Wastewater-based monitoring of COVID-19

Presented by:
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University of Miami

UNIVERSITY OF MIAMI

Weill Cornell Medicine

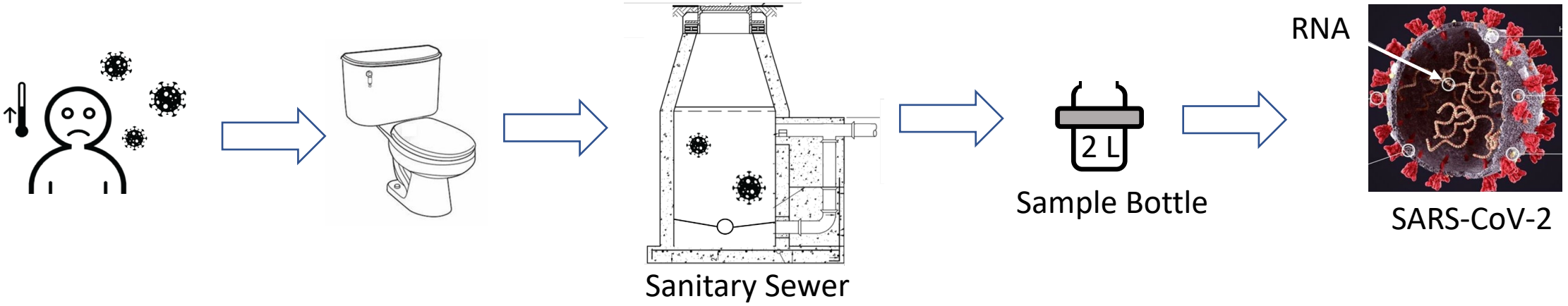
Funded by
NIH RADx-rad Grant
1U01DA053941-01

SF-RAD

https://covidsfrad.org/

Motivation & Objectives

Infected humans excrete COVID-19 virus in feces and urine

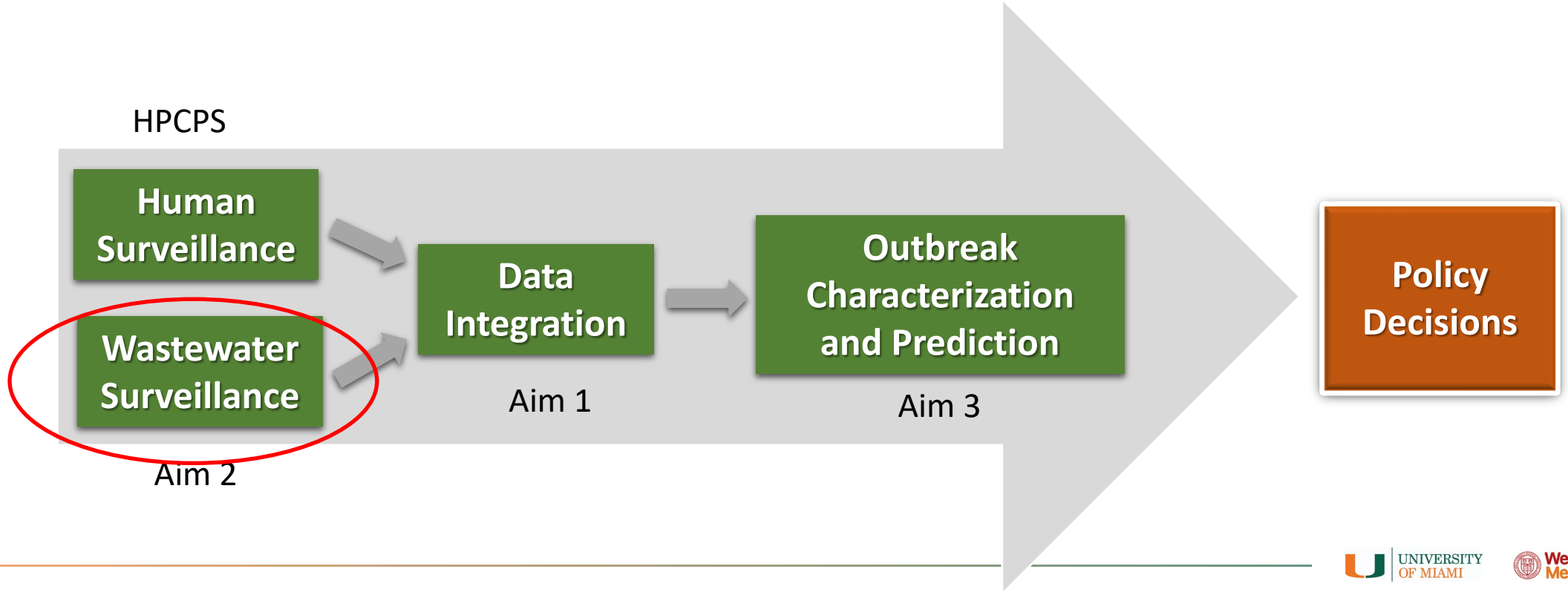


Ultimate objective: Relate wastewater measurements to predict COVID-19 cases.

SF-RAD: SARS-CoV-2 Wastewater-Based Surveillance

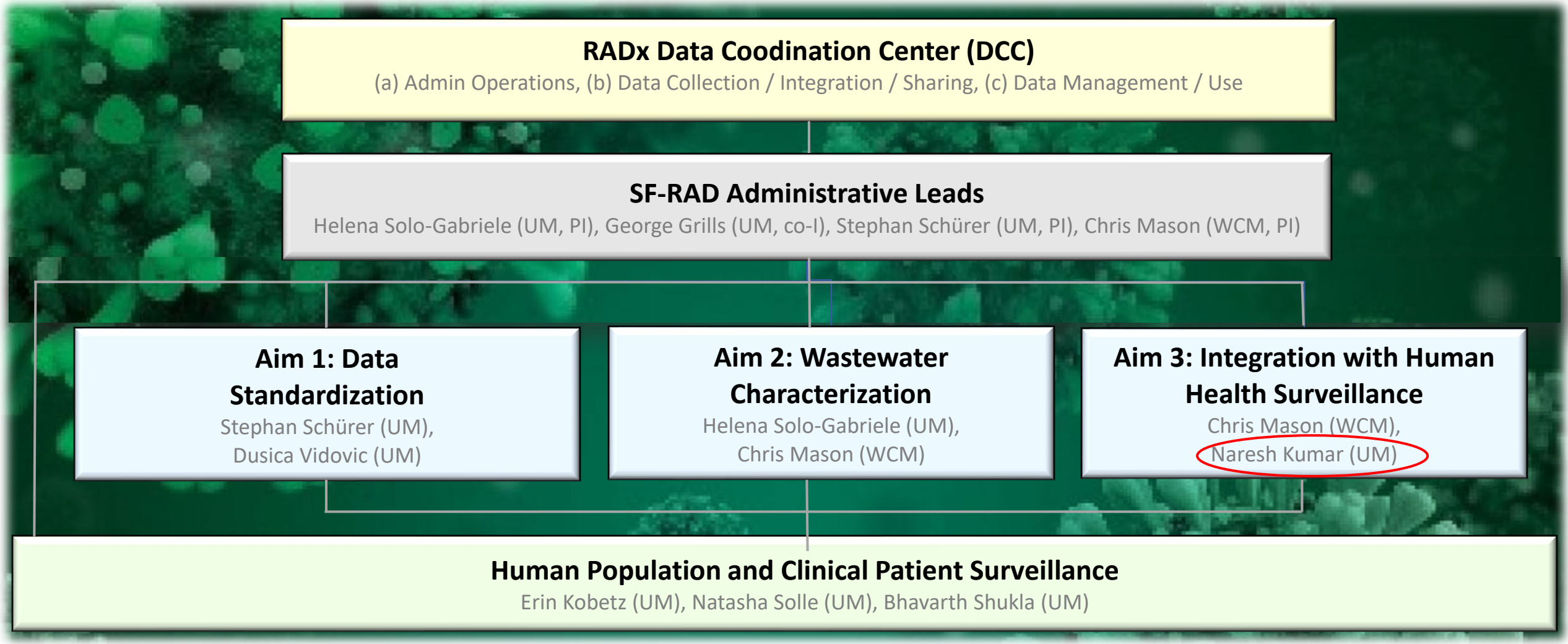
Aims

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



SF-RAD: SARS-CoV-2 Wastewater-Based Surveillance

Administrative Organization and Leadership



Aim 2:
Wastewater Characterization

Human Surveillance

Student Residents

Fall'20/Spring'21

- Students tested weekly (nasal swab, qPCR) Supplemented by 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

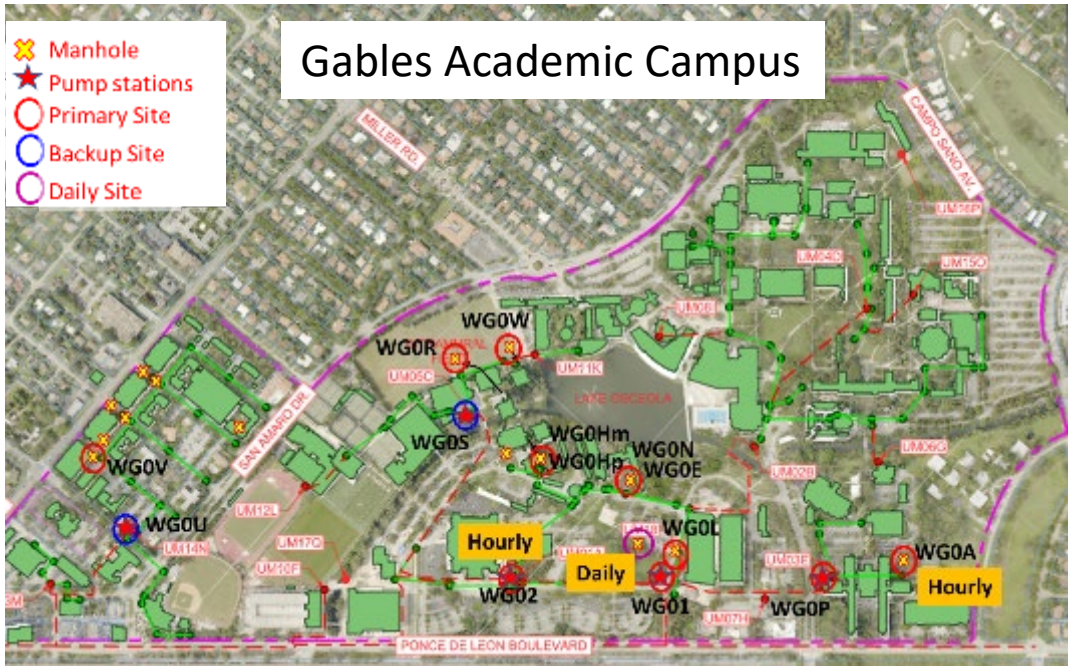
- Treat known COVID patients
- Electronic medical records pulled regularly

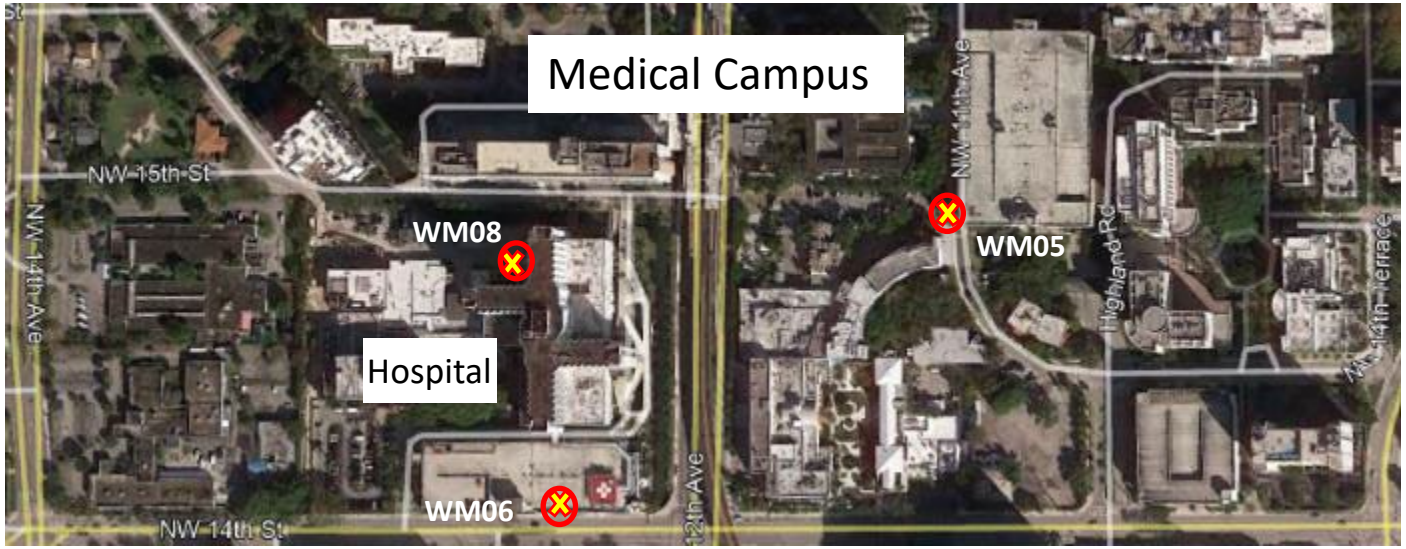
Miami-Dade County Residents (FDOH)

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

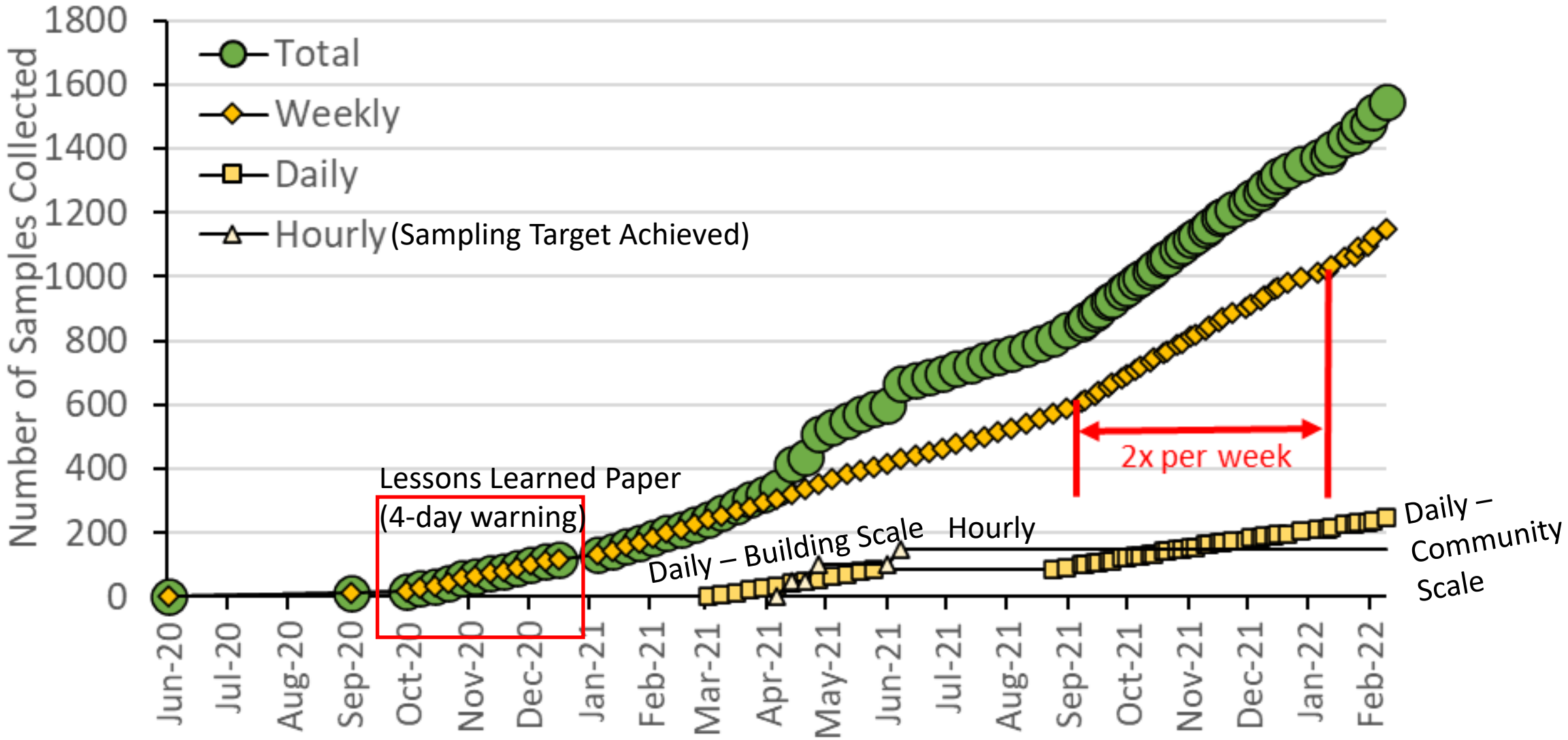
Sample Collection Plans

Community Scale
850,000





Sample Collection



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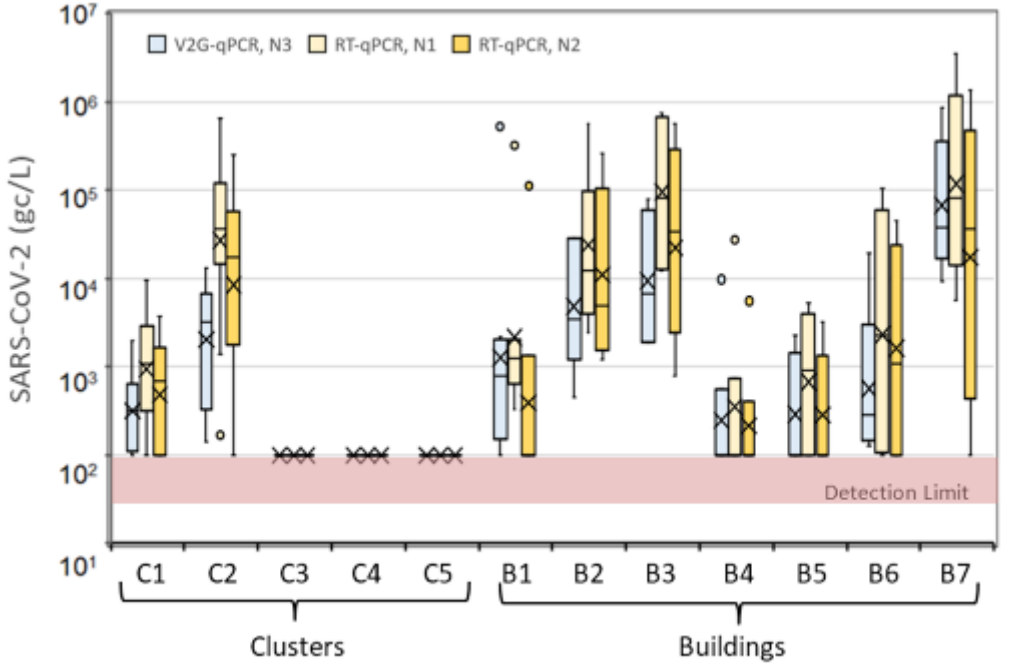
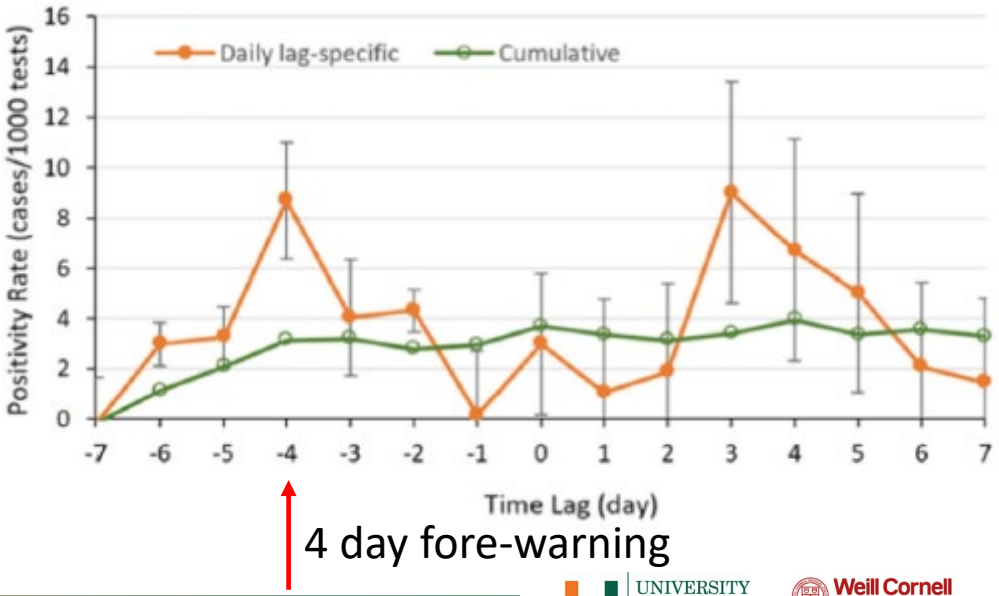
journal homepage: www.elsevier.com/locate/scitotenv

Lessons learned from SARS-CoV-2 measurements in wastewater

- A new innovative detection method (V2G-qPCR) was successful
- 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%

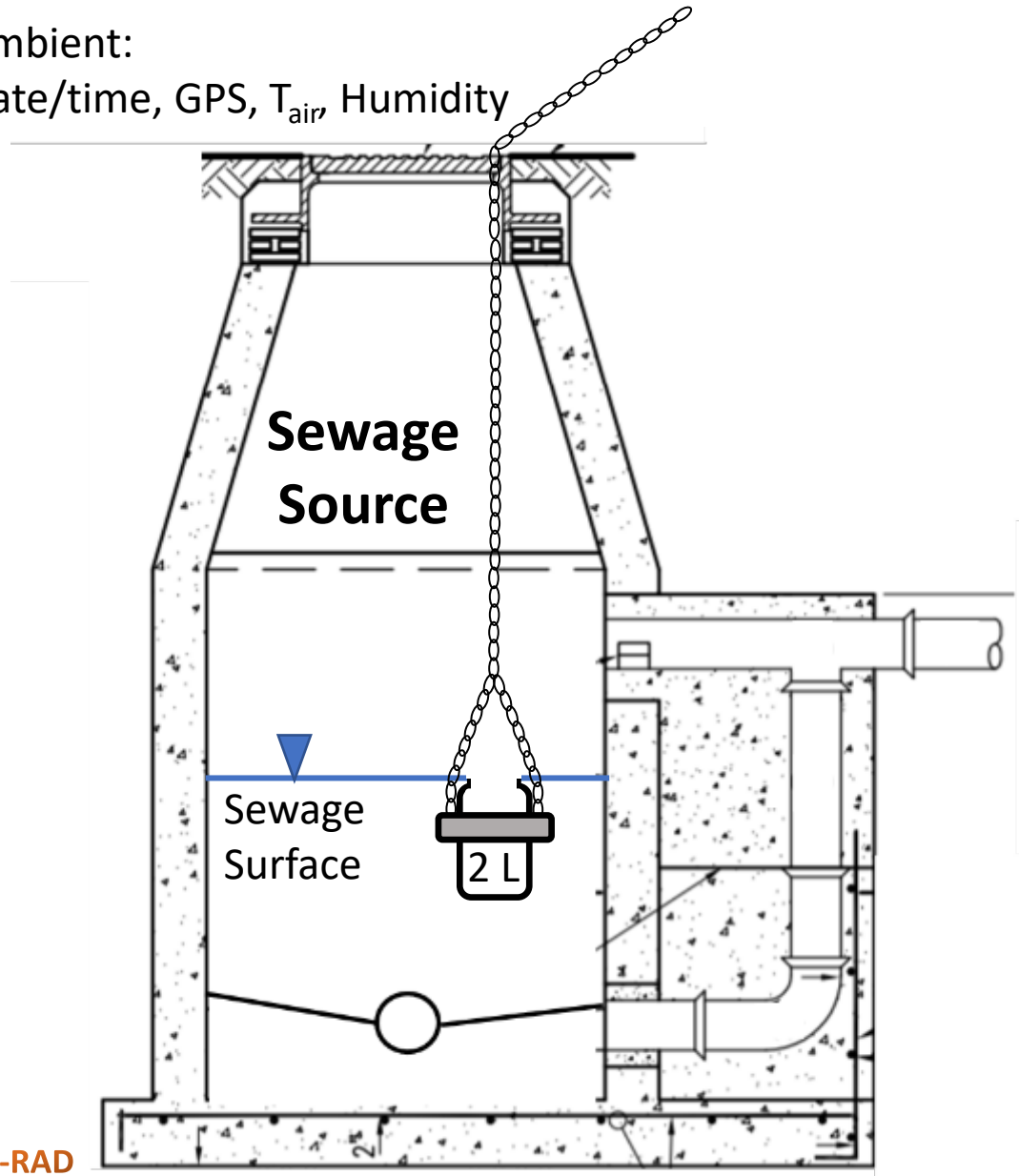
V2G – qPCR works and efficient



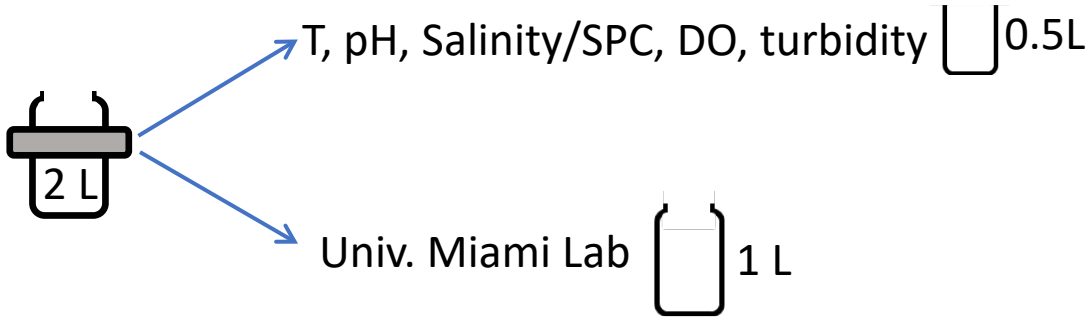


SAMPLING at UMiami

Ambient:
Date/time, GPS, T_{air}, Humidity

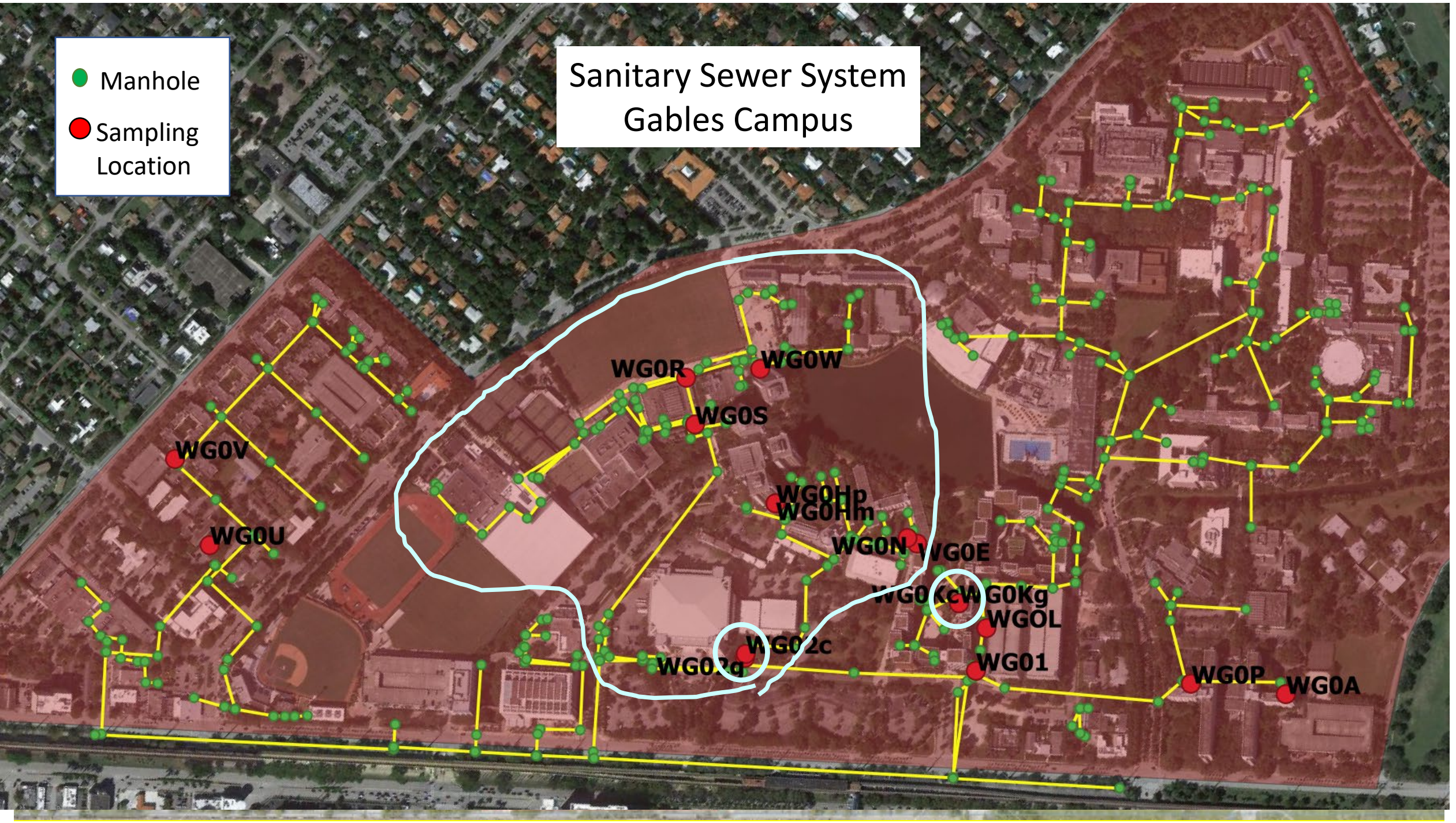


Collect Samples Weekly
Results available in 12 hours



Sampling Sites (Currently at 18 per day)

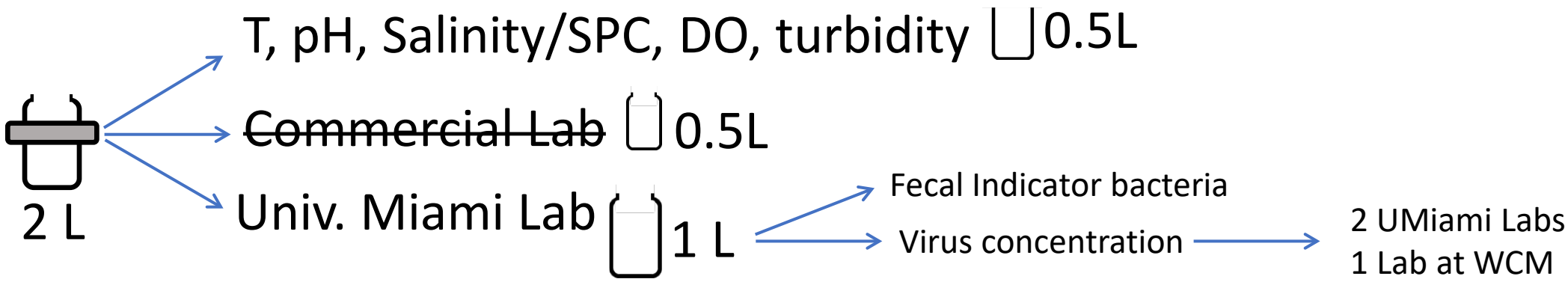
- 13 samples at Gables campus (undergraduate dorms)
- 1 at Marine campus
- 2 at University of Miami hospital
- 2 at the County Central District Wastewater Treatment Plant


Manhole
Sampling Location
Sanitary Sewer System
Gables Campus
WG0R
WG0W
WG0S
WG0V
WG0U
WG0Hp
WG0Hm
WG0N
WG0E
WG0Kg
WG0L
WG01
WG0P
WG0A

Sampling

0.5 L
1 L
T, pH, SPC, DO, turb.

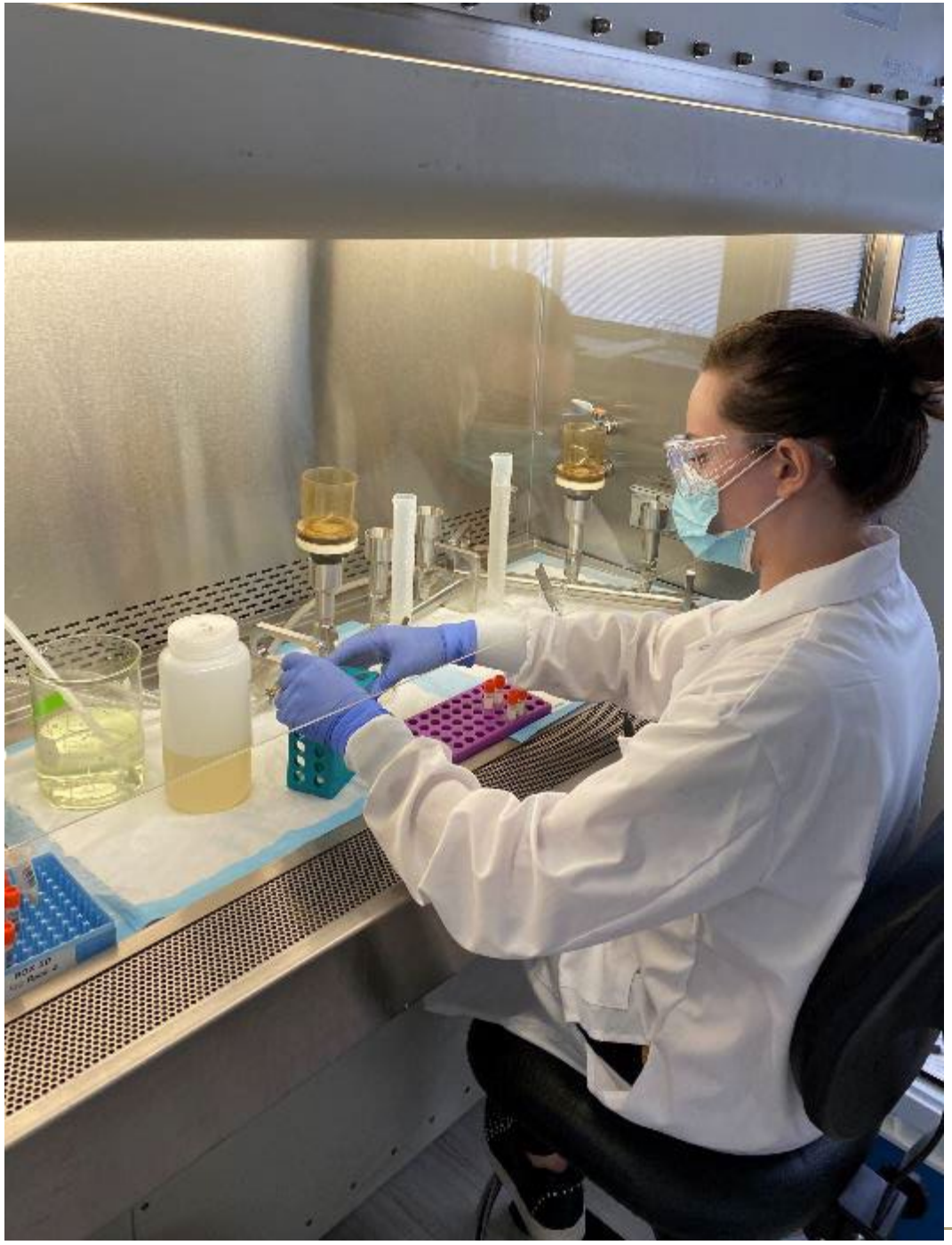
2 L


T, pH, Salinity/SPC, DO, turbidity
0.5L
Commercial Lab
0.5L
2 L
Univ. Miami Lab
1 L
Fecal Indicator bacteria
Virus concentration
2 UMiami Labs
1 Lab at WCM

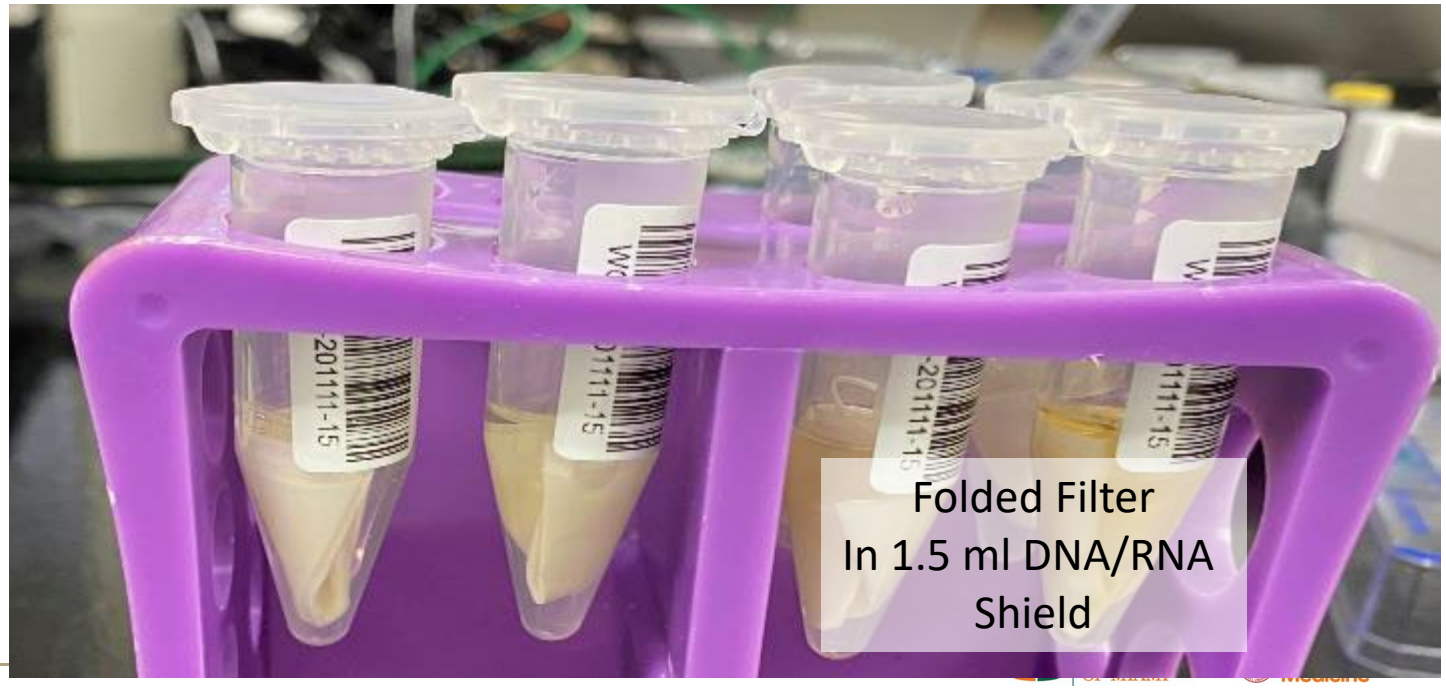
1. Add OC43 spike, recovery control
2. Add $MgCl_2$ (50 mM)
3. Acidify to pH 3.5-4.5

pH meter

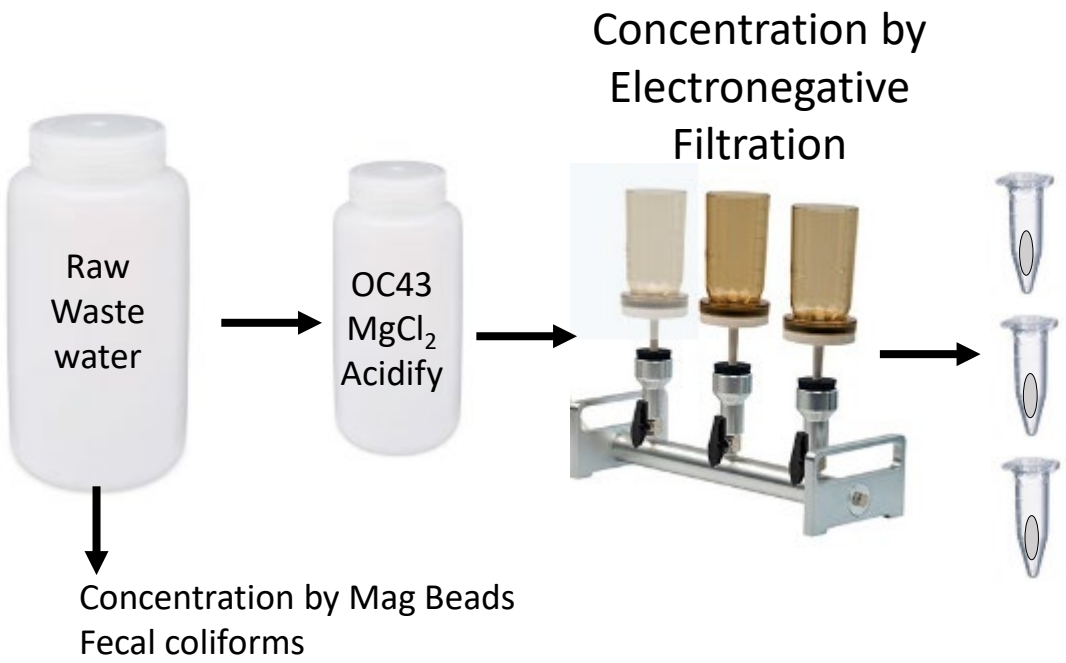
Continuous Stirring



Electronegative
Filter


Folded Filter
In 1.5 ml DNA/RNA
Shield

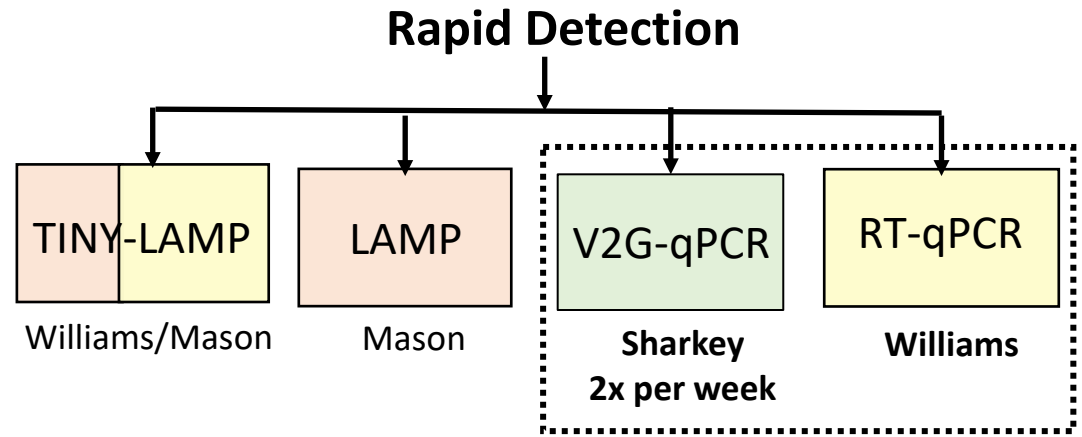
Sample Analysis Plan (Weekly Sampling)

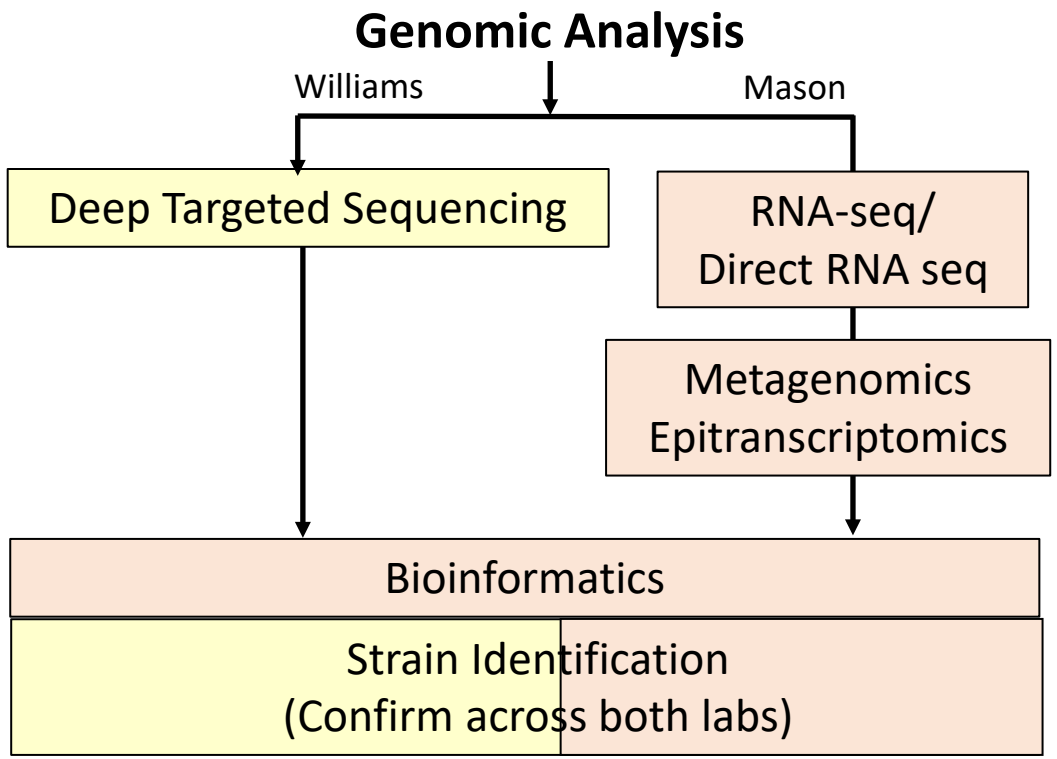


M. Sharkey,
Center for AIDS Research, UM

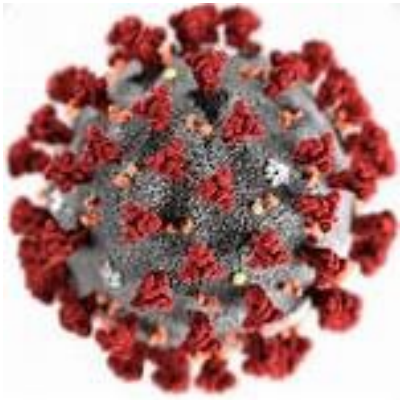
S. Williams
Onco-Genomics Shared Resource, UM

C. Mason,
Integrated Genomics Lab, WCM/MetaSub

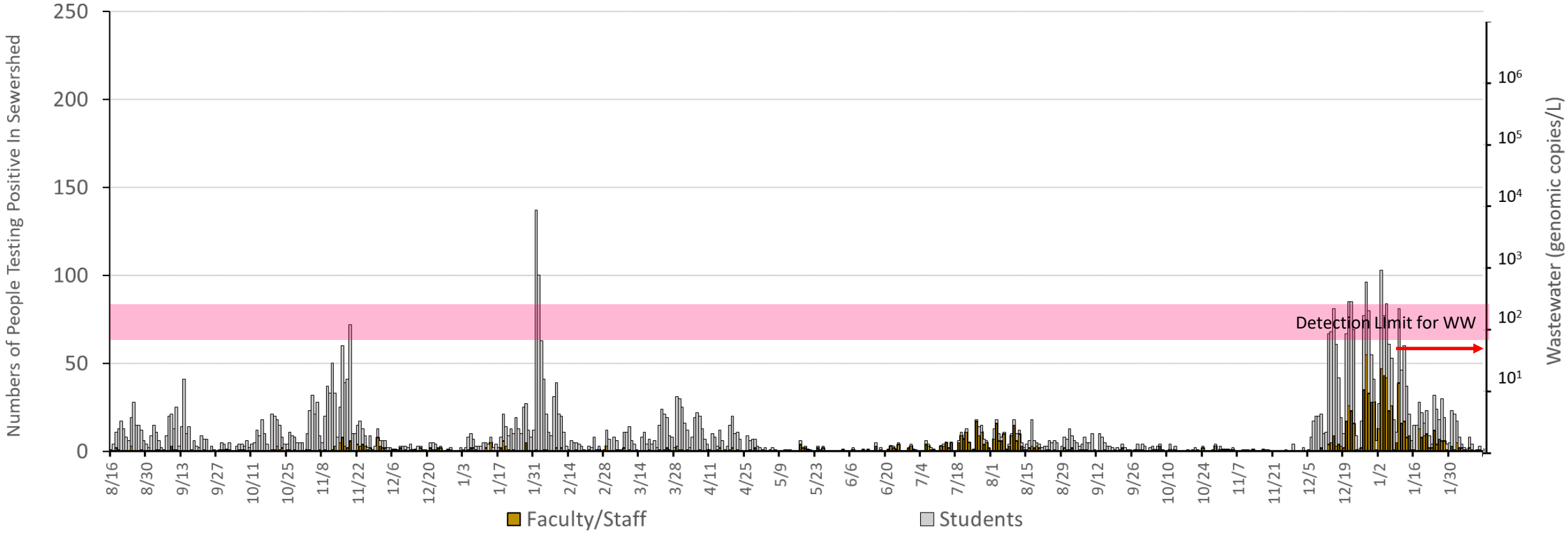




SARS-CoV-2 Results



University Surveillance



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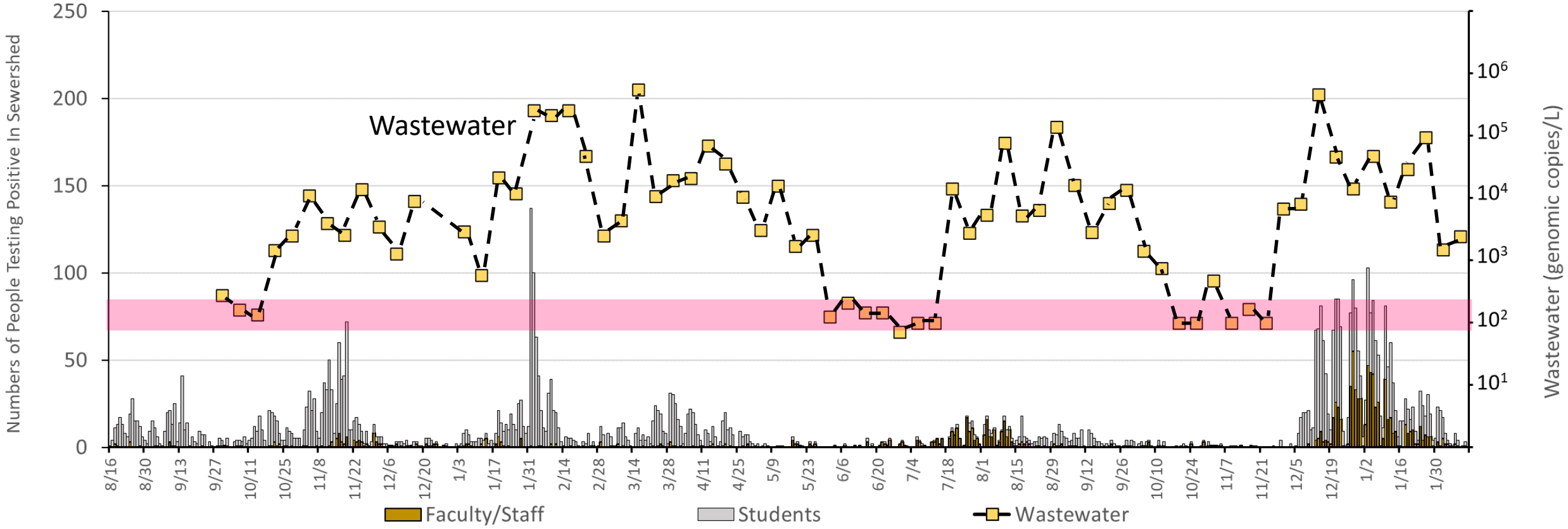

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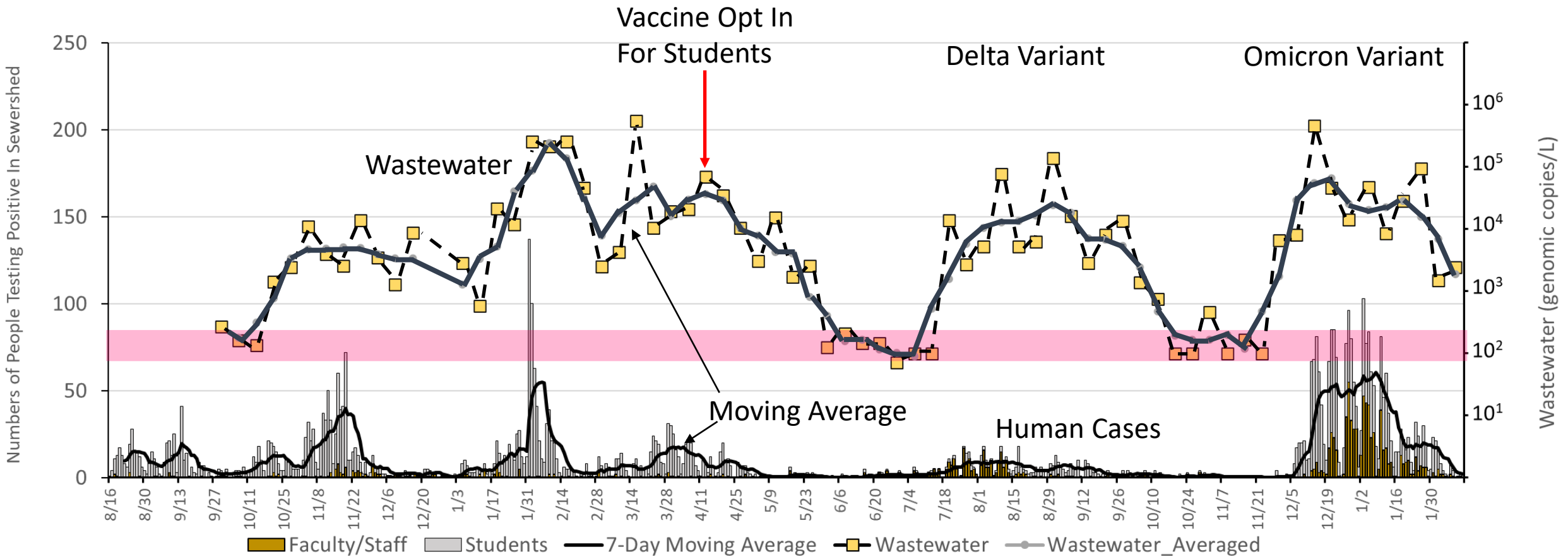


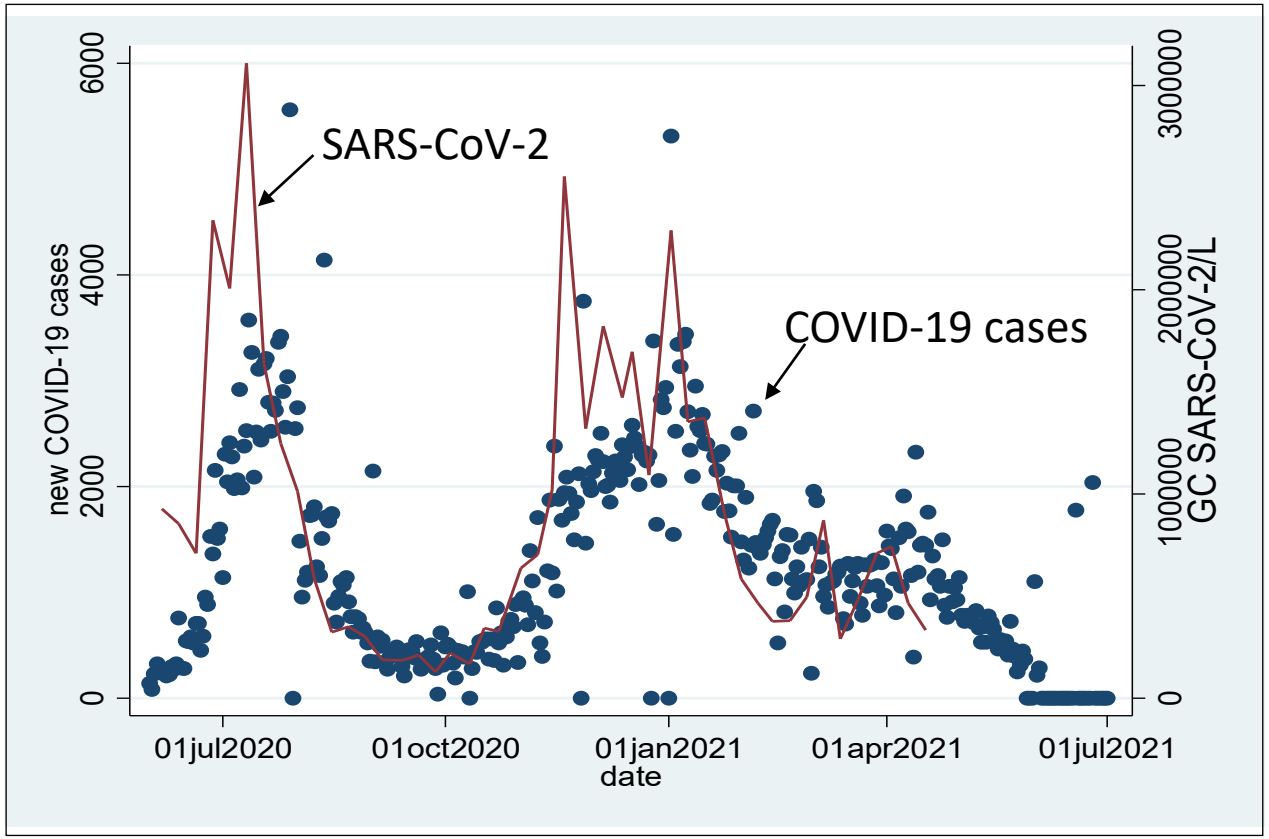
County Scale
(Miami-Dade County, FL)

Log-log association between SARS-Cov-2 and new COVID-19 cases shows that

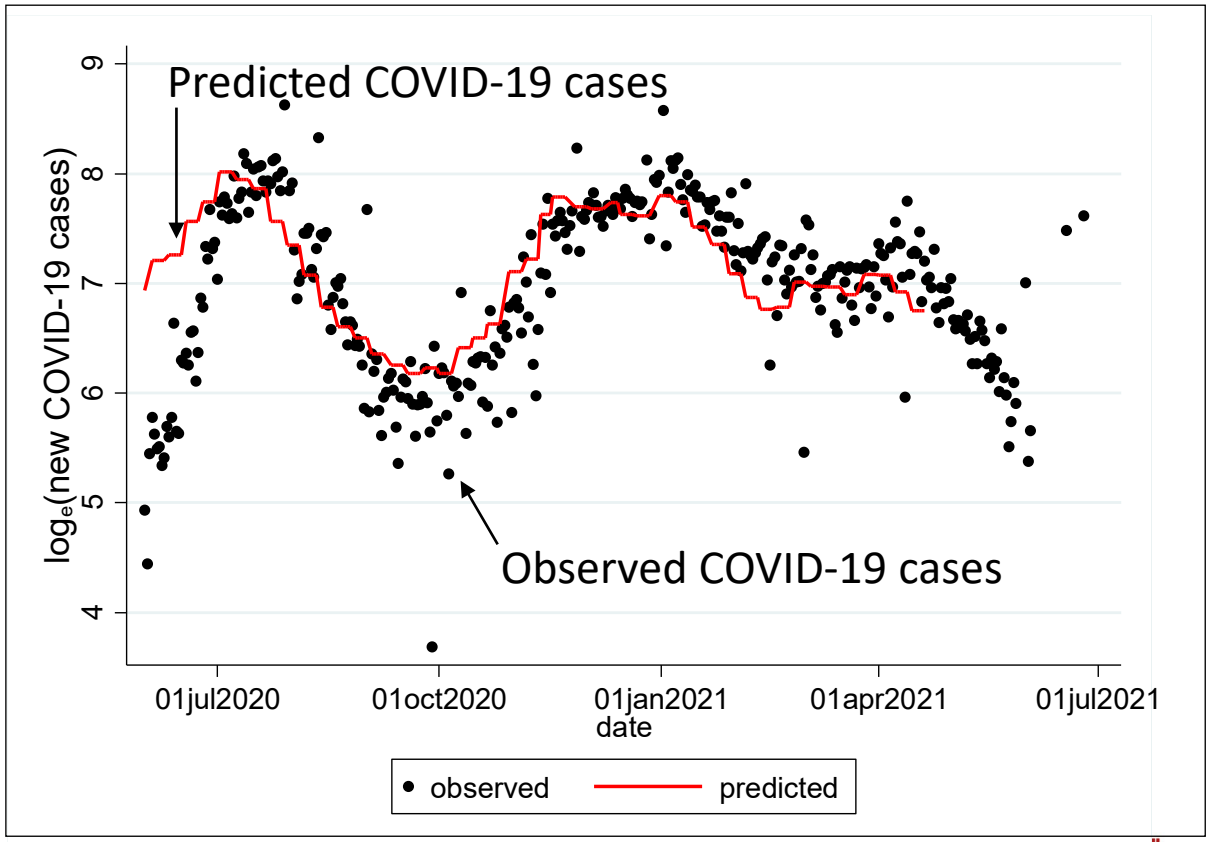
a 1% increase in SARS-CoV-2 was associated with 0.69% increase in COVID-19 new cases, June 2020 to May 2021

Coefficient = 0.69, 95% CI = 0.53 - 0.85; p < 0.01)

Association between SARS-Cov-2 and new COVID-19 cases in Miami-Dade



Association between observed and predicted new COVID-19 cases in Miami-Dade



Pilot study with Miami-Dade Public Schools

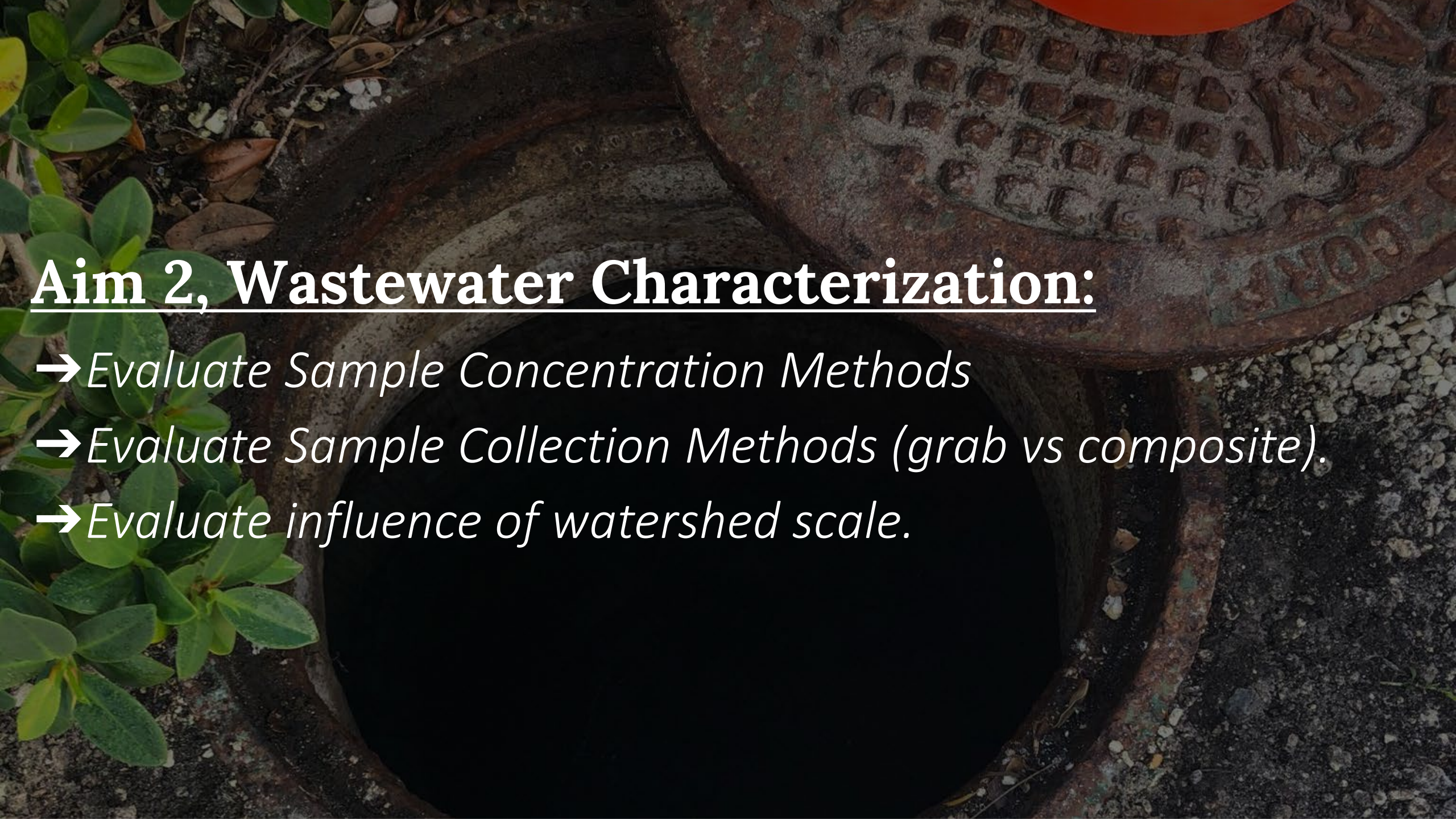
- UMiami also leading RADx-UP initiative (Dr Lisa Gwynn, PI)
- COVID-19 testing at 9 Miami-Dade public schools

  Elementary Schools: Arch Creek, Greynolds, Fulford, Sabal Palm

  Middle Schools: North Miami, JFK

  High Schools: N. Miami, N. Miami Beach, Booker T. Washington

"Maximizing Child Health and Learning Potential: How to Promote a School Culture of Safety in the Era of COVID-19."



Aim 2, Wastewater Characterization:

➔*Evaluate Sample Concentration Methods*

➔*Evaluate Sample Collection Methods (grab vs composite).*

➔*Evaluate influence of watershed scale.*

Sample Collection Methods

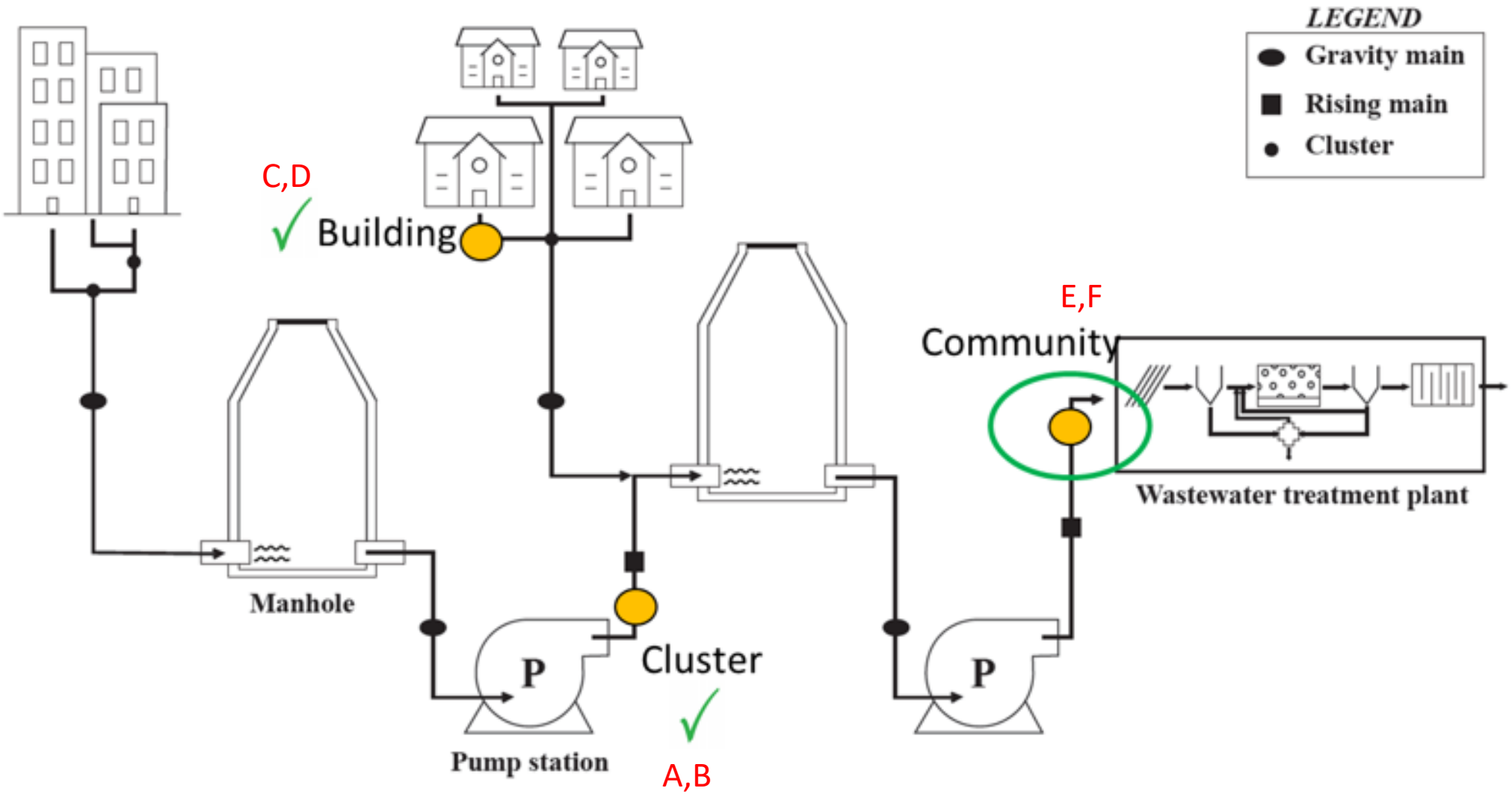
- **Grab** = Instant in time and space (fresh sample)
- **Composite** = Samples collected over time at one location (sample sits)

Wastewater Sampling

- ***Where do we sample wastewater from?***
  - Manholes (building scale)
  - Pump Stations (cluster)
  - Wastewater Treatment Plant
- ***How* do we collect the wastewater?**
  - Chain and bottle (grab)
  - Automatic sampler (composite)
    - Samples on each hour, the entire day- 24 hours

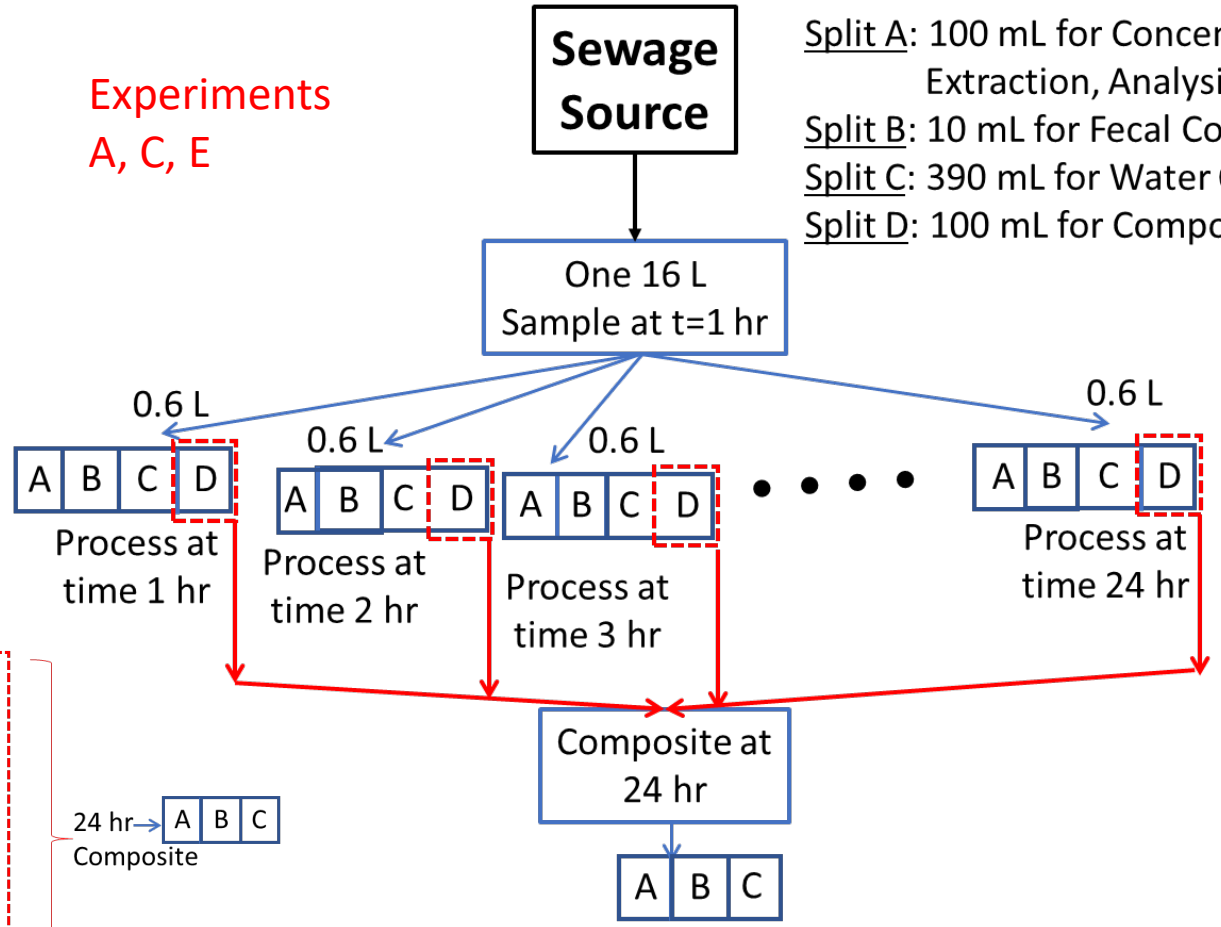
ISCO

Watershed Scales

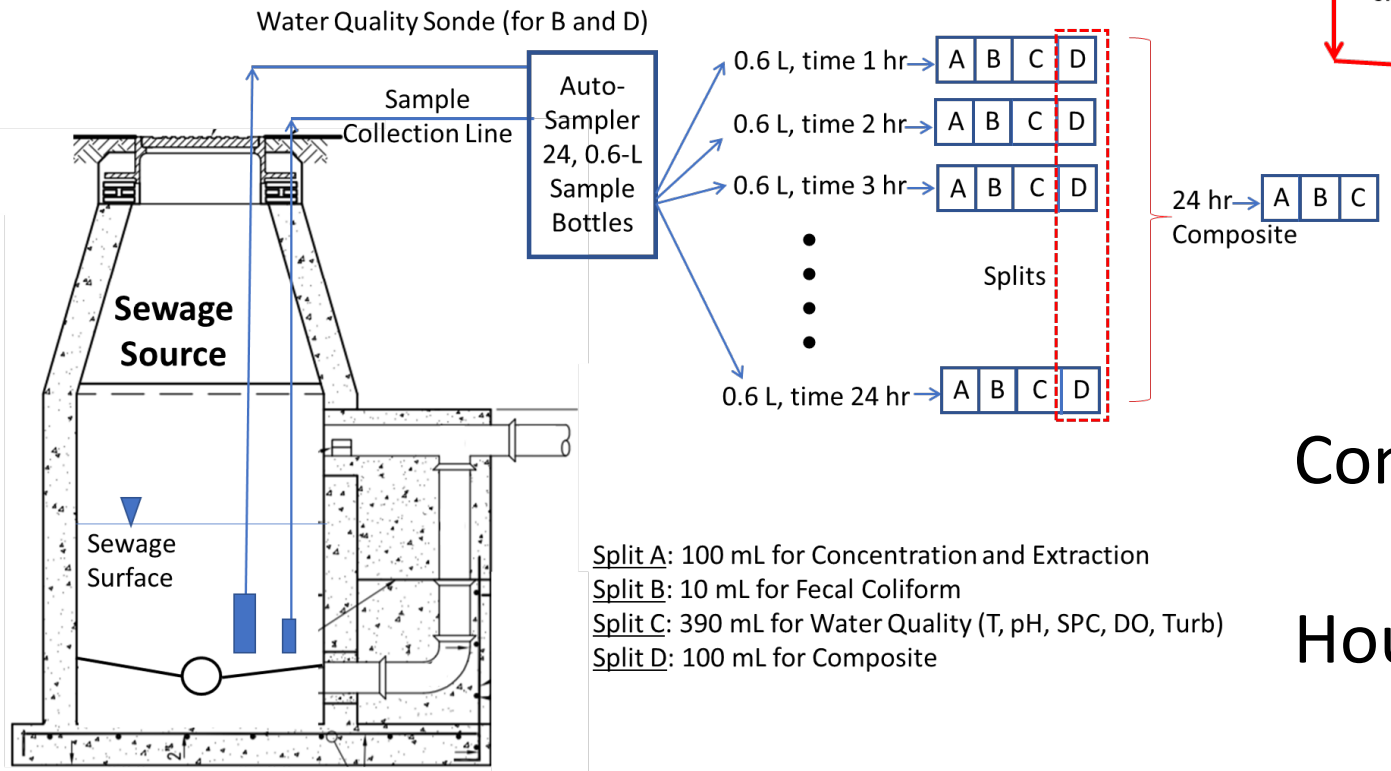


Experimental Design

Experiments
A, C, E



Experiments
B, D, F



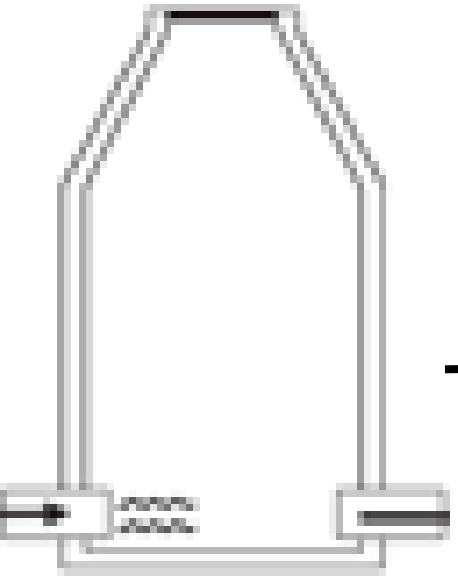
Split A: 100 mL for Concentration and Extraction
Split B: 10 mL for Fecal Coliform
Split C: 390 mL for Water Quality (T, pH, SPC, DO, Turb)
Split D: 100 mL for Composite

Compare degradation of SARS-CoV-2

Hour-to-hour variability

PALL
Pall Corporation
MicroFunnel Filter Funnels
5000 mL
KIMAX
KIMBLE

Sample Analysis Plan (Hourly Sampling)



Continuous Flow and continuous water quality measurements

Water Quality
T, pH, SPC, DO, Turb

Fecal Coliform

Composite

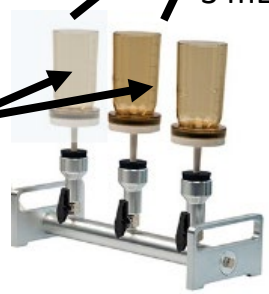
250 mL

OC43 & SIV

$MgCl_2$

Acidify (HCl)

Electronegative Filtration



2 Filters in 3 mL DNA/RNA Shield

Integrated Genomics Lab, Weill Cornell Medicine/MetaSub
Chris Mason

Sylvester Cancer Center OncoGenomics Shared Resource
Sion Williams

Center for AIDS Research
Mark Sharkey

Extraction

HIV Spike → Pure RNA

SARS-CoV-2 (NC gene)

OC43

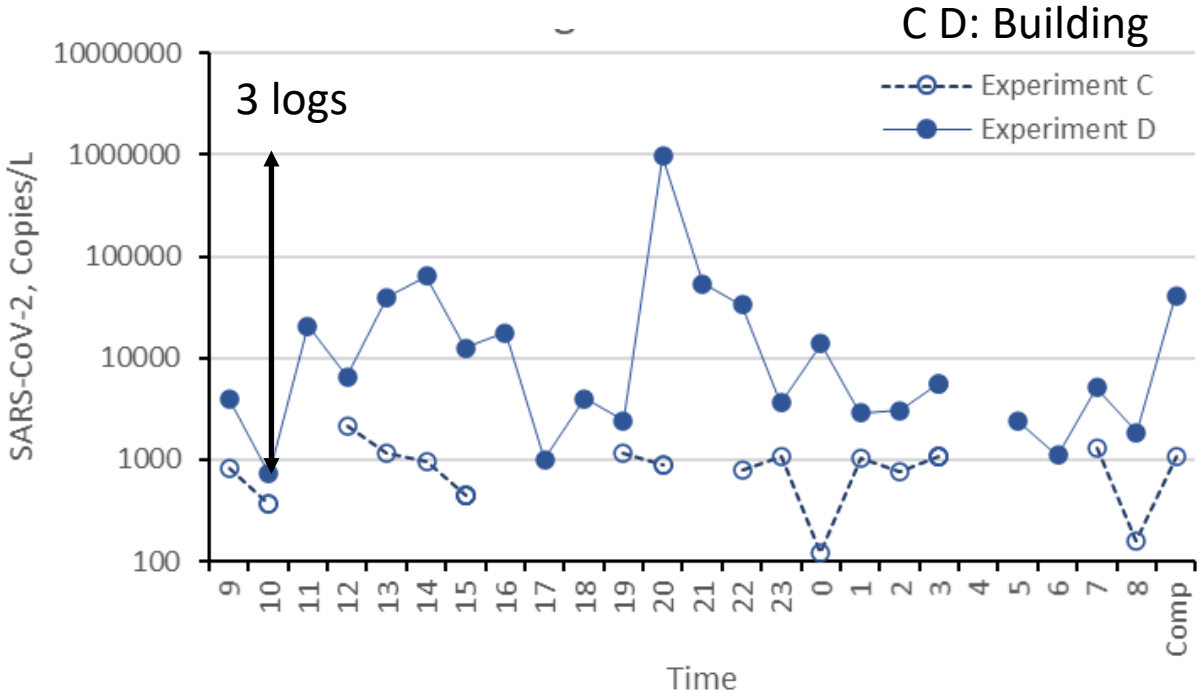
HIV Extract control

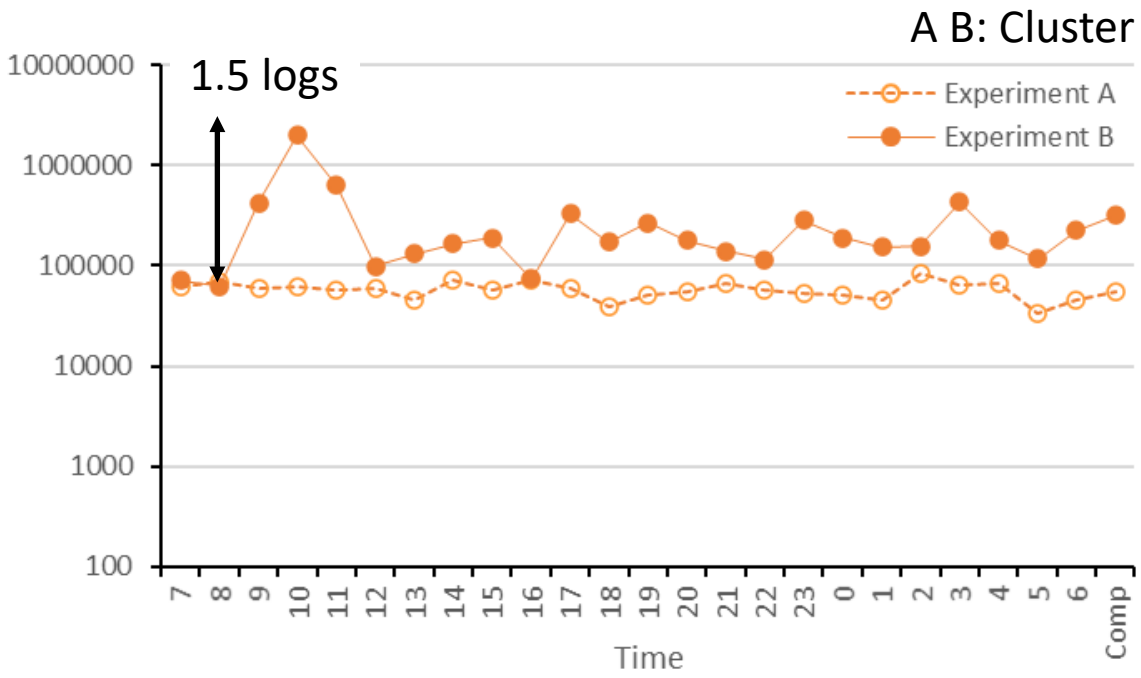
B2M

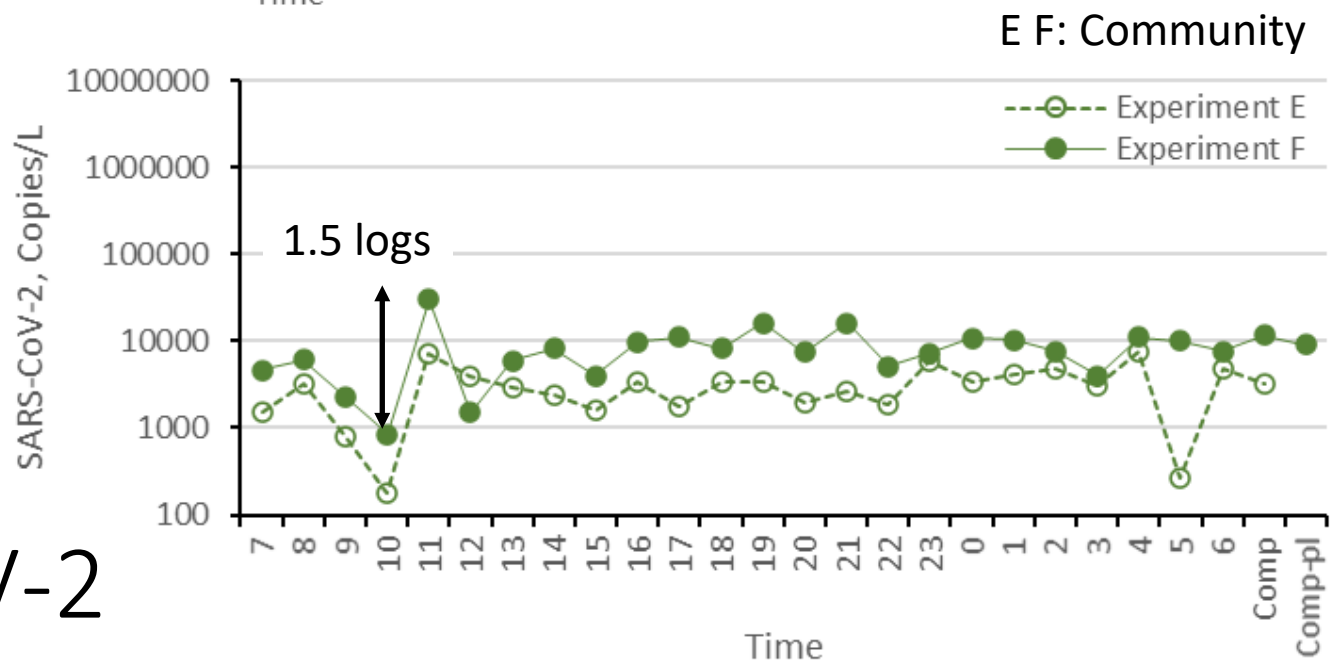
PMMoV

SIV

V2G-qPCR


C D: Building
3 logs
Experiment C
Experiment D
SARS-CoV-2, Copies/L
10000000
1000000
100000
10000
1000
100
9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 0 1 2 3 4 5 6 7 8 Comp
Time


A B: Cluster
1.5 logs
Experiment A
Experiment B
10000000
1000000
100000
10000
1000
100
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 0 1 2 3 4 5 6 Comp
Time


E F: Community
1.5 logs
Experiment E
Experiment F
SARS-CoV-2, Copies/L
10000000
1000000
100000
10000
1000
100
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 0 1 2 3 4 5 6 Comp Comp-pl
Time

SARS-CoV-2

Conclusions

- Wastewater in sewer variable.
  - 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, secondary
- Fecal coliform shows evidence of multiplication in sewer
- Specific conductivity correlated with fecal coliform at building scale

Next Steps

- Complete analysis of remaining targets including metagenomics
- Run statistics

Aim 3:
Integration with Human Health Surveillance

Data and Methods Overview

- **Analysis pipeline** customized for metagenomic sampling
  - SARS-CoV-2 reads identified and filtered (kraken2)
  - Aligned to Wuhan reference with very sensitive local alignment (bowtie2)
  - Trim adapters (ivar trim) and deduplicate reads (Picard)
  - Compile coverage per base (samtools mpileup)
  - Variants called with liberal filters (min depth = 3; VAF > 0.1) (ivar variants)
  - Variants of Concern (VOC) per WHO-defined lineages examined
- Applicable to wastewater and clinical samples

Weill Cornell Medicine

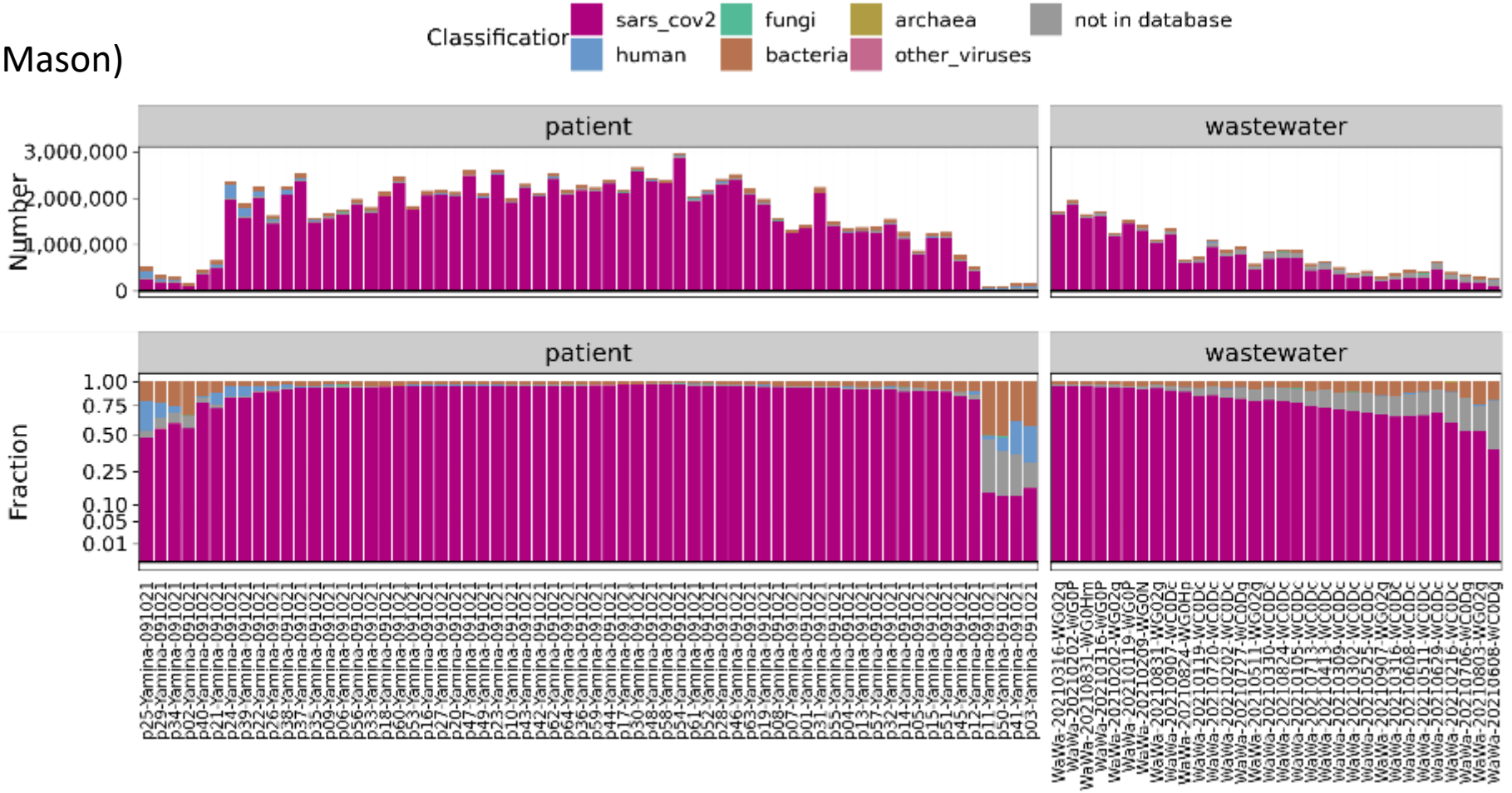
UNIVERSITY OF MIAMI

Oncogenomics Shared Resource Lab
Sylvester Comprehensive Cancer Center
(Sion Williams)

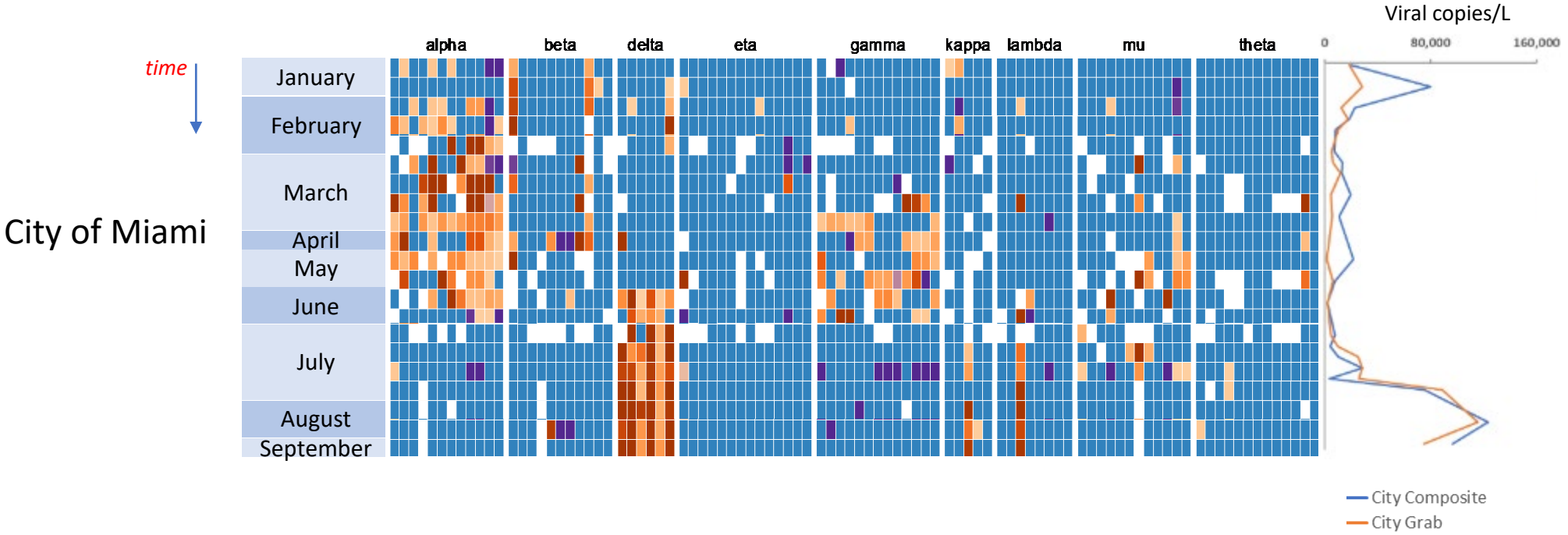
Weill Cornell Medicine

Integrated Genomics Lab (Chris Mason)

Performance of ARTIC protocol on clinical and wastewater samples: good library efficiency to amplify SARS-CoV-2



Detection of SARS-CoV-2 Lineages in Wastewater



Coverage of reference

Mutant allele (<10%)

Mutant allele (10%, 20%, etc.)

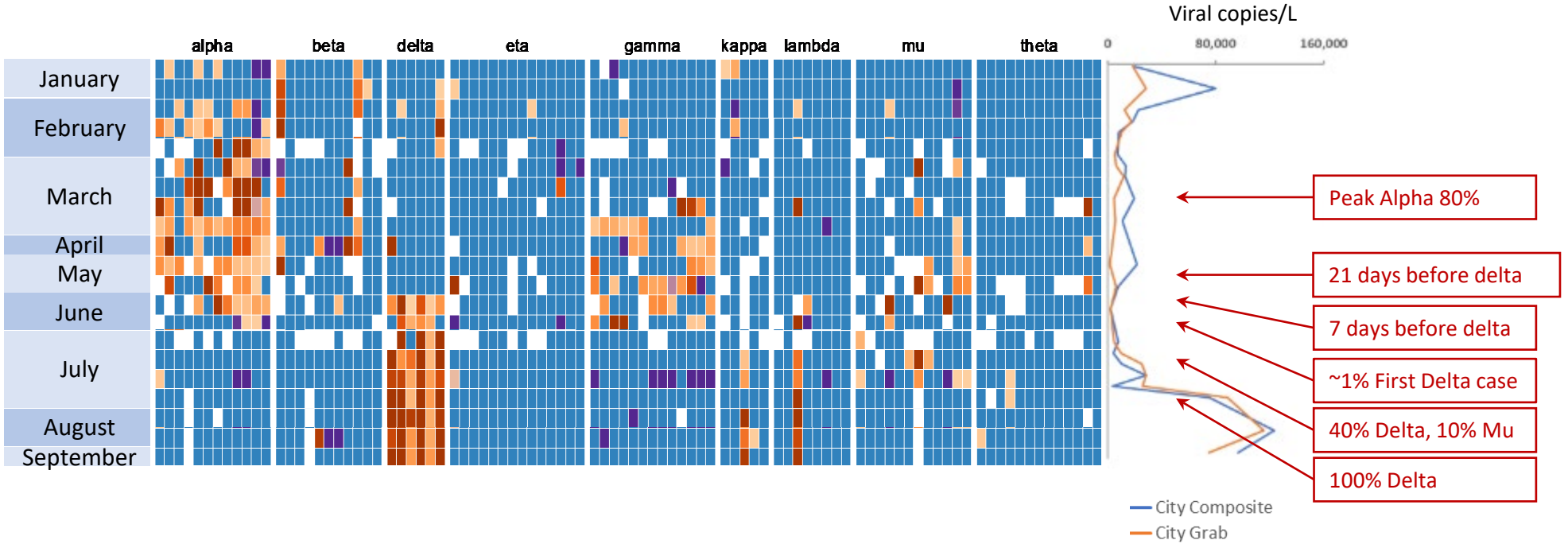
Mutant allele (100%)

No coverage of that site

- SARS-CoV-2 lineages in City wastewater mirror patient data
- Even at low viral load lineages can be discerned
- High diversity followed by Alpha, Gamma, Mu, then Delta
- Delta detectable at -7 days before first sequenced case

Detection of SARS-CoV-2 Lineages in Wastewater

City of Miami



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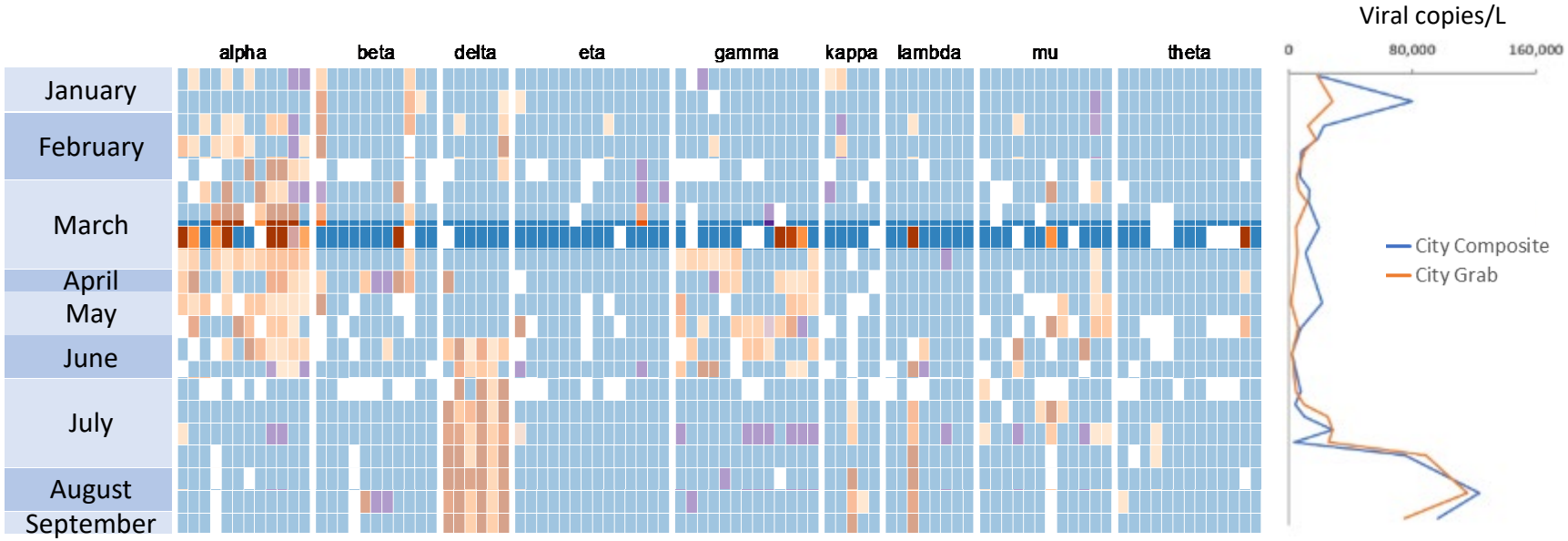
Detection of SARS-CoV-2 Lineages in Wastewater

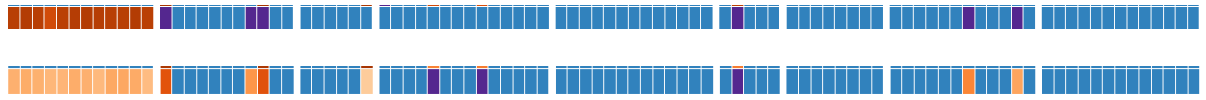
City of Miami



March 16th

South Gables Campus

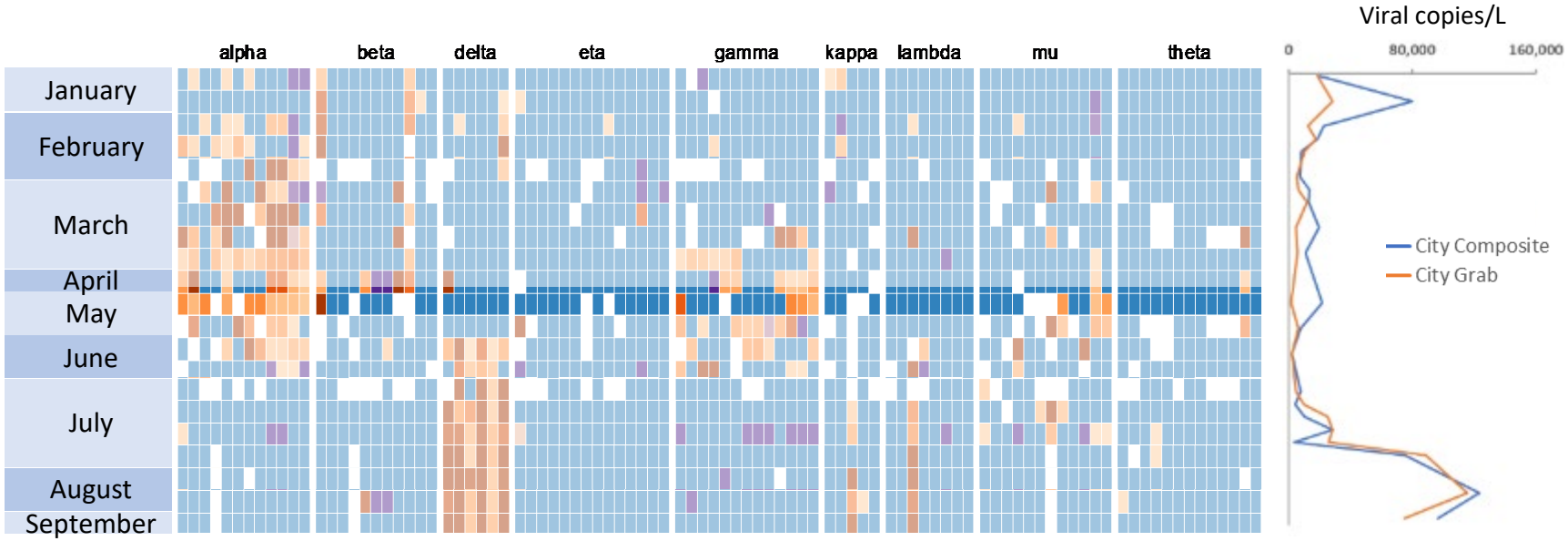
Building Cluster



- Peaks on Gables Campus reflect the abundance of Alpha in the City and patients

Detection of SARS-CoV-2 Lineages in Wastewater

City of Miami



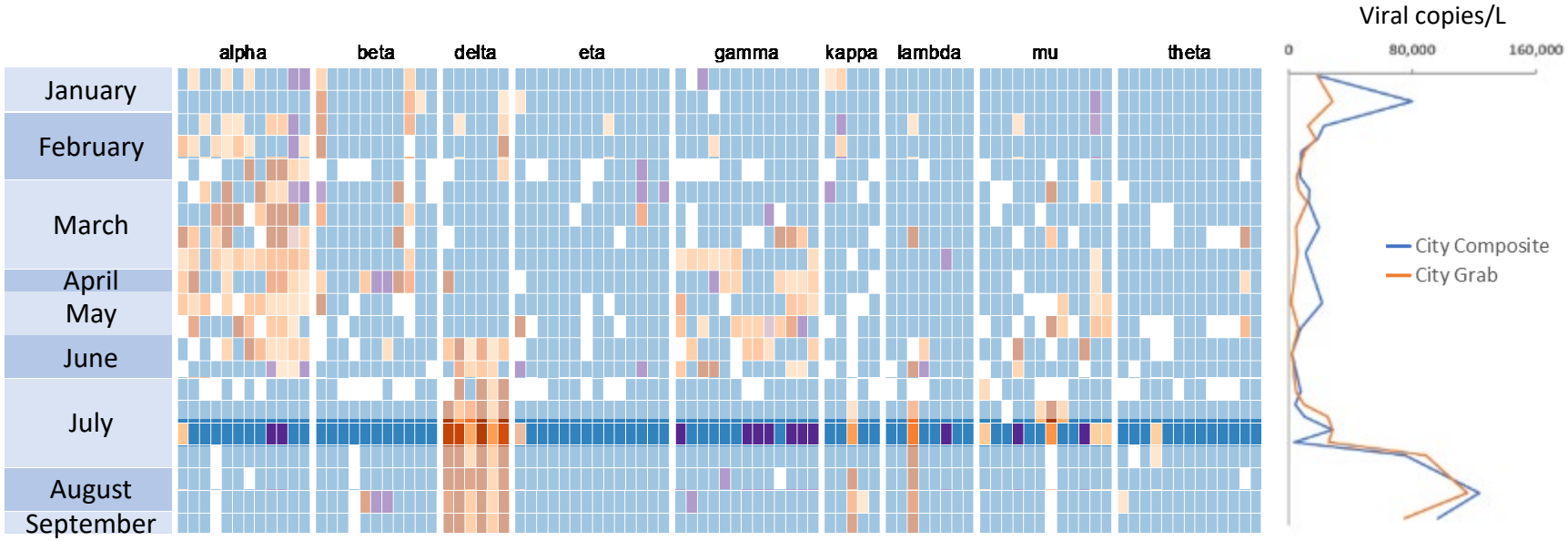
May 11th

South Gables Campus

- Low level Gamma cluster on campus
- Distinct from City lineage distribution

Detection of SARS-CoV-2 Lineages in Wastewater

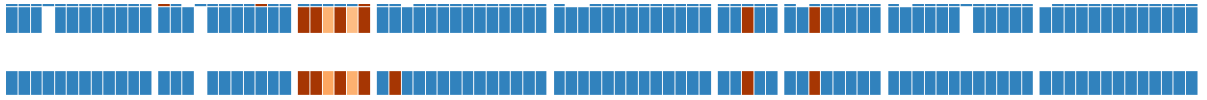
City of Miami



July 20th

North Gables Campus

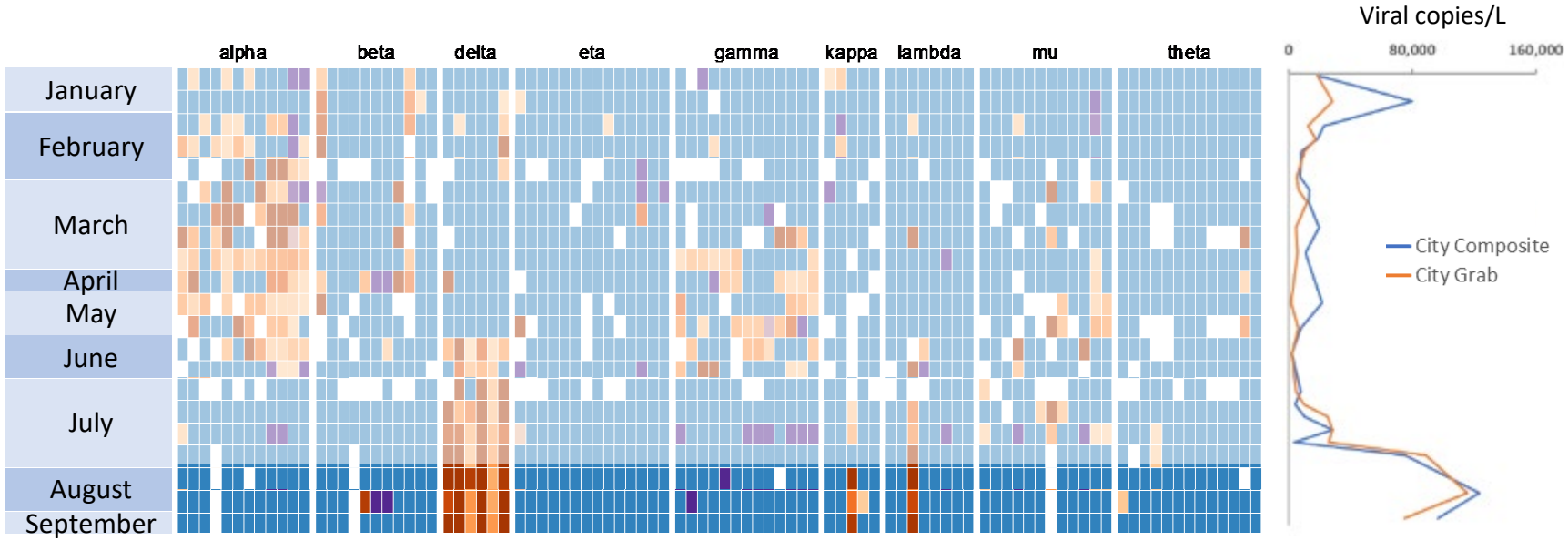
Building 12



- Building 12, 072021, all students tested, all negative
- Lineage matches abundant Delta strain

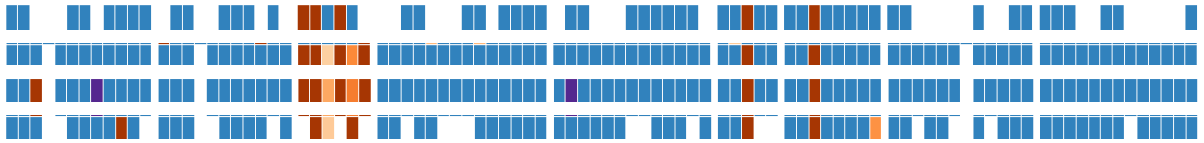
Detection of SARS-CoV-2 Lineages in Wastewater

City of Miami

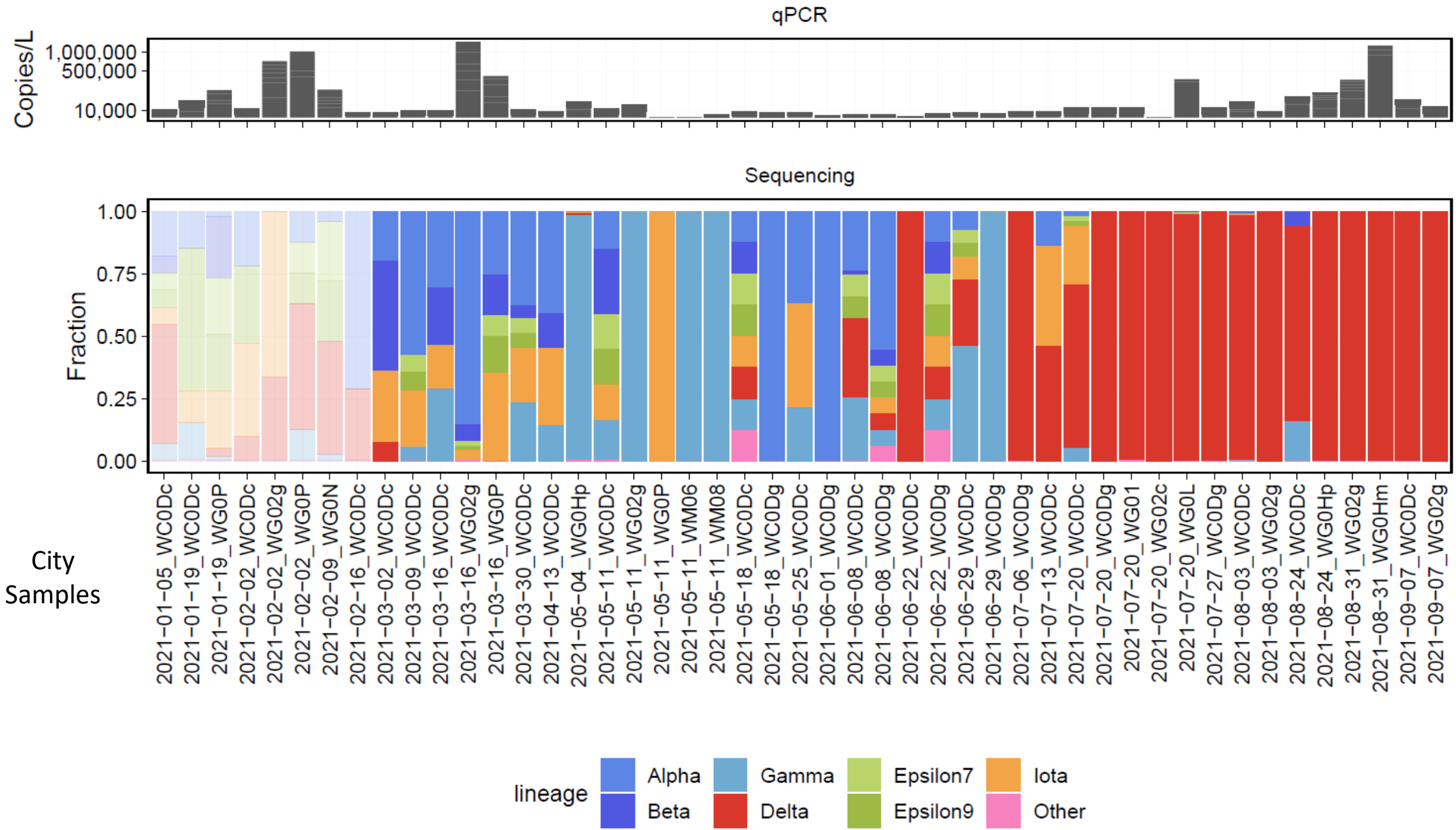


August 3rd – September 7th

South Gables Campus & Building Clusters



- Delta predominant lineage on campus in alignment with City and patients


qPCR
Copies/L
1,000,000
500,000
10,000
Sequencing
Fraction
1.00
0.75
0.50
0.25
0.00
City
Samples
2021−01−05_WC0Dc
2021−01−19_WC0Dc
2021−01−19_WG0P
2021−02−02_WC0Dc
2021−02−02_WG02g
2021−02−02_WG0P
2021−02−09_WG0N
2021−02−16_WC0Dc
2021−03−02_WC0Dc
2021−03−09_WC0Dc
2021−03−16_WC0Dc
2021−03−16_WG02g
2021−03−16_WG0P
2021−03−30_WC0Dc
2021−04−13_WC0Dc
2021−05−04_WG0Hp
2021−05−11_WC0Dc
2021−05−11_WG02g
2021−05−11_WG0P
2021−05−11_WM06
2021−05−11_WM08
2021−05−18_WC0Dc
2021−05−18_WC0Dg
2021−05−25_WC0Dc
2021−06−01_WC0Dg
2021−06−08_WC0Dc
2021−06−08_WC0Dg
2021−06−22_WC0Dc
2021−06−22_WC0Dg
2021−06−29_WC0Dc
2021−06−29_WC0Dg
2021−07−06_WC0Dg
2021−07−13_WC0Dc
2021−07−20_WC0Dc
2021−07−20_WC0Dg
2021−07−20_WG01
2021−07−20_WG02c
2021−07−20_WG0L
2021−07−27_WC0Dg
2021−08−03_WC0Dc
2021−08−03_WG02g
2021−08−24_WC0Dc
2021−08−24_WG0Hp
2021−08−31_WG02g
2021−08−31_WG0Hm
2021−09−07_WC0Dc
2021−09−07_WG02g
lineage
Alpha
Beta
Gamma
Delta
Epsilon7
Epsilon9
Iota
Other

Action Items

- Complete analysis of the changes in variant dominance in wastewater and patient populations
- Analyze daily data to decrease uncertainty in case predictions
- Evaluate normalization of SARS-CoV-2 using PMMoV, B2M, fecal coliform
- Analyze hourly data to evaluate representativeness of grab versus composite samples
- Evaluate magnetic bead concentration methods
- Continue data standardization
- Complete operational informatics infrastructure

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Thank you
https://covidsfrad.org

MIAMI

Weill Cornell Medicine

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President Frenk
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George Grills

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- Krista Ryon
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