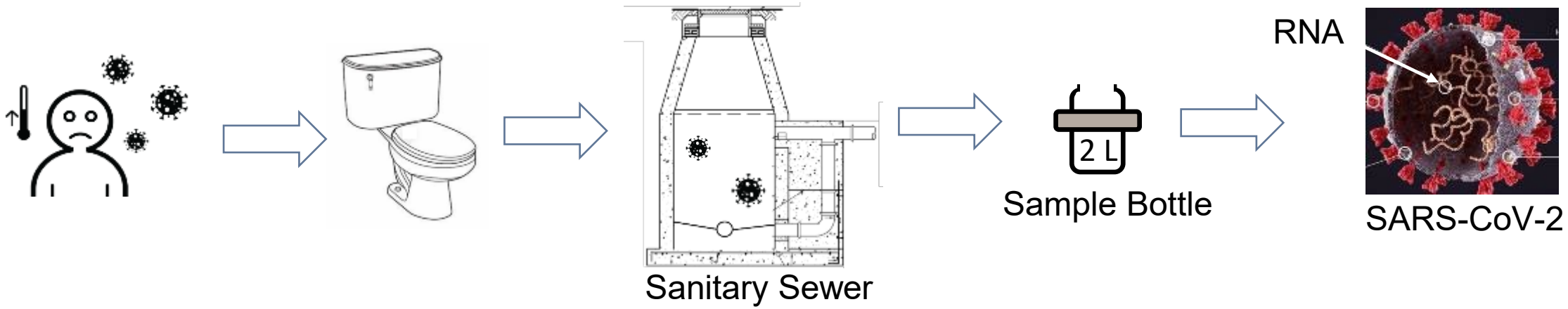
(PIs: Mason, Schürer, Solo-Gabriele)

covidsfrad.org

Motivation & Objectives

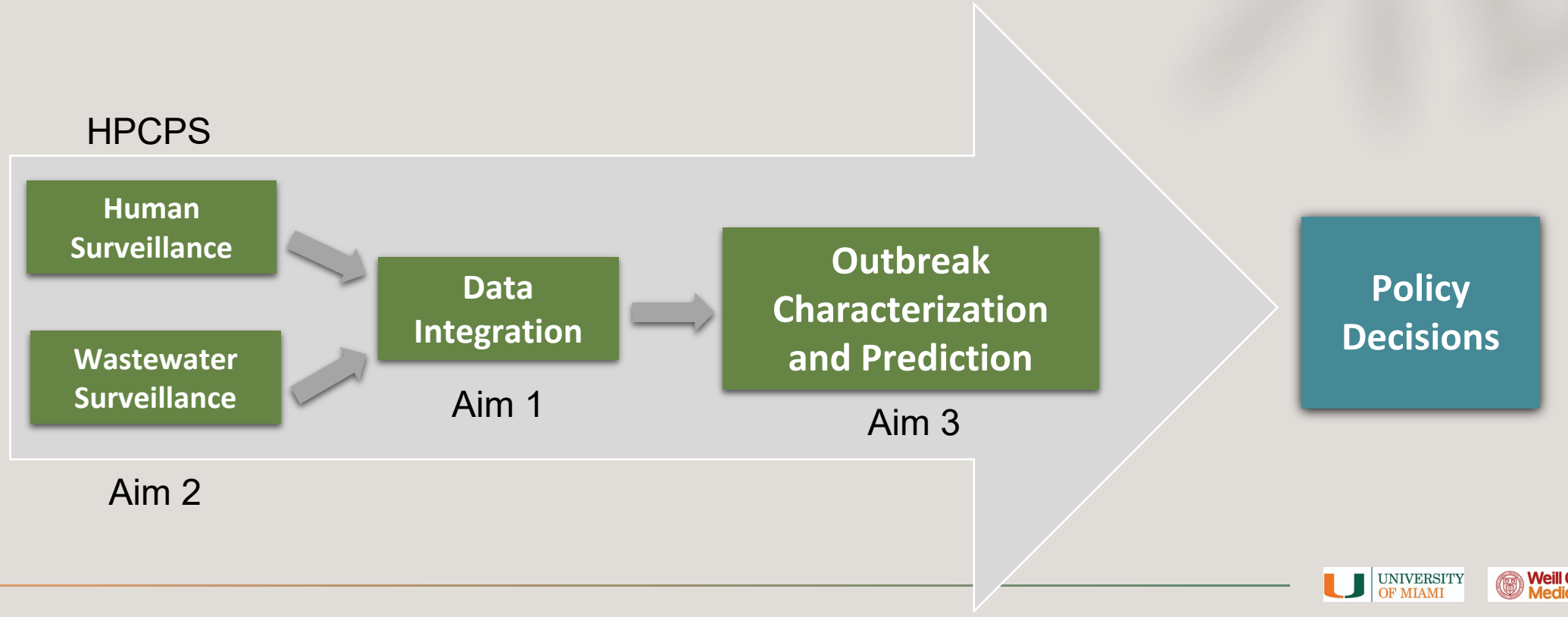
Infected humans excrete COVID-19 virus in feces and urine



Ultimate objective: Relate wastewater measurements to predict COVID-19 cases.

Aims of the Study

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



Human Surveillance

Student Residents

Fall'20/Spring'21
- Students tested weekly
- Nasal swab, qPCR & breath test
- COVID results and total tests by building/dorm room

Summer/Fall'21
- Unvaccinated students tested weekly
- All students tested when wastewater exceeds

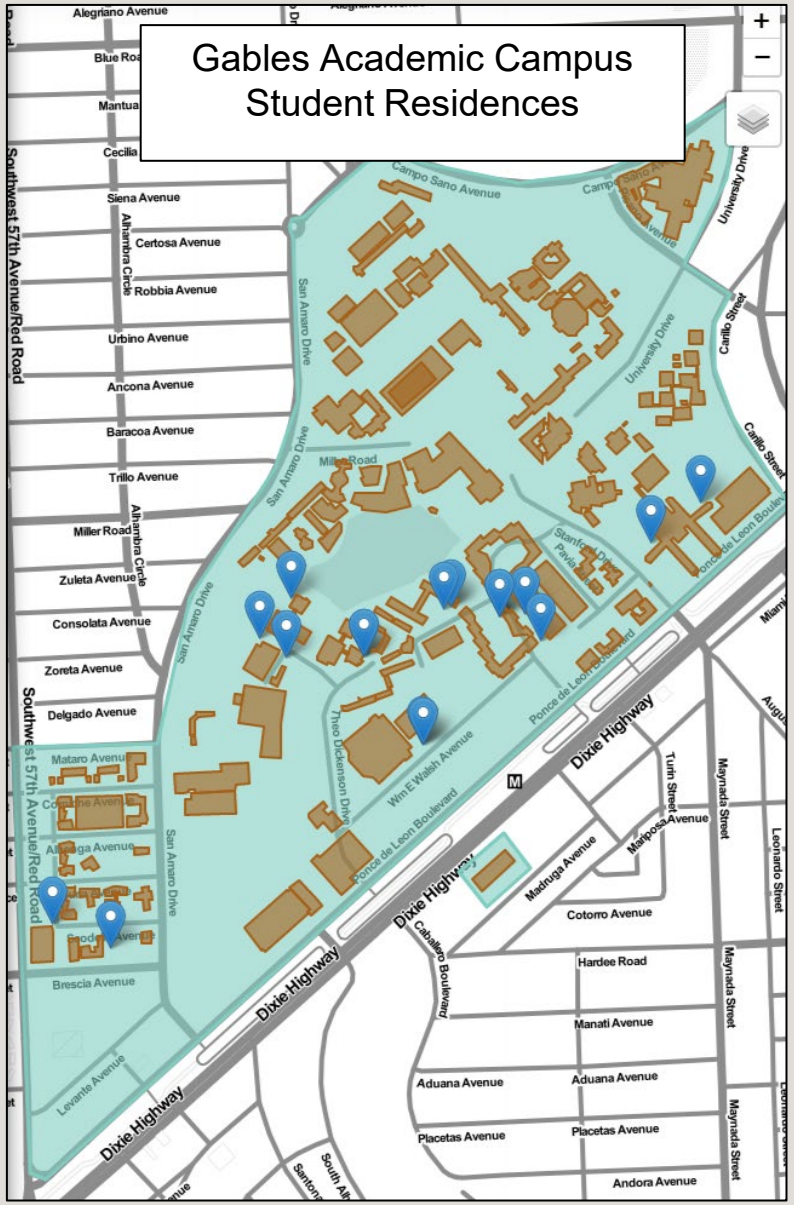
University Hospital

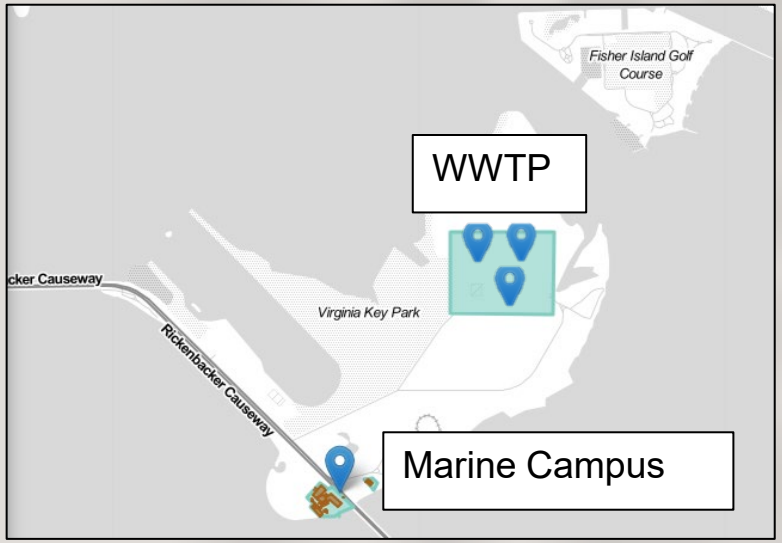
- Treat known COVID patients
- Electronic medical records pulled regularly

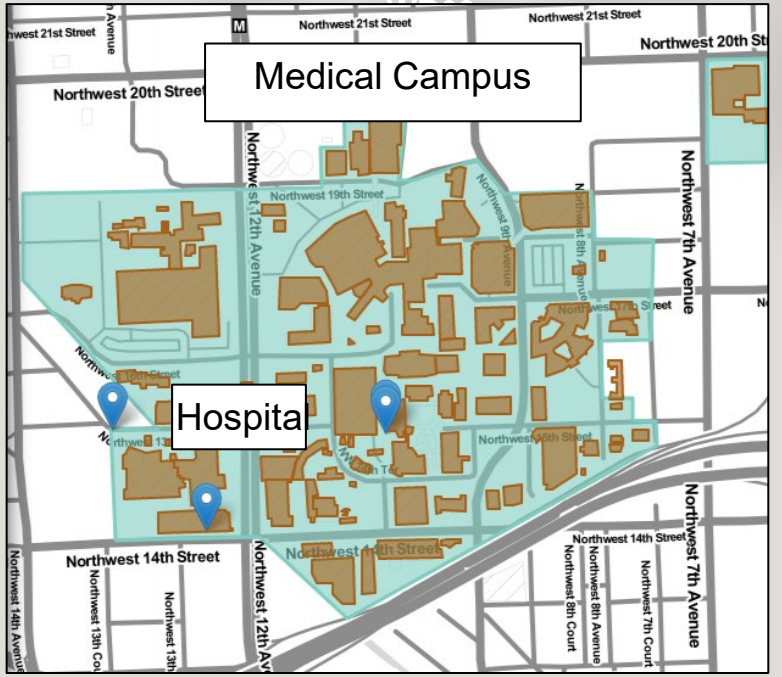
Miami-Dade County Residents (FDOH)

- Positives by zip code
- Number of tests by zip code

Sample Collection Plans







Sample Frequency: weekly, 2x per week, daily, hourly

Wastewater-Based Surveillance Experimental Overview

- Wastewater Sampling (How/Where?)
- Watershed Scales (Building, Cluster, Community)
- Overall Wastewater Processing Workflow
- Molecular Detection of SARS-CoV-2
- Weekly Samples: Evaluate predictive ability of wastewater
  - Can normalization of results improve predictive ability?
- Daily Samples: Evaluate the most effective time frequency of sample collection
- Hourly Samples: Evaluate SARS-CoV-2 degradation relative to hour-to-hour variability. Are composite samples better than grab samples?

Wastewater Sampling

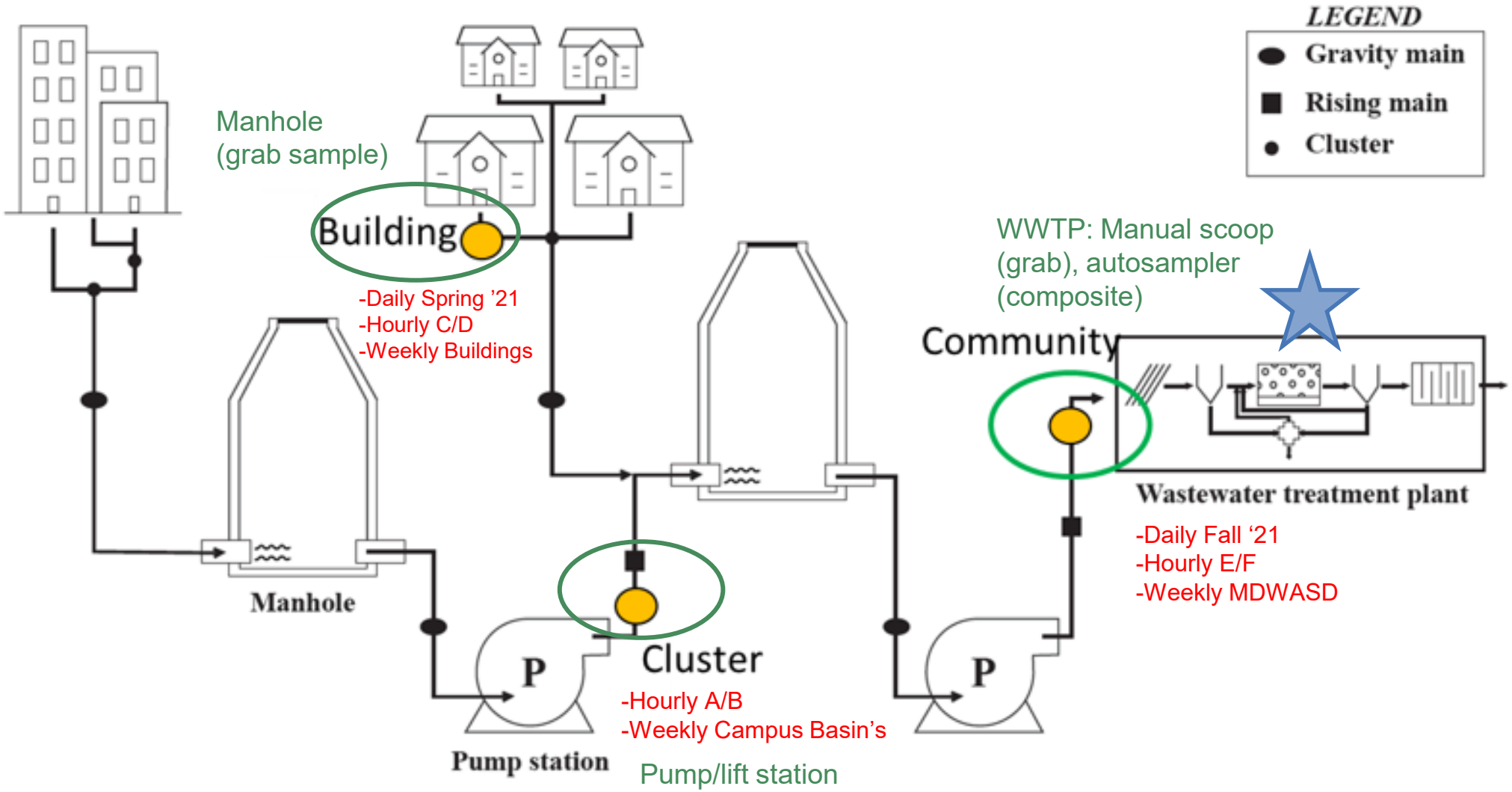
Where do we sample wastewater from?

- Manholes (building scale)
- Pump/Lift Stations (cluster scale)
- Wastewater Treatment Plant (community scale)

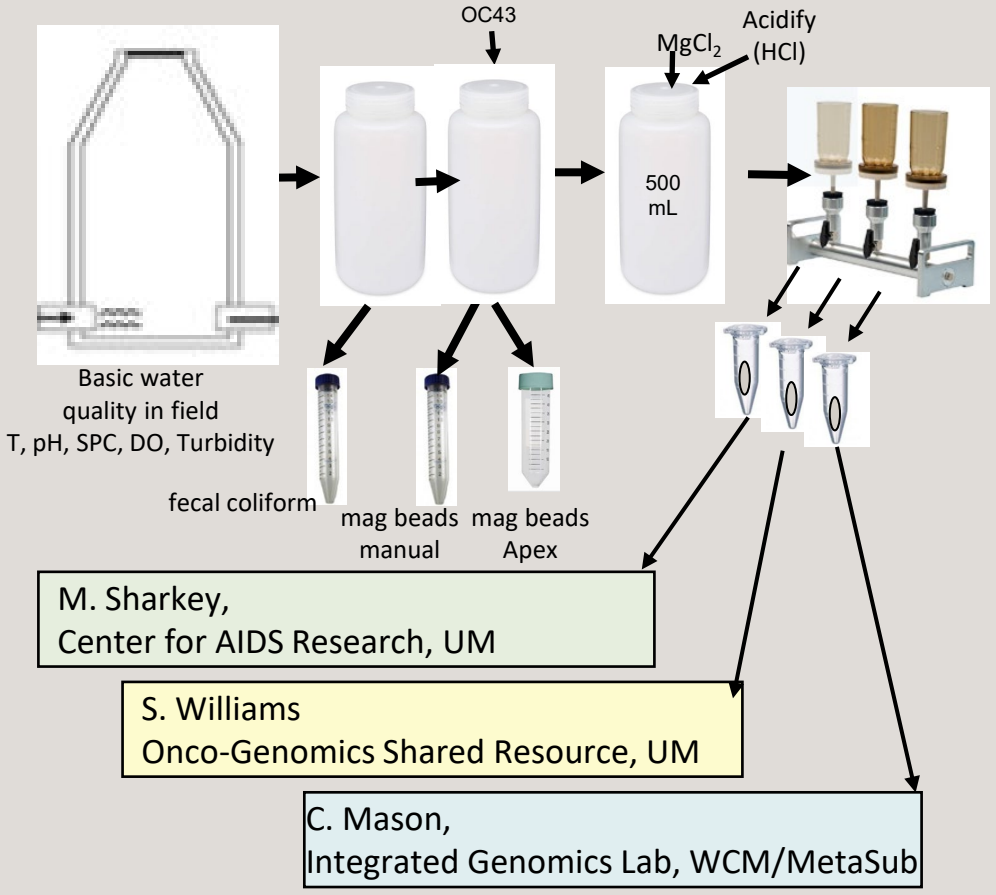
How do we collect the wastewater?

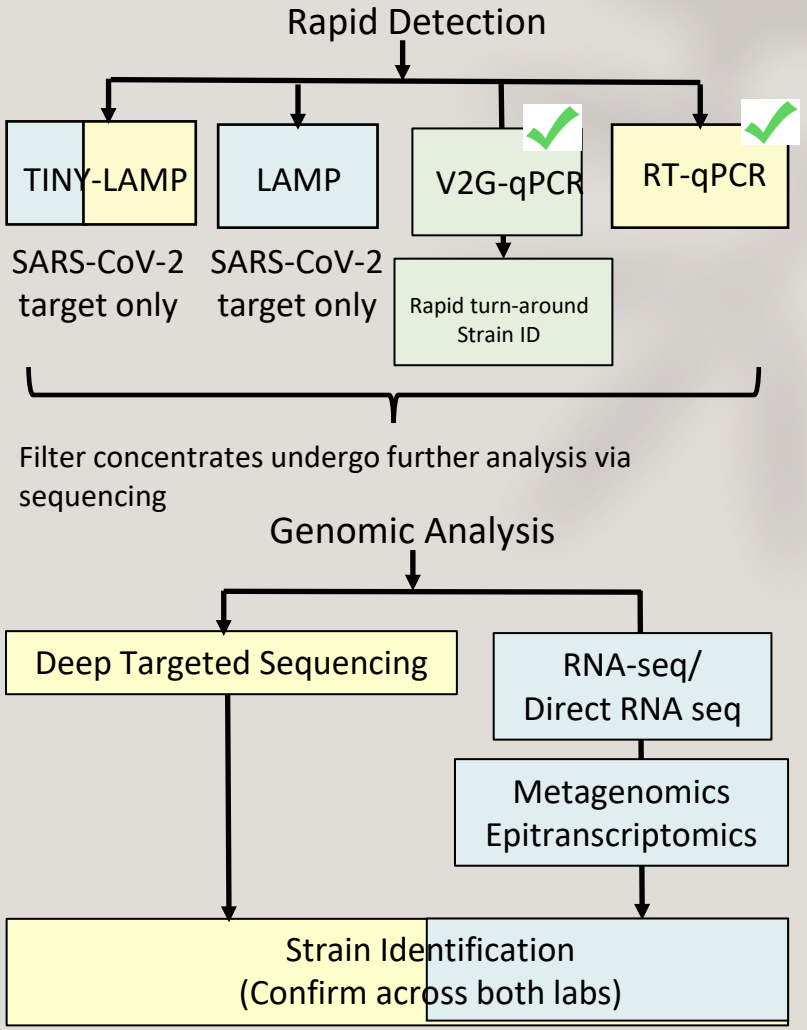
- Chain and bottle (grab)
- Manual or battery powered pump (grab)
- Automatic sampler (composite)
- Wastewater treatment plant (composite; grab)

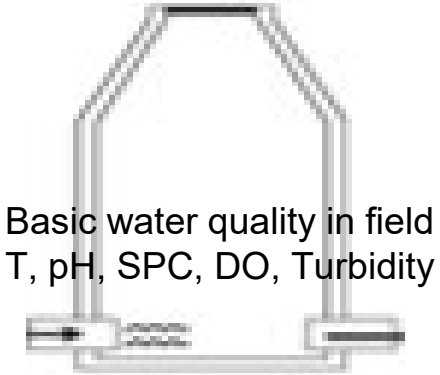
Watershed Scales Visualized



University of Miami & Weill Cornell Medicine Collaborative Sample Analysis







Basic water quality in field
T, pH, SPC, DO, Turbidity

Wastewater Collection

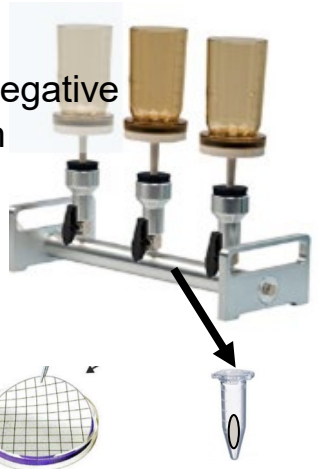
Viral Spike's

$MgCl_2$

500 mL

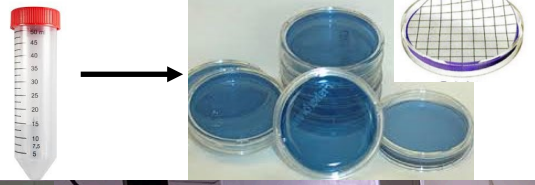
Acidify (HCl)

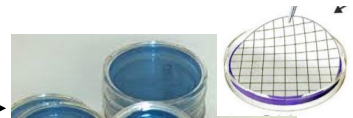
Electronegative Filtration



Bacterial Analysis

1:100 dilution
W.W. + PBS





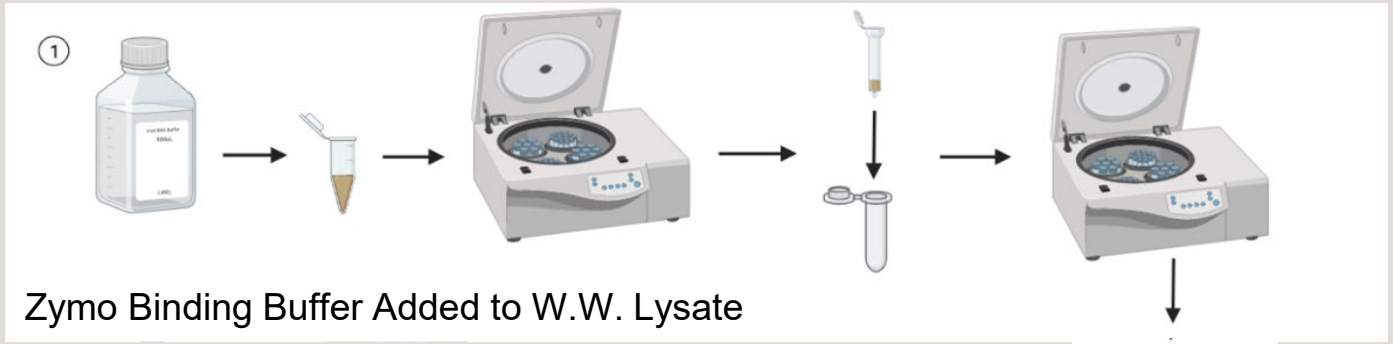
Overall Wastewater Sample Processing

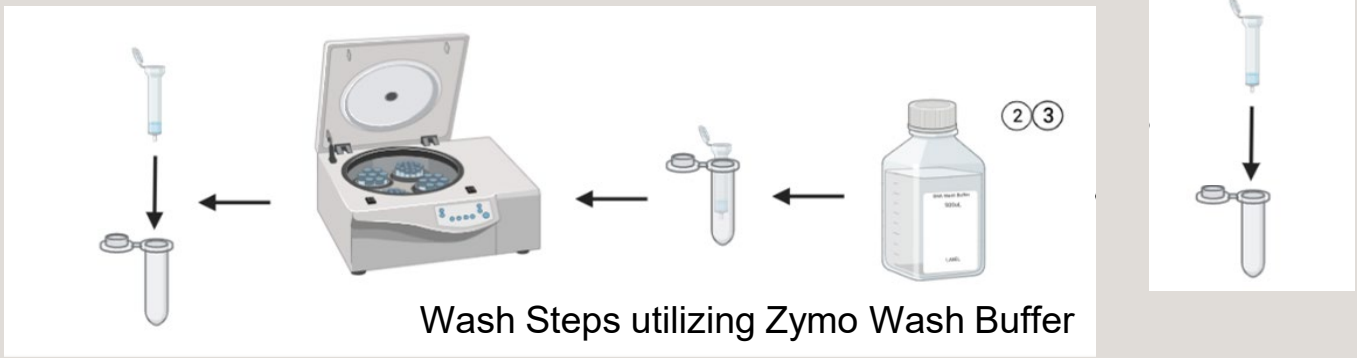
Pall Corporation

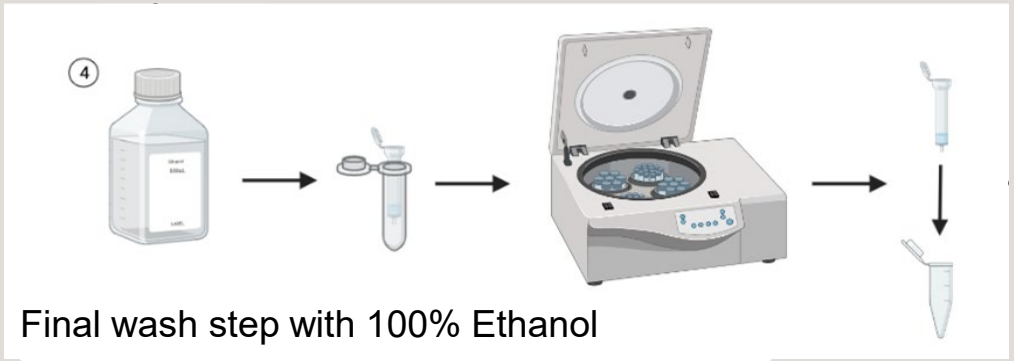
Molecular Analysis of Processed Wastewater Samples

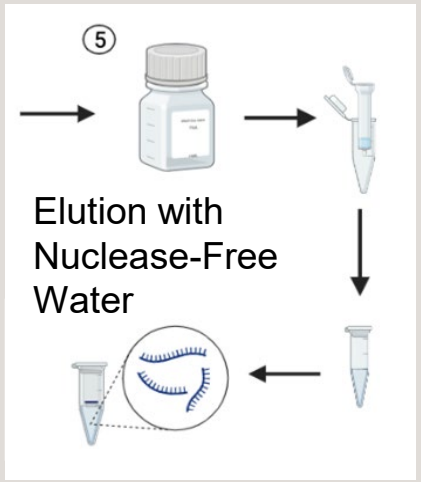
SARS-CoV-2 Detection via V2G-qPCR

Nucleic Acid Extraction & Purification from EN Filters









RNA Extraction/Purification

- Zymo Research QuickRNA Viral Kit
- Silica spin column vs. chemical-based
- Wastewater EN filter lysate samples

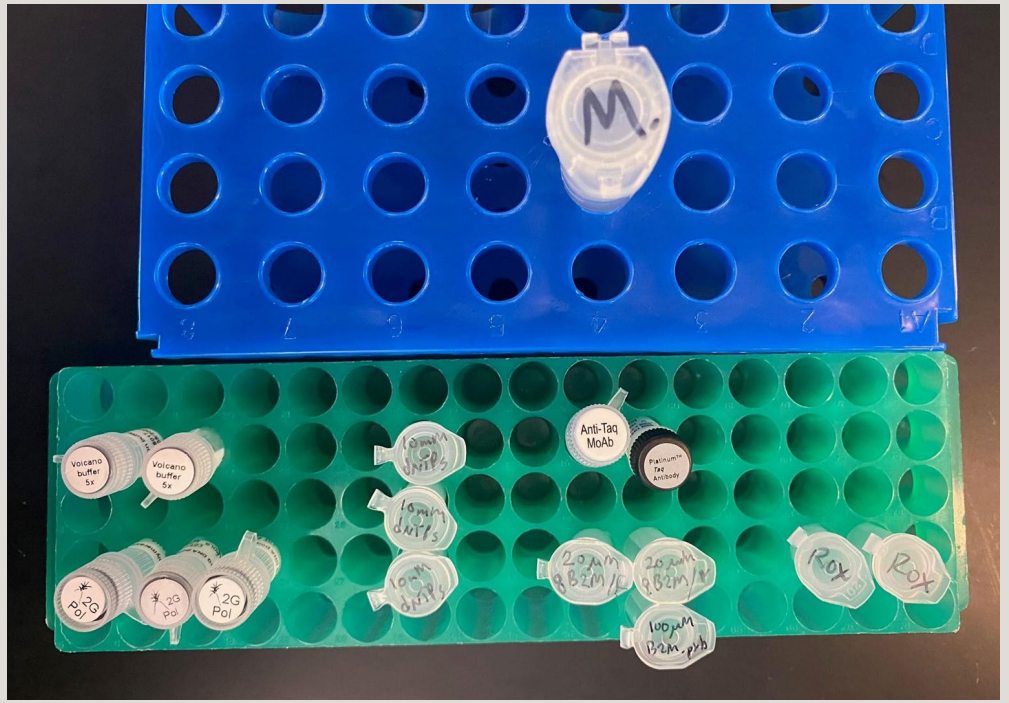
Main Steps of RNA Extraction (for qPCR quantification):

- Isolation
- Purification
- Concentration

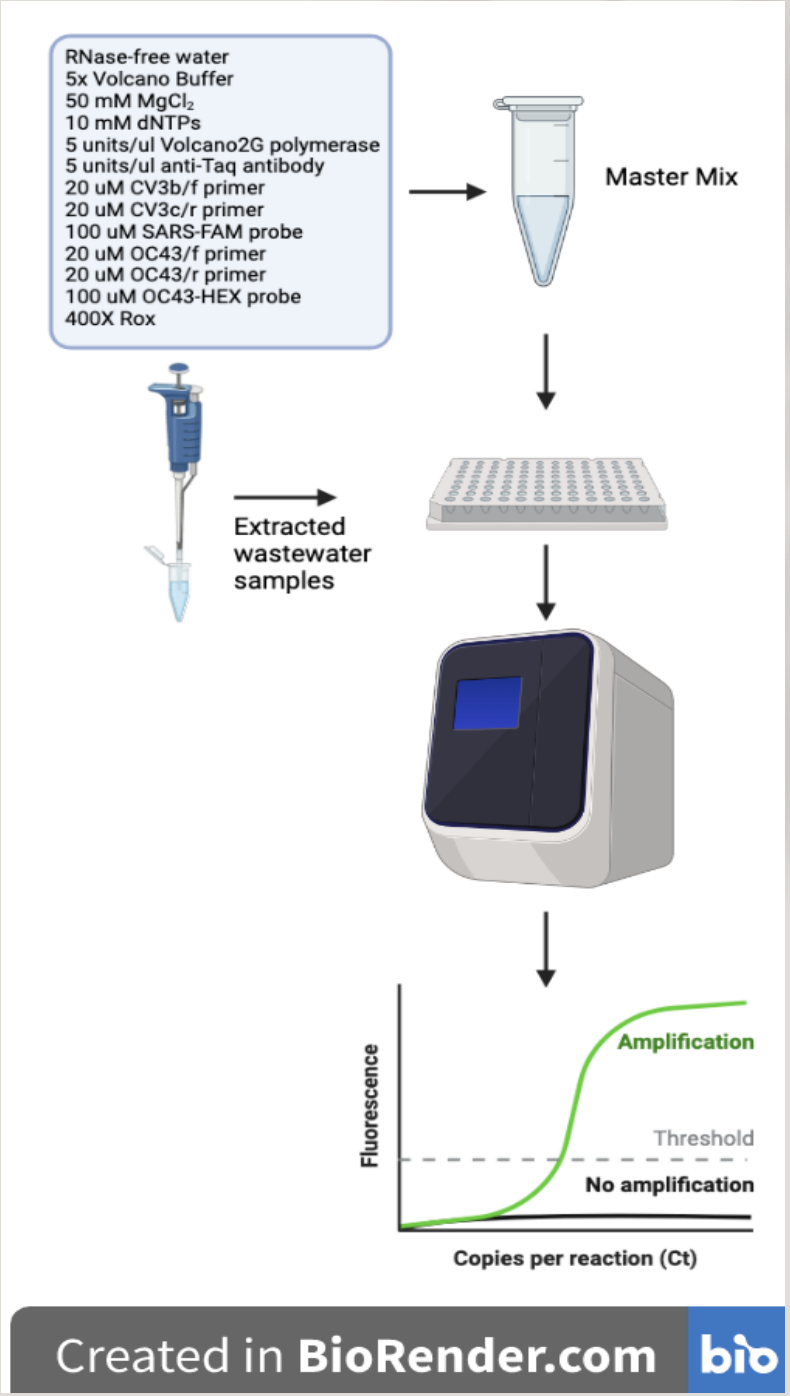
Created in BioRender.com

Image by: Samantha Abelson

V2G-qPCR Reaction Setup

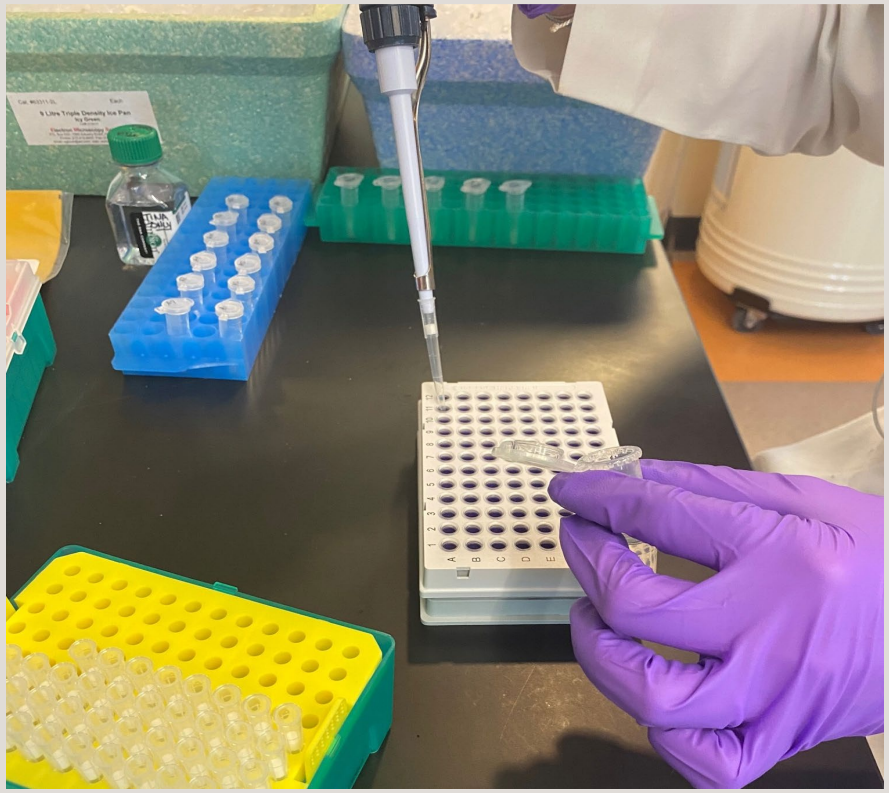


1. Pull out appropriate reagents and thaw/place on ice
2. Create master mix by combining appropriate reagents for specified V2G-qPCR target (SARS-CoV-2, B2M, OC43, HIV, PMMoV, SIV)
3. Load the 96-well plate with master mix (keep plate on ice-block)
4. Load extracted/purified RNA from wastewater concentrate sample, standards (10^1 – 10^5), and nuclease-free water per well for NTC's
5. Seal and centrifuge plate briefly to lift bubbles from bottom of wells

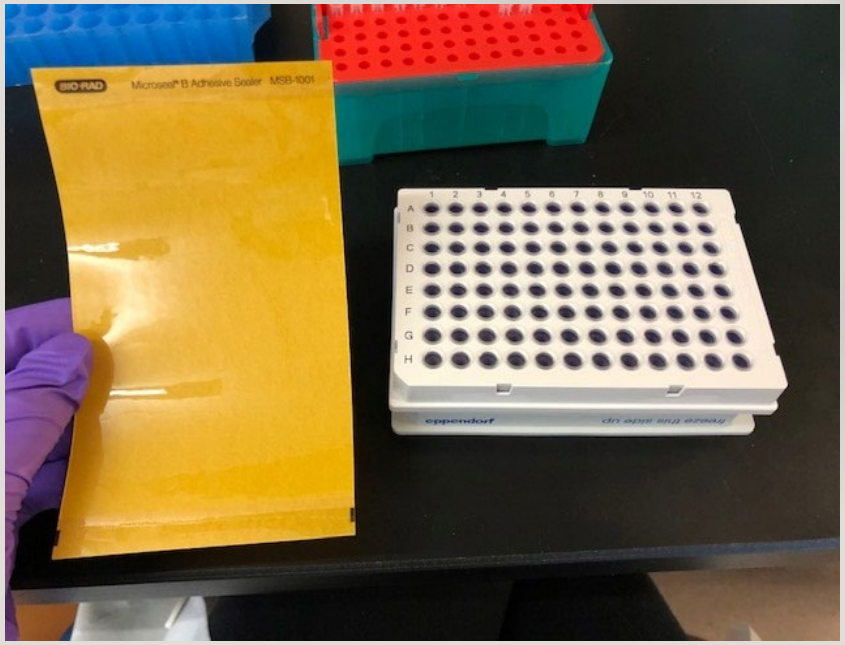


V2G-qPCR Plating Visualized

Load the plate with master mix first



Then add RNA, Standards, and NTC’s



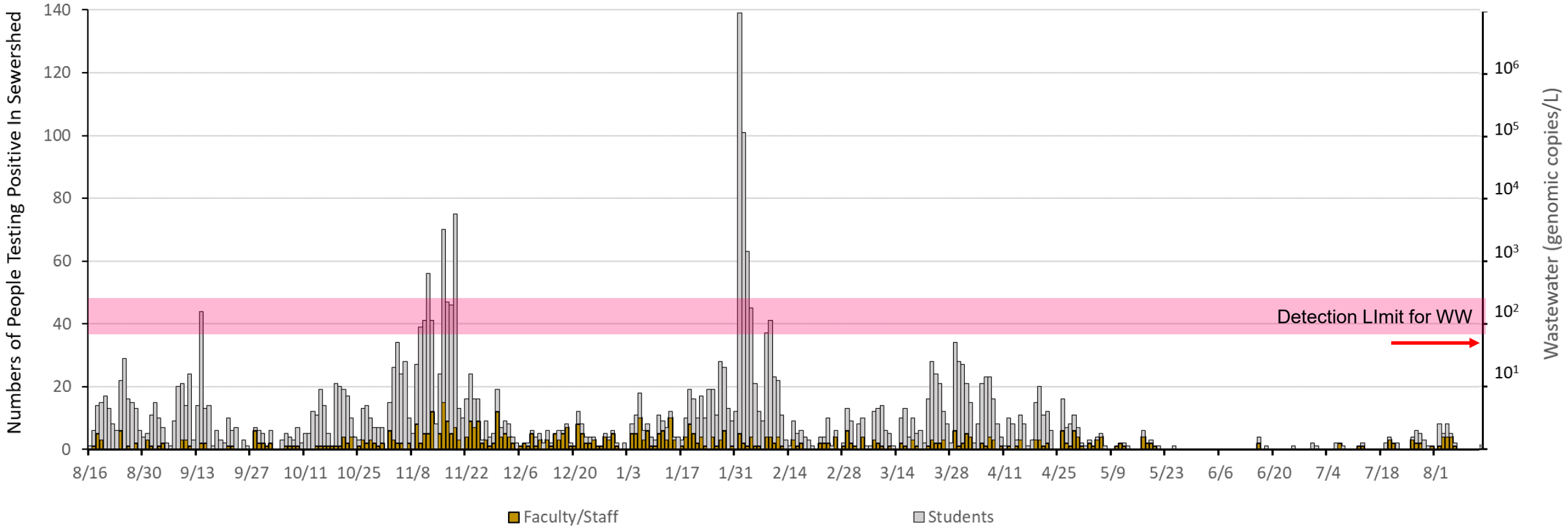
Apply adhesive seal

Weekly, Hourly, & Daily Sampling Results

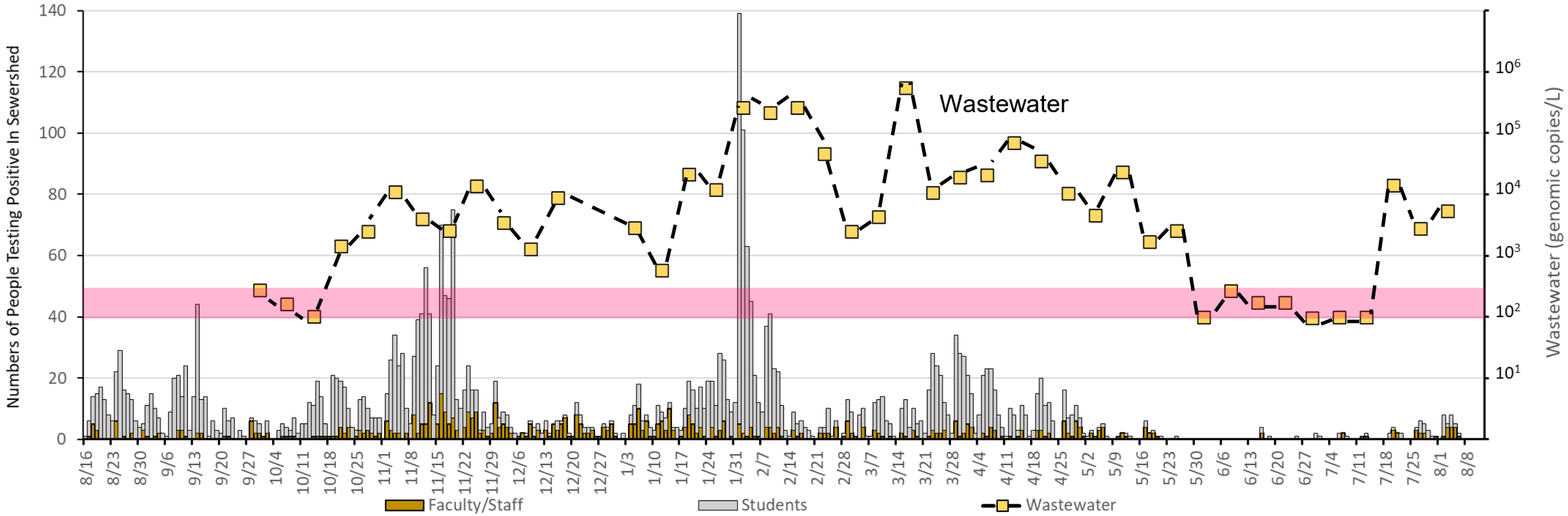
University Scale Sampling alongside Human Health

Building, Cluster, & Community Scale

University Surveillance



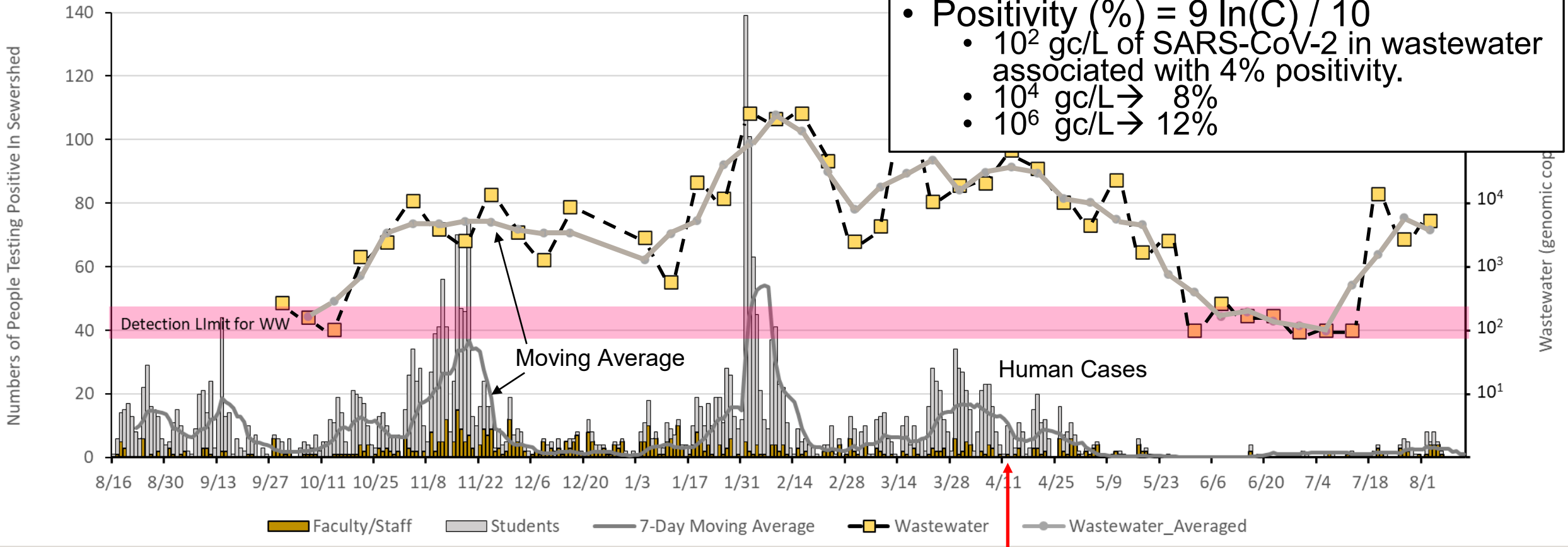
University Surveillance



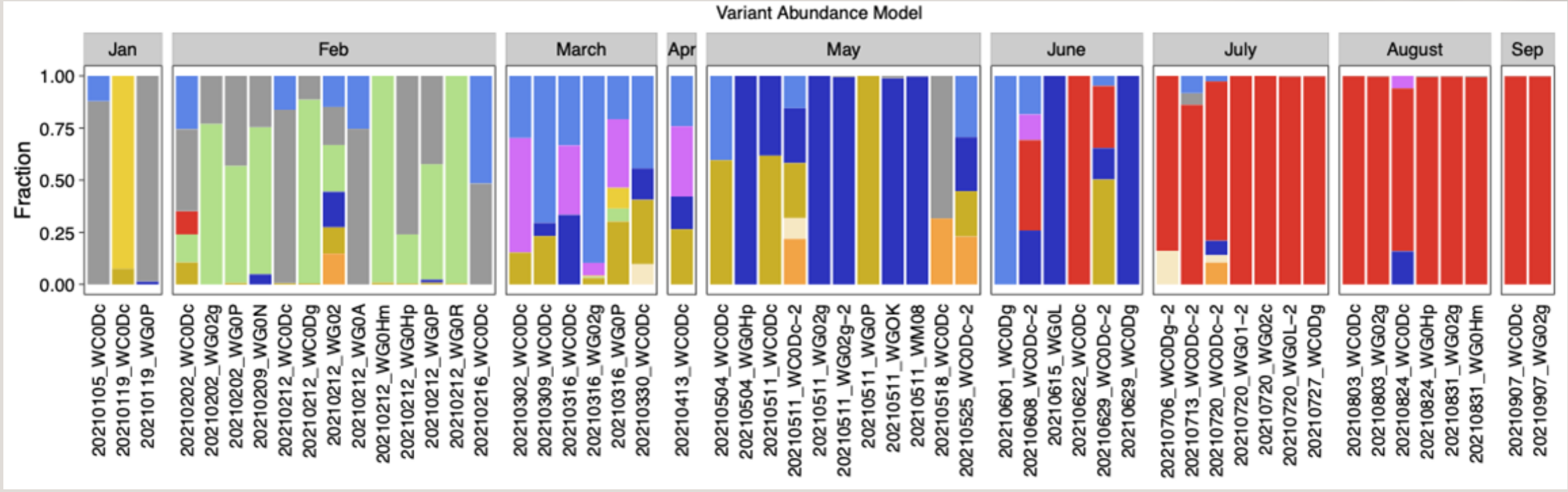
University Surveillance

Naresh Kumar & Alejandro Mantero

- SARS-CoV-2 in wastewater was a 4-day lead indicator
- Positivity (%) = 9 ln(C) / 10
  - 10^2 gc/L of SARS-CoV-2 in wastewater associated with 4% positivity.
  - 10^4 gc/L→ 8%
  - 10^6 gc/L→ 12%



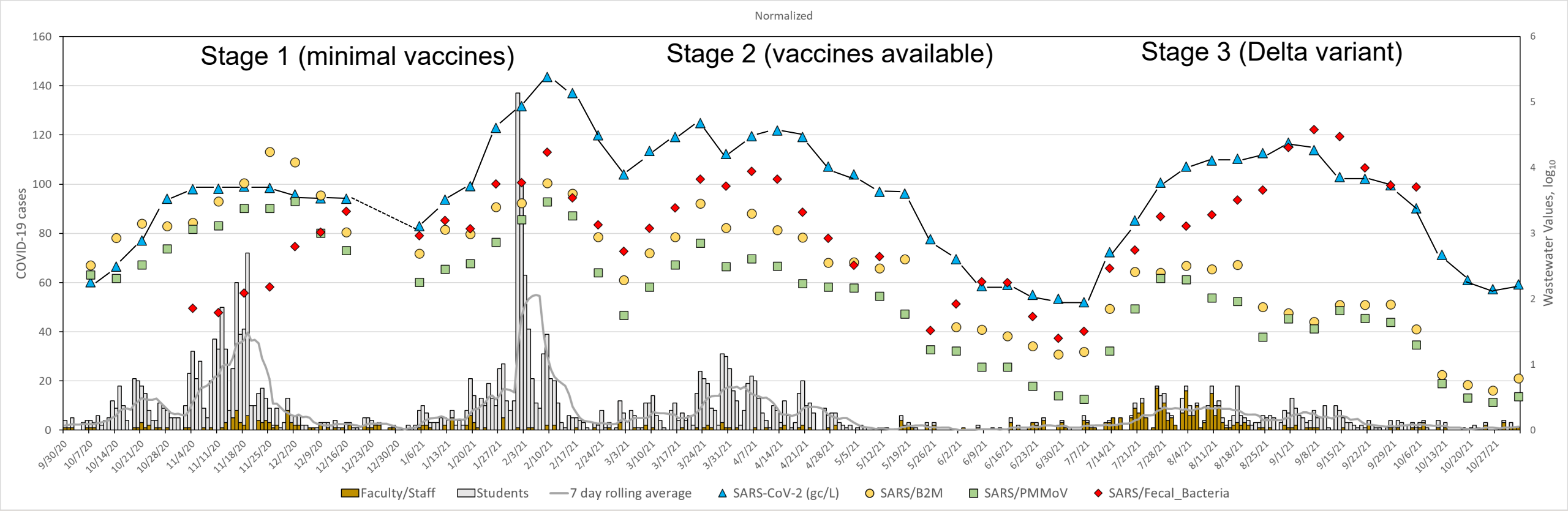
Variant Monitoring (Weill Cornell Medicine): SARS CoV-2 presence over time (Weekly Sampling)



Normalization – Why It's Important

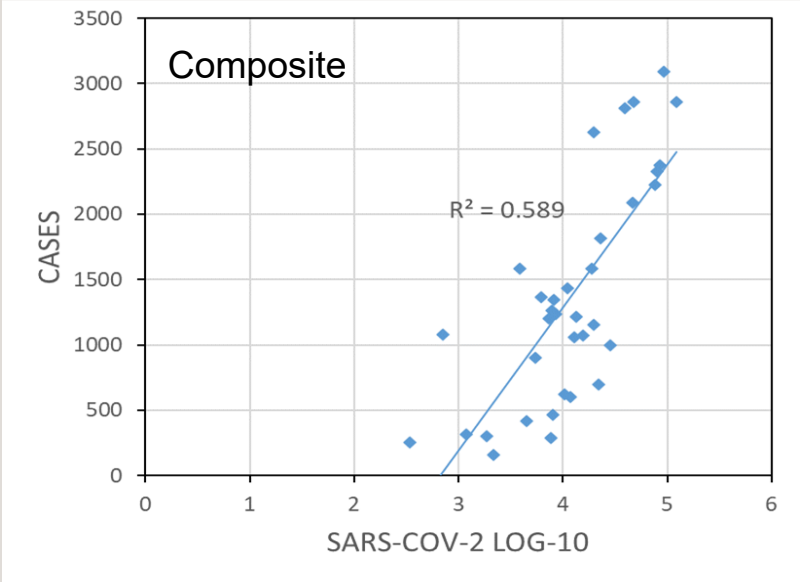
- Wastewater: black water and grey water
- Will normalization by a fecal indicator help improve the COVID-19 predictive capability of wastewater water?
  - PMMoV (Pepper Mild Mottled Virus) by V2G-qPCR
  - B2M (Beta-2-Microglobulin, protein coding gene) by V2G-qPCR
  - Fecal coliform bacteria by culture
- Evaluate at campus and community scale
- At community scale evaluate grab versus composites

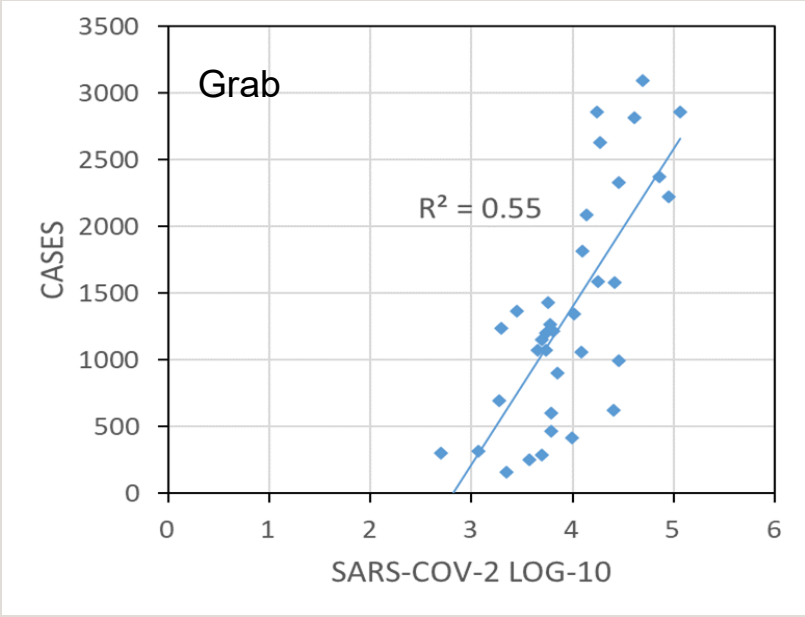
Comparison of Normalized B2M, PMMoV, Fecal Bacteria, Raw Wastewater and Human Cases

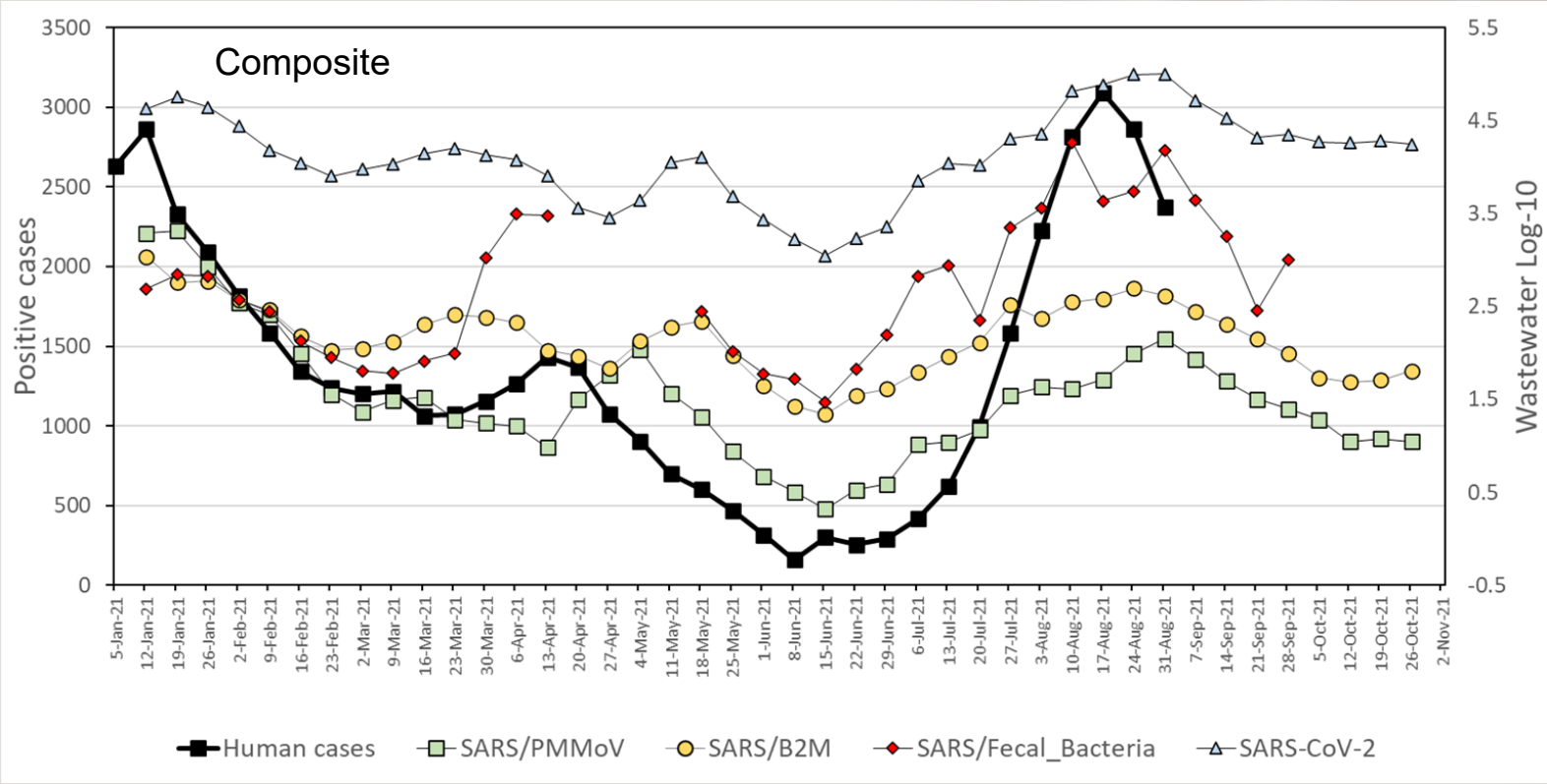


University Scale – Weekly Sampling

Weekly Sampling Results: Community Scale (Central District WWTP)







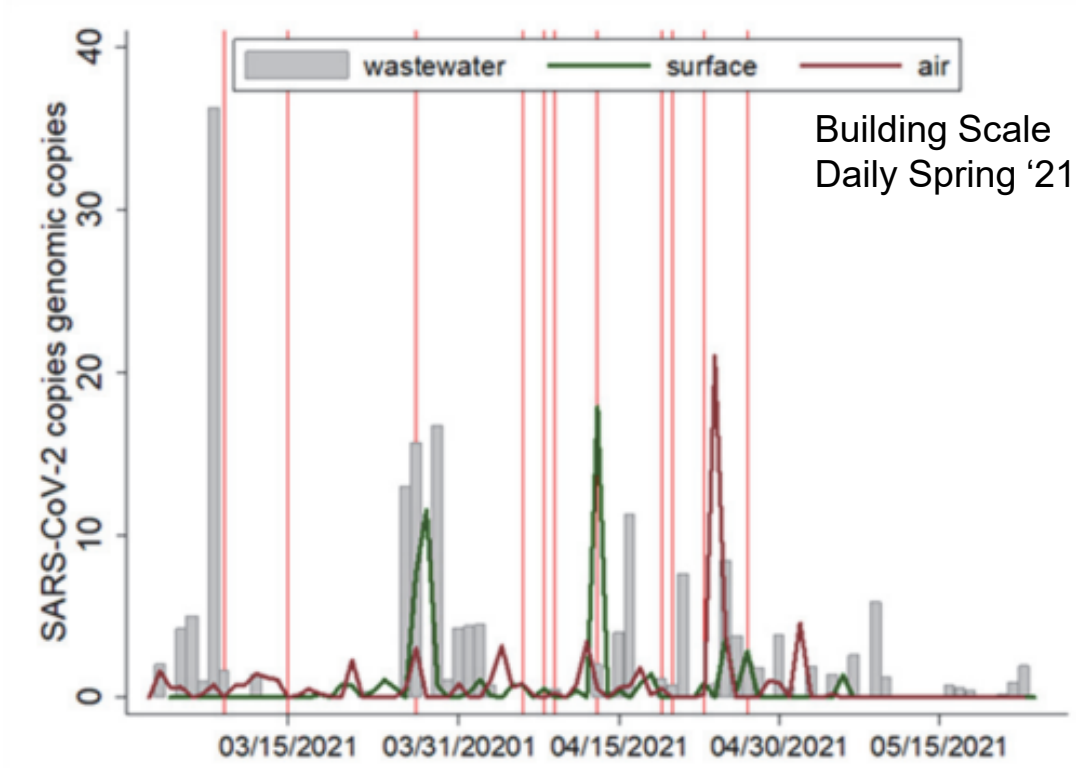
	Composite R^2	Grab R^2
SARS-CoV-2	0.59	0.55
SARS/PMMoV	0.45	0.35
SARS/B2M	0.56	0.40
SARS/Fecal coliform	0.25	0.16

Normalization – What we've Found

- At community scale, normalization with fecal indicators does not provided improvement for COVID-19 predictions
- Composite samples overall better than grab samples for COVID-19 predictions
- At campus scale, normalization (with PMMoV and B2M) appears to provide some improvement

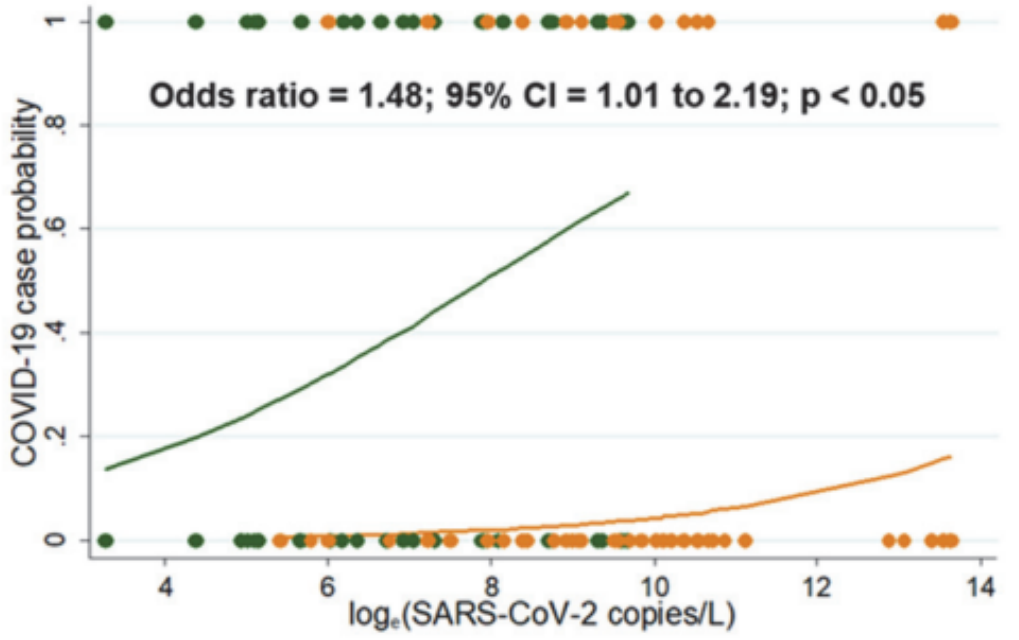
Daily Sampling – UM Campus

Fig. 10. SARS-CoV-2 distribution and COVID-19 cases in a UM dorm March-May 2021 (vertical red line indicates diagnosis of a COVID-19 case).



Building Scale
Daily Spring '21

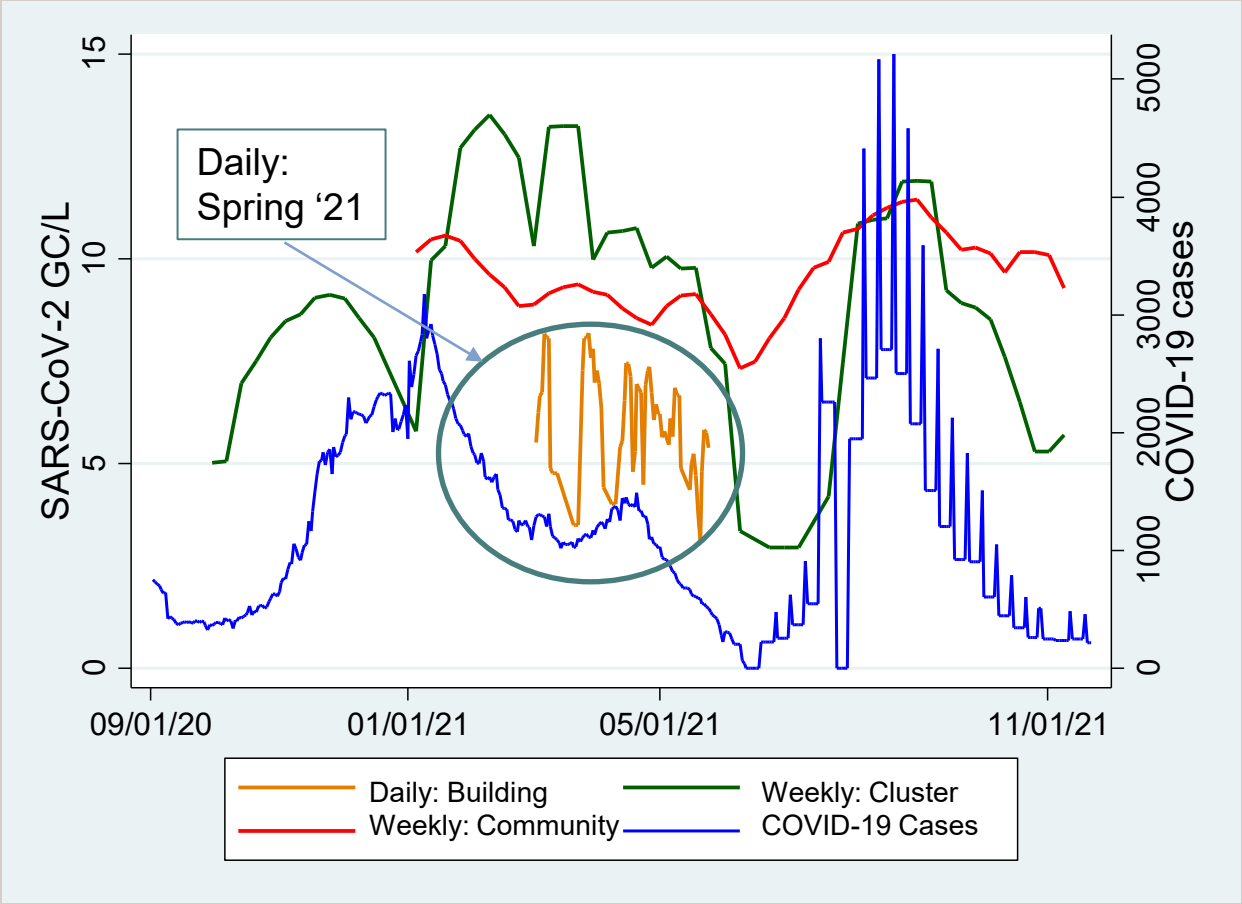
Fig .11. Probability of a COVID-19 case at the UM campus with respect to four day lagged wastewater SARS-CoV-2 concentration, 2020 to 2021.



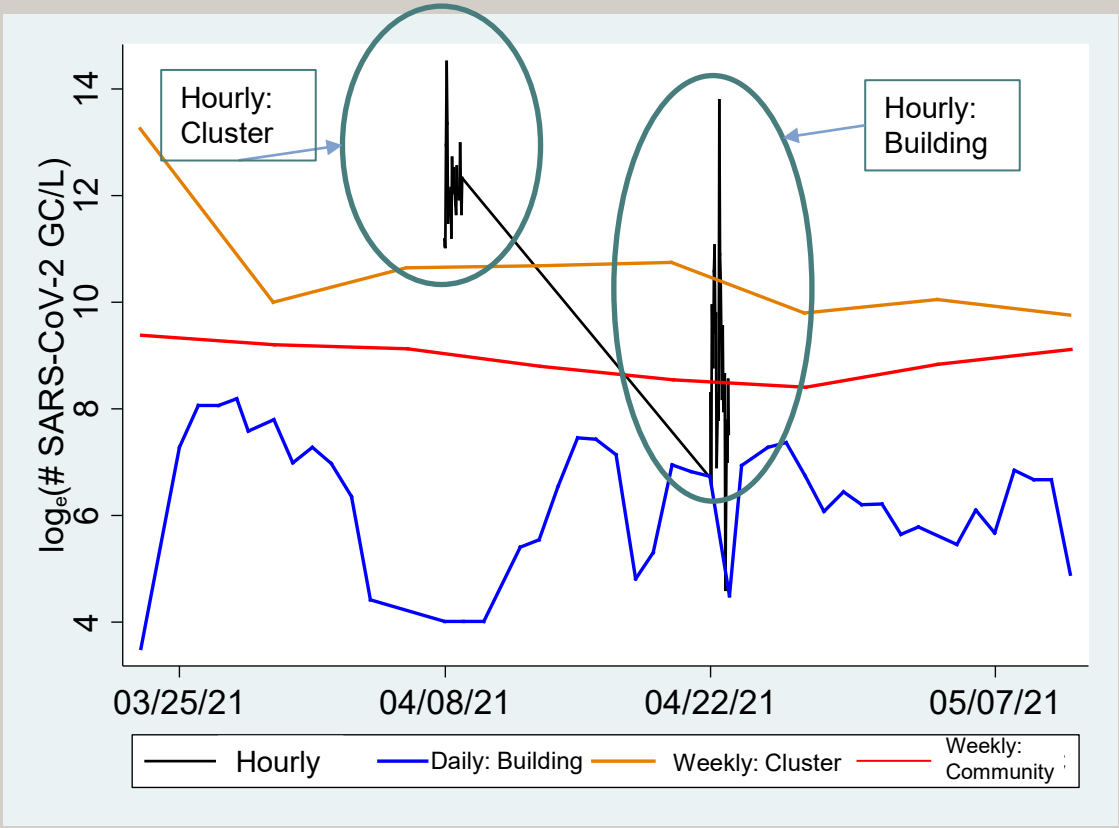
Building Scale
Fall '20 & Spring '21

SARS-CoV-2 Presence: Daily/Weekly/Hourly

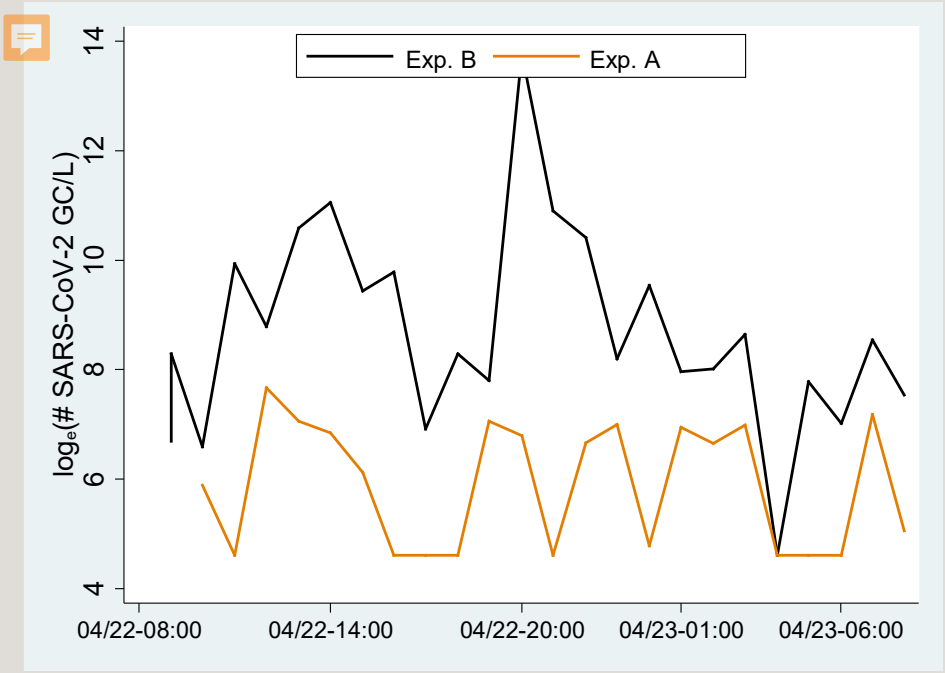
- Graph showing SARS-CoV-2 concentration wastewater at different sites and new COVID-19 cases in Miami-Dade county



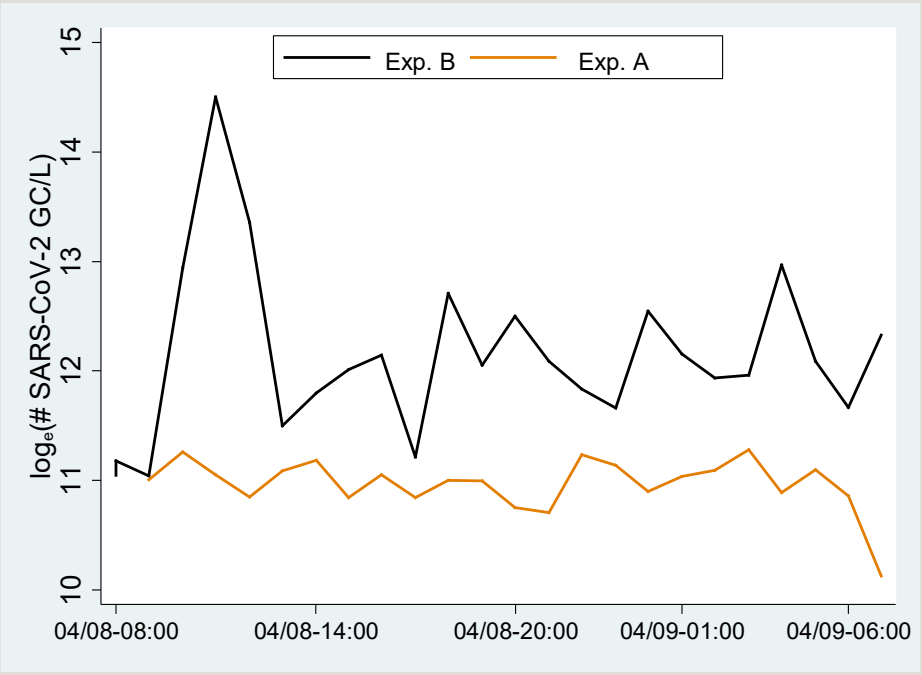
- Comparison of SARS-CoV-2 concentration in hourly, daily and weekly samples



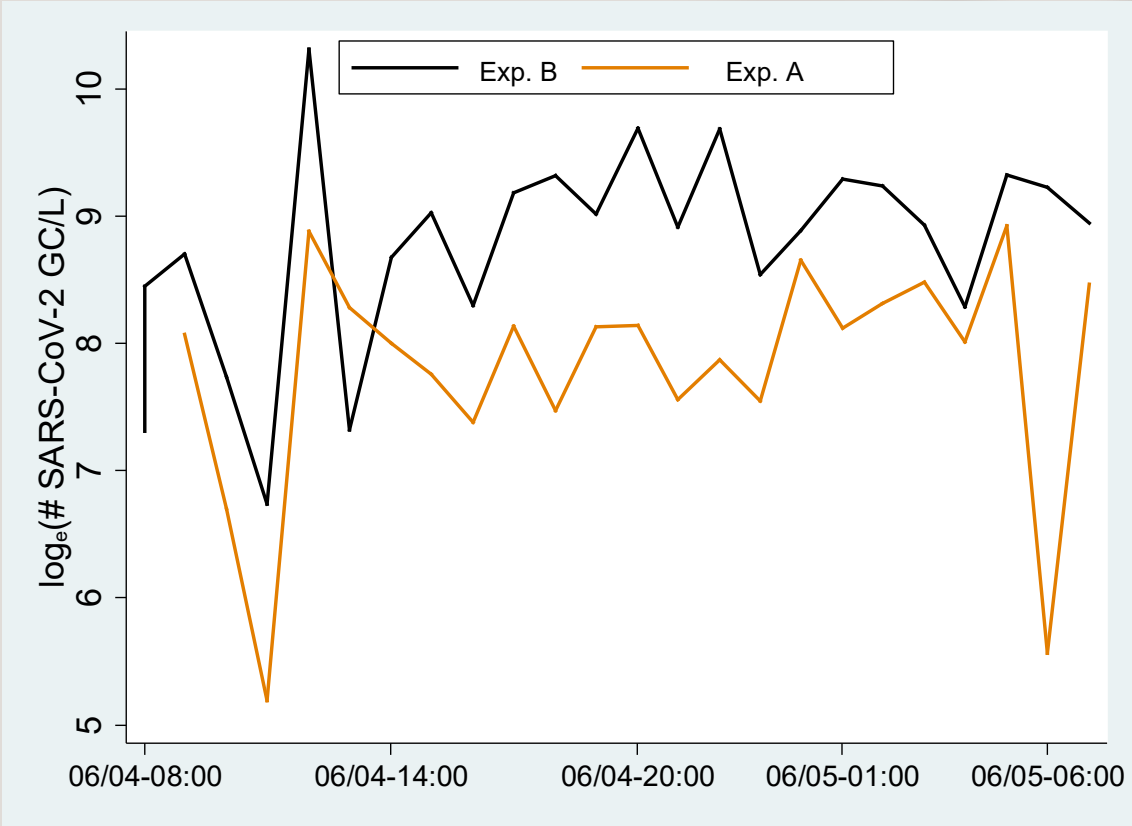
Hourly Sampling Time Lagged SARS-CoV-2 Abundance



Hourly Experiment #2 (Building) performed April 22-23, 2021: UM



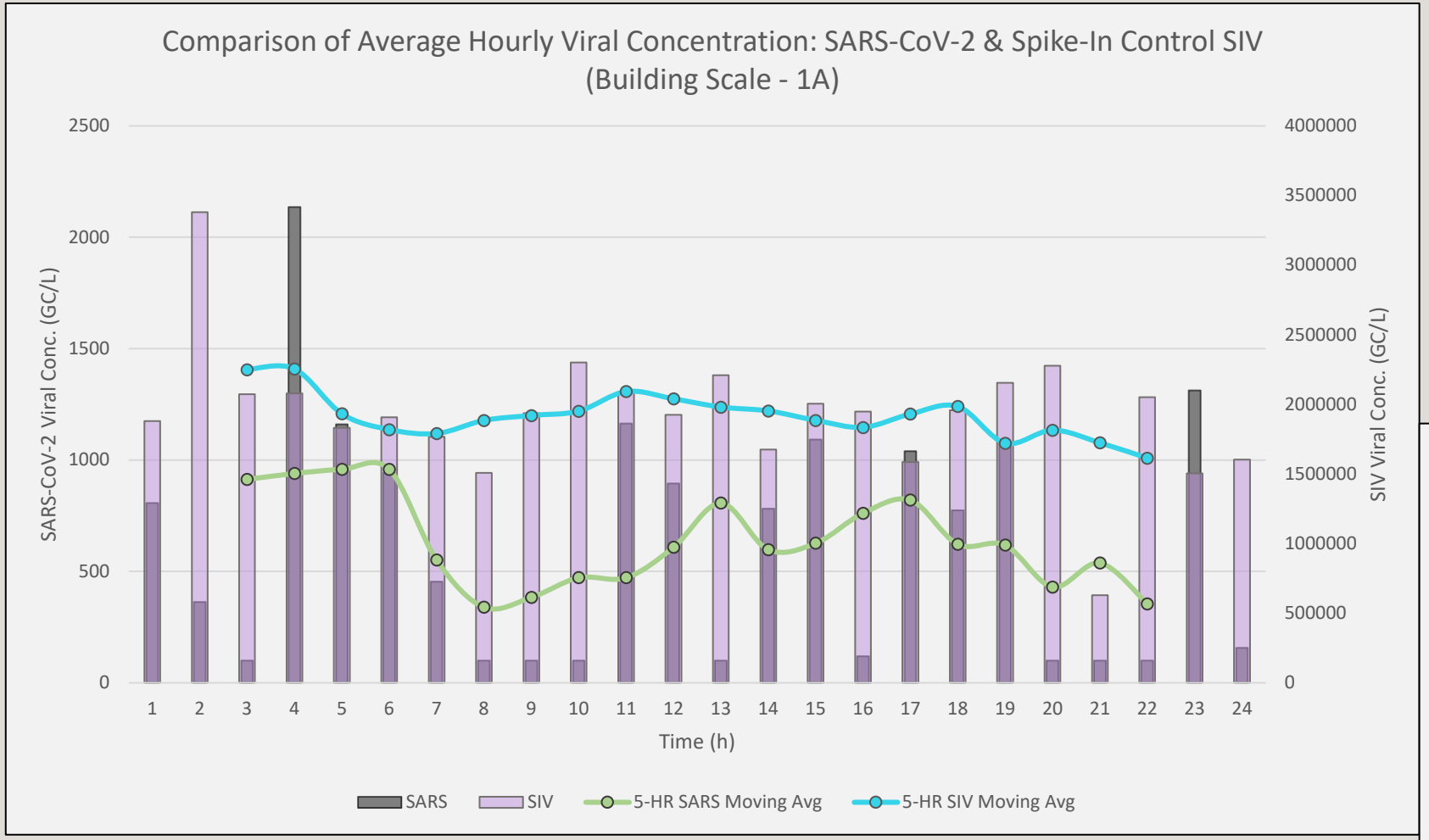
Hourly Experiment #1 (Cluster) performed April 8-9, 2021: UM

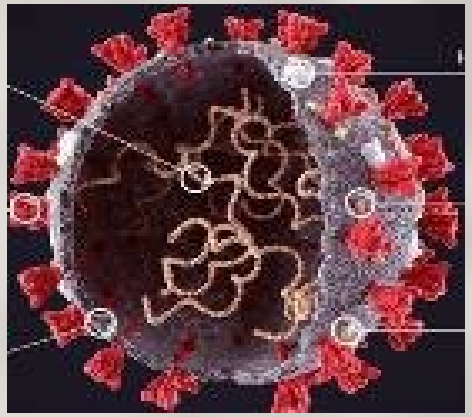


Hourly Experiment #3 (Community) performed June 4-5, 2021: CDWWTP

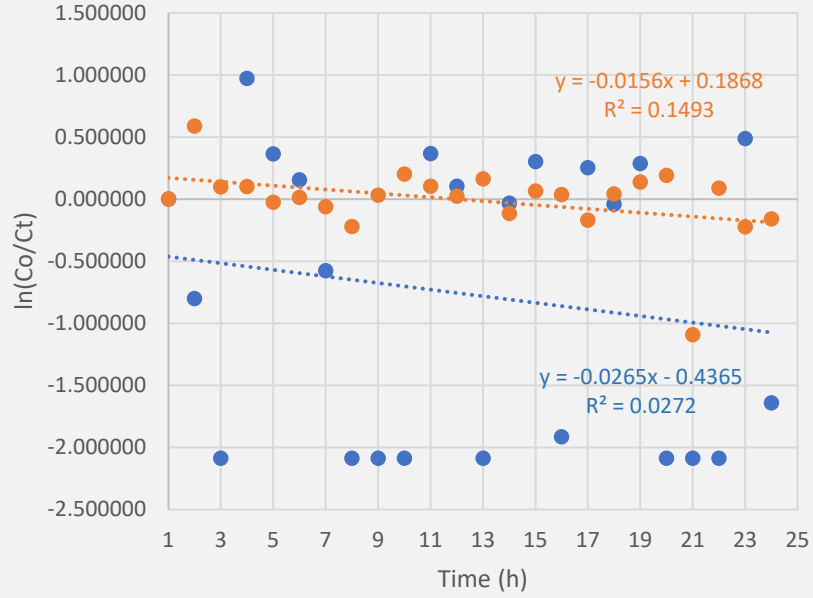
Experiment A: assessing viral degradation over hourly lag-time
Experiment B: exploring hour-to-hour variability of viral abundance

Building Scale: Hourly Degradation of Viral Presence



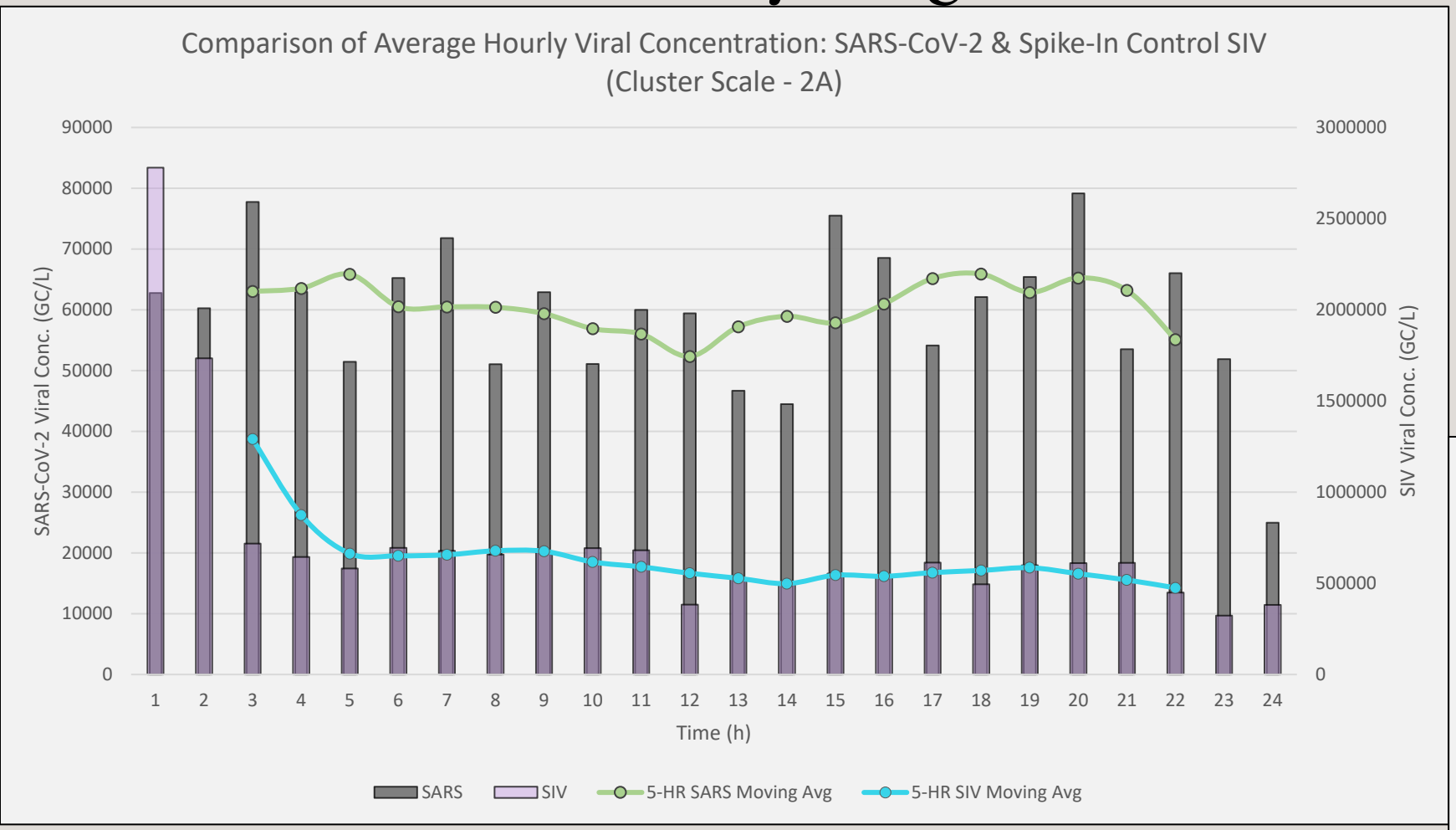


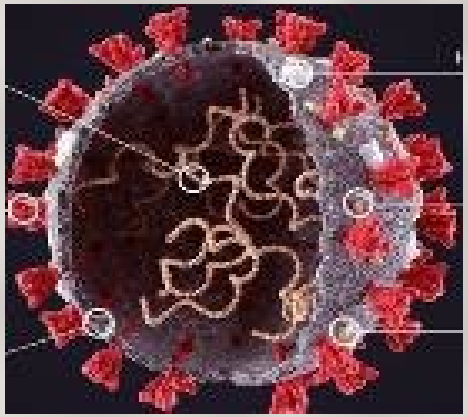
SARS-CoV-2 SIV



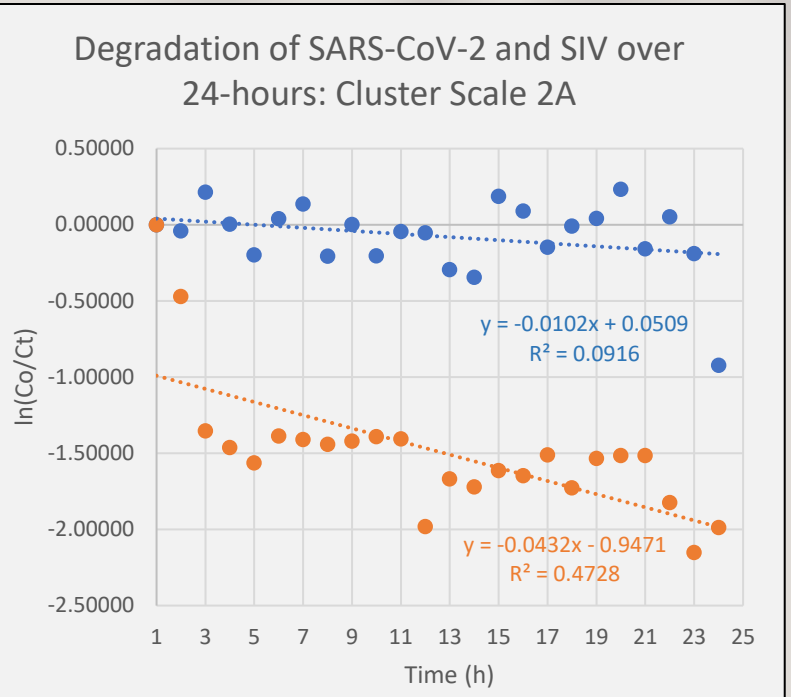
Statistical Overview (95% Confidence)	SARS-CoV-2		SIV	
Experiment Scale	R^2	p-value	R^2	p-value
Building – Exp. 1A	0.027192	0.441291	0.149265	0.062205

Cluster Scale: Hourly Degradation of Viral Presence



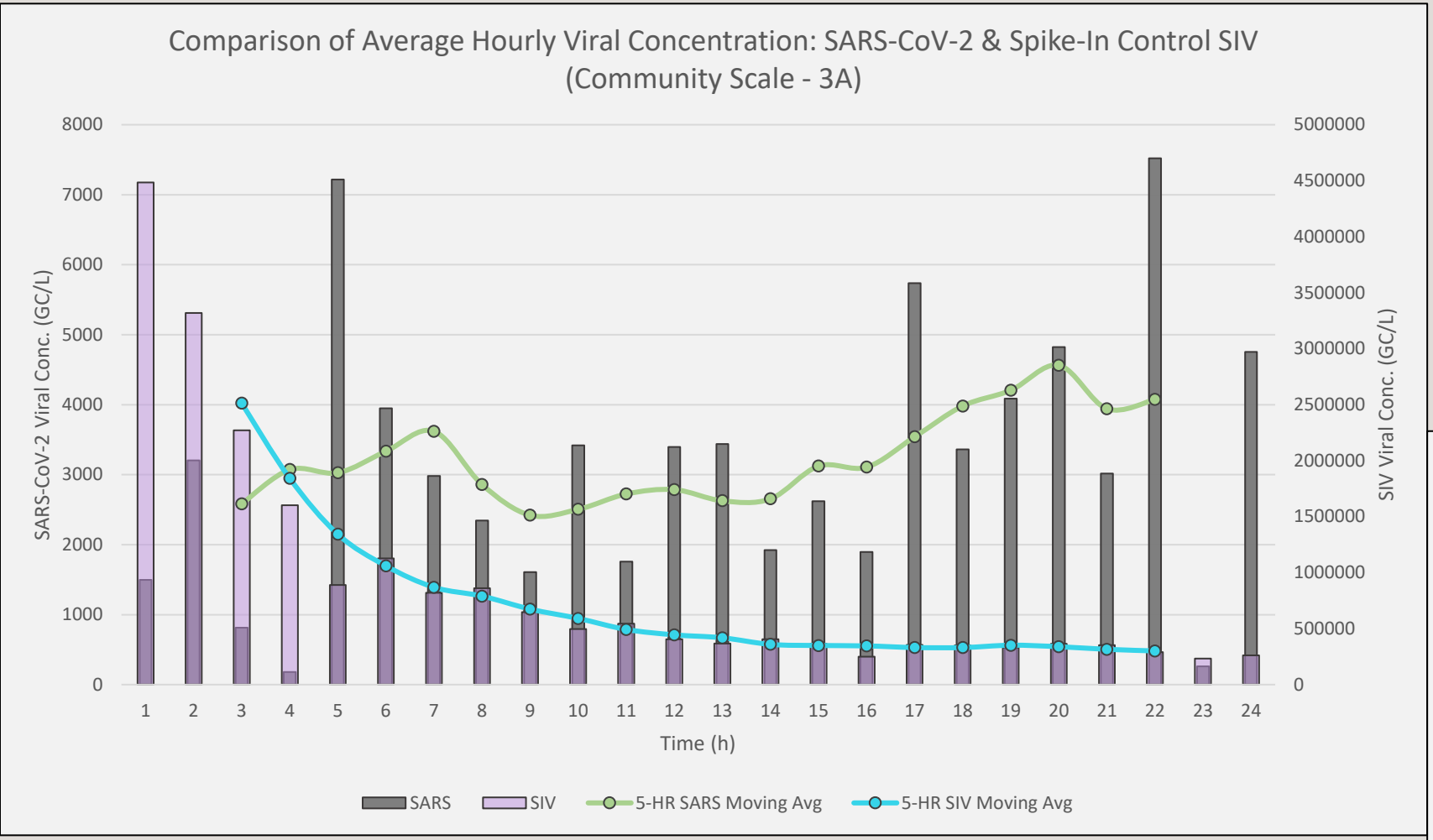


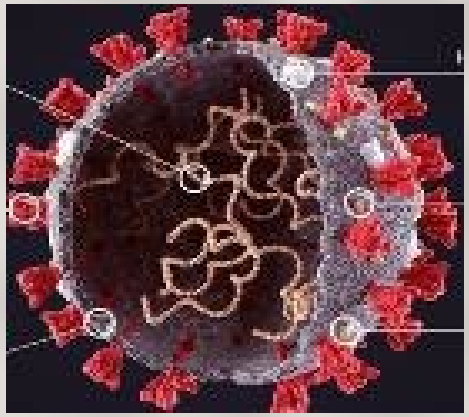
SARS-CoV-2 SIV

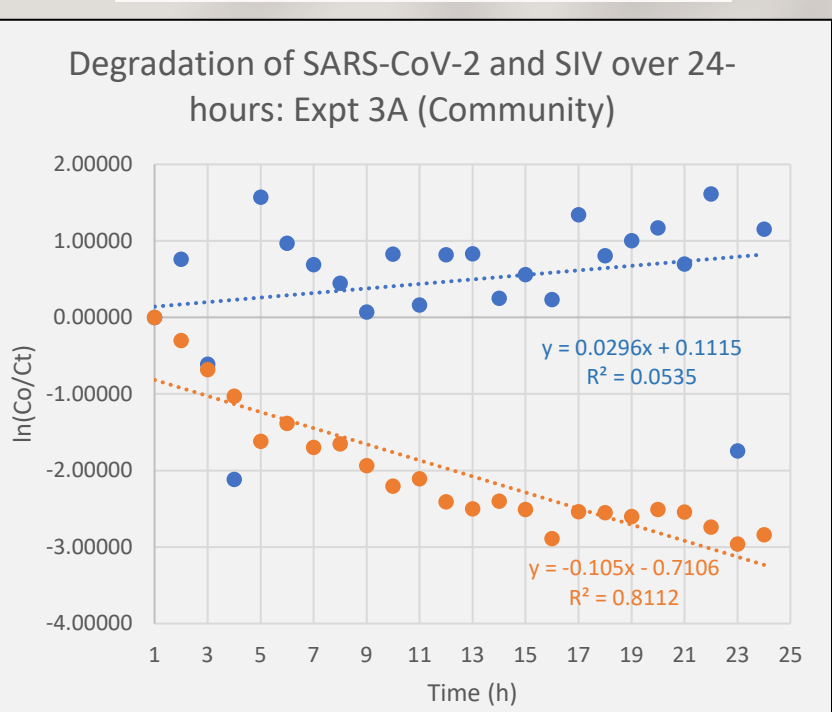


Statistical Overview (95% Confidence)	SARS-CoV-2		SIV	
Experiment	R^2	p-value	R^2	p-value
Cluster – Exp. 2A	0.091584	0.150609	0.472842	0.000205

Community Scale: Hourly Degradation of Viral Presence

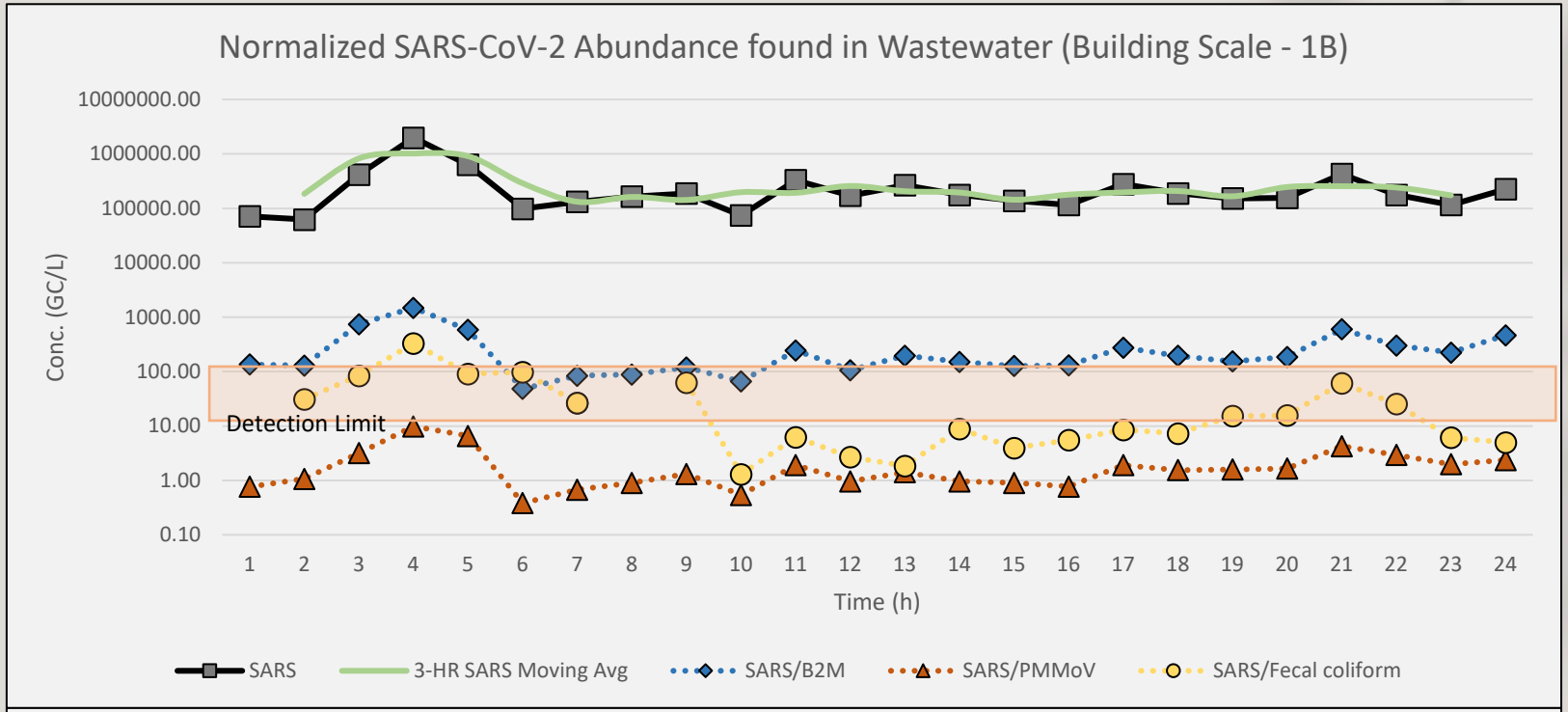


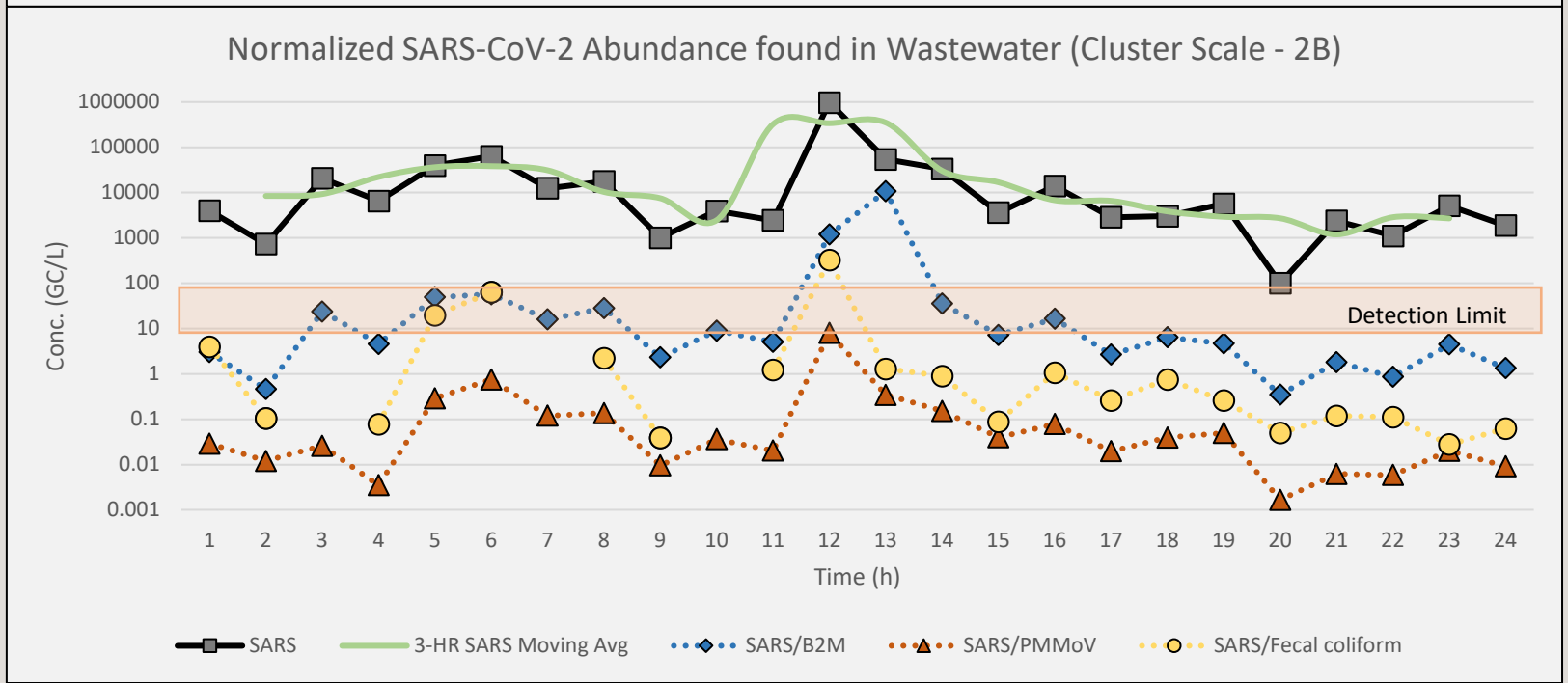




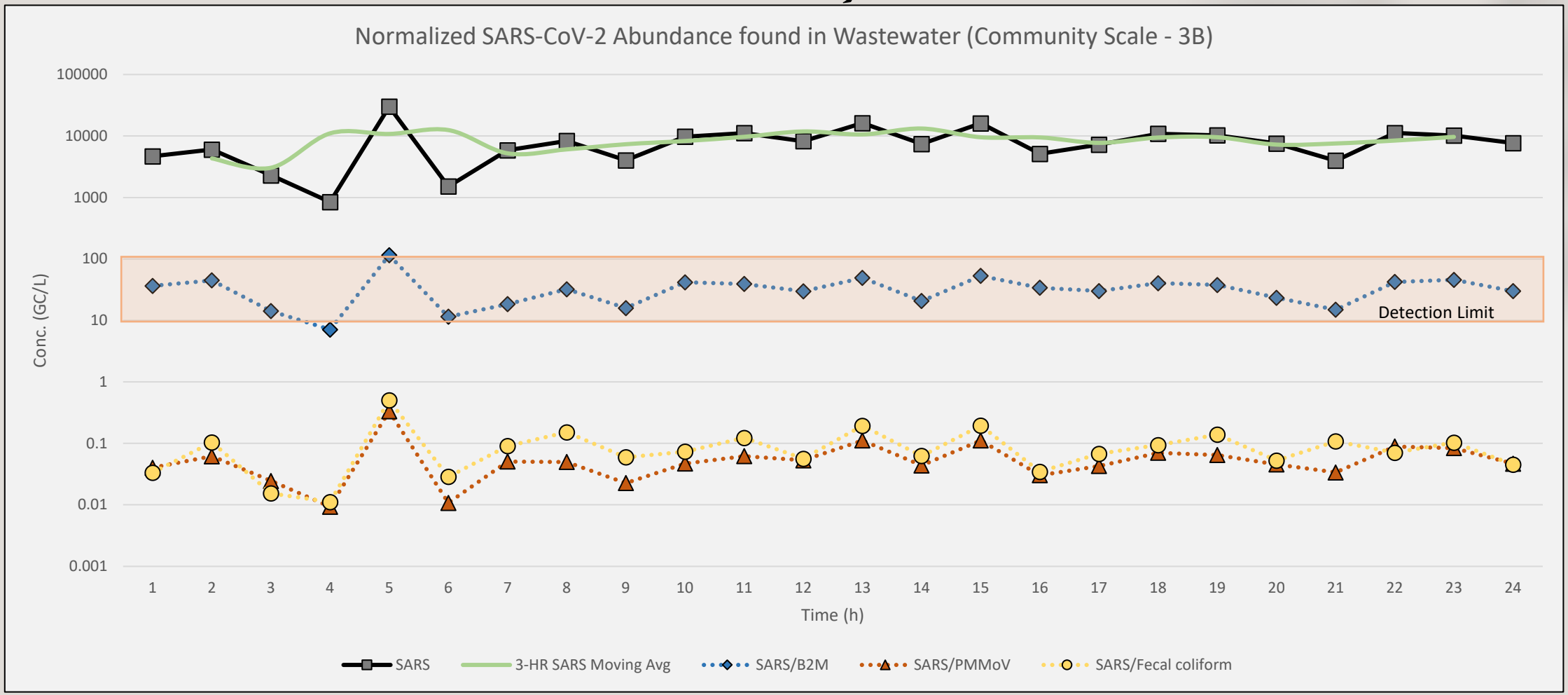
Statistical Overview (95% Confidence)	SARS-CoV-2		SIV	
Experiment Scale	R^2	p-value	R^2	p-value
Community – Exp. 3A	0.053458	0.277017	0.811224	2.01E-09

Hour-to-Hour Variability and Normalization Factors: Building and Cluster Scale





Hour-to-Hour Variability and Normalization Factors: Community Scale



Conclusions found from Hourly Sampling Experiments

- Wastewater in sewer variable over time
  - 3 log-10 variation in SARS-CoV-2 observed at building scale
  - 1.5 log-10 variation in SARS-CoV-2 observed at cluster and community scale
- Degradation at room temperature over time, secondary for SARS-CoV-2
  - Degradation of SIV statistically correlated with time
- B2M & PMMoV better normalizers of SARS-CoV-2 viral trends than Fecal coliform by culture
- Specific conductivity correlated with fecal coliform at building scale (only physical water parameter of significance)
  - Fecal coliform shows evidence of multiplication in sewer

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