Lessons Learned from SARS-CoV-2 Measurements in Wastewater

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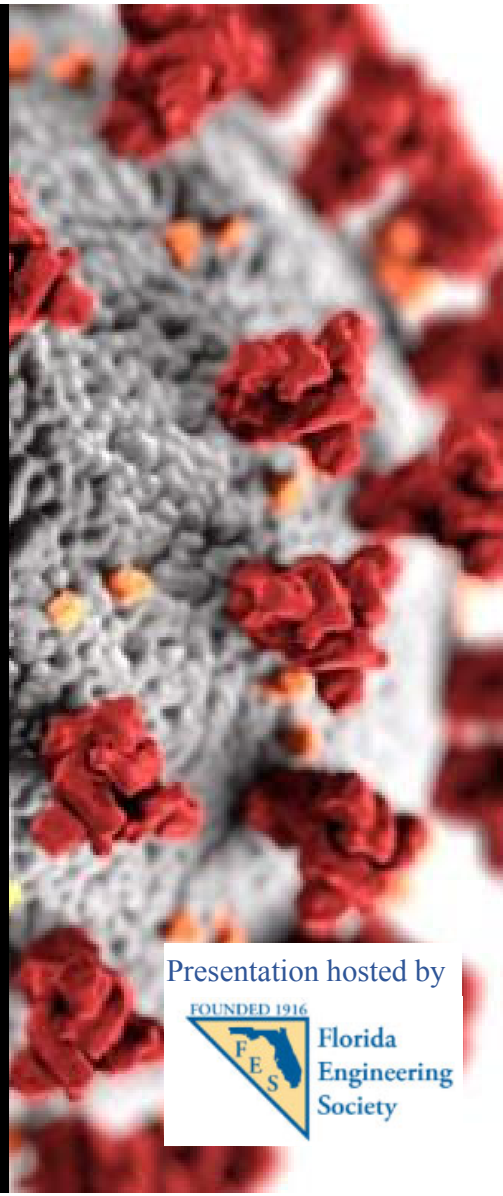

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Outline

- My background
- Study design
- Water quality results
- Concentration and Detection for SARS-CoV-2
- SARS-CoV-2 results
- Lessons learned
- Acknowledgments

My Background

- Microbes at beaches, sewage as a source
- Oceans and Human Health Center, 2005 – 2015
- Teach Water Analysis (drinking water and wastewater)

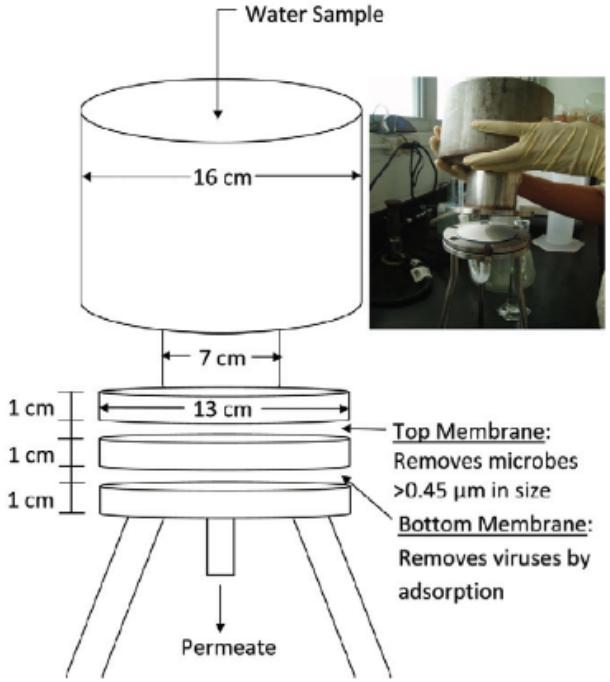
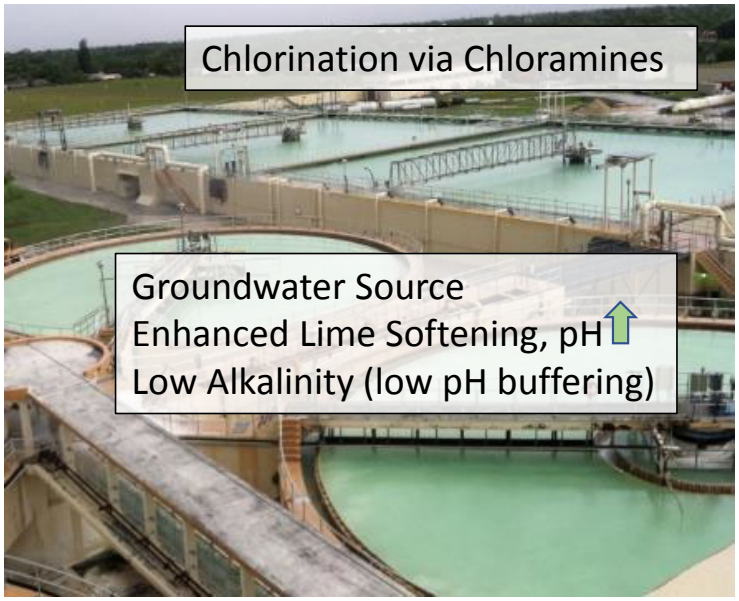
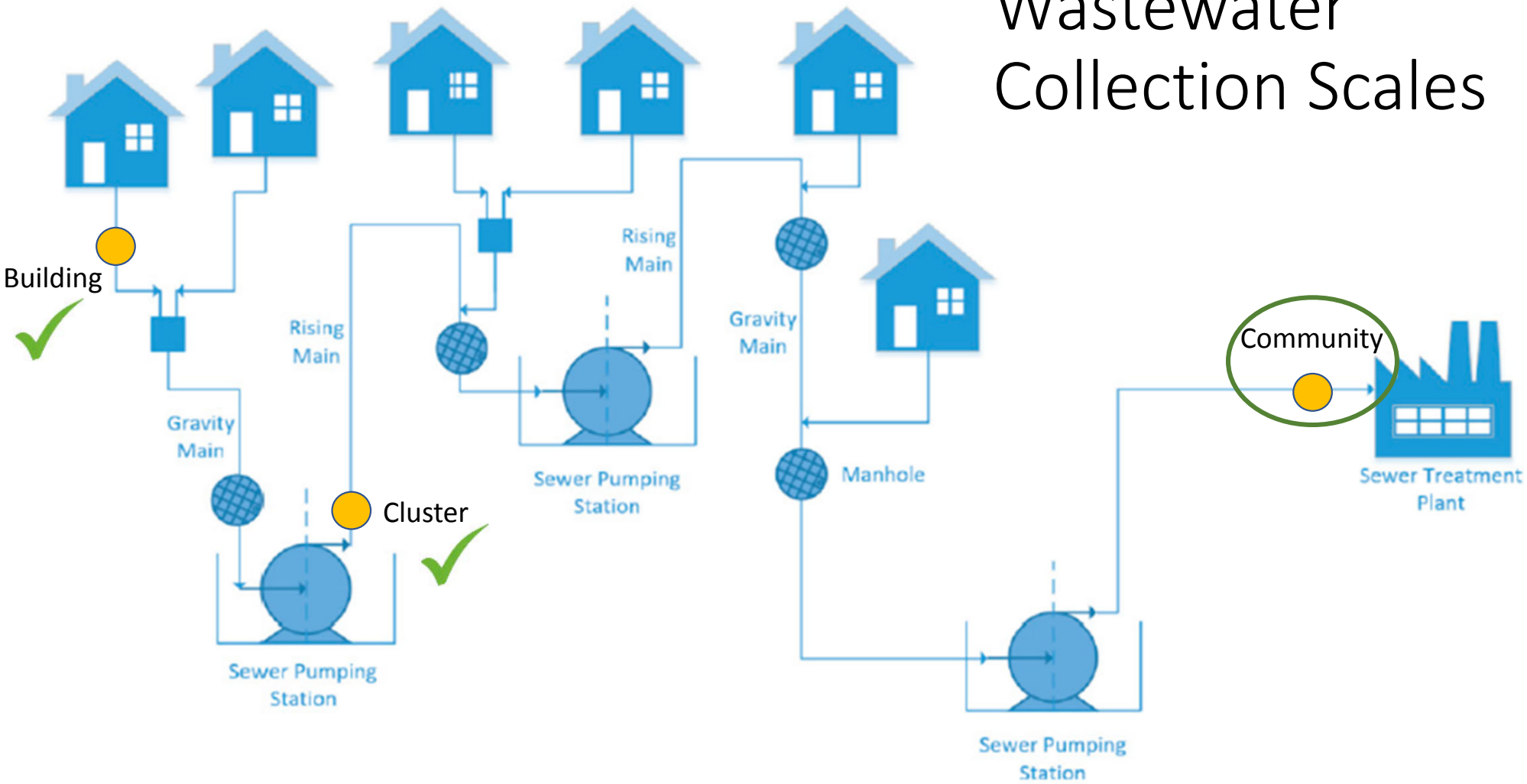


Fig. 1. Simplified Schematic and Photograph of the 90-mm stainless steel filtration apparatus.



Wastewater Collection Scales



Base image from Drenoyanis et al. 2019

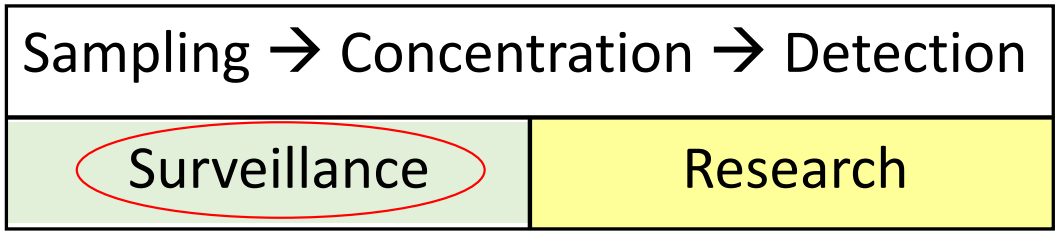
Motivation & Objectives

SARS-CoV-2 RNA excreted in feces and urine from symptomatic and asymptomatic individuals (4 to 10 day early warning).

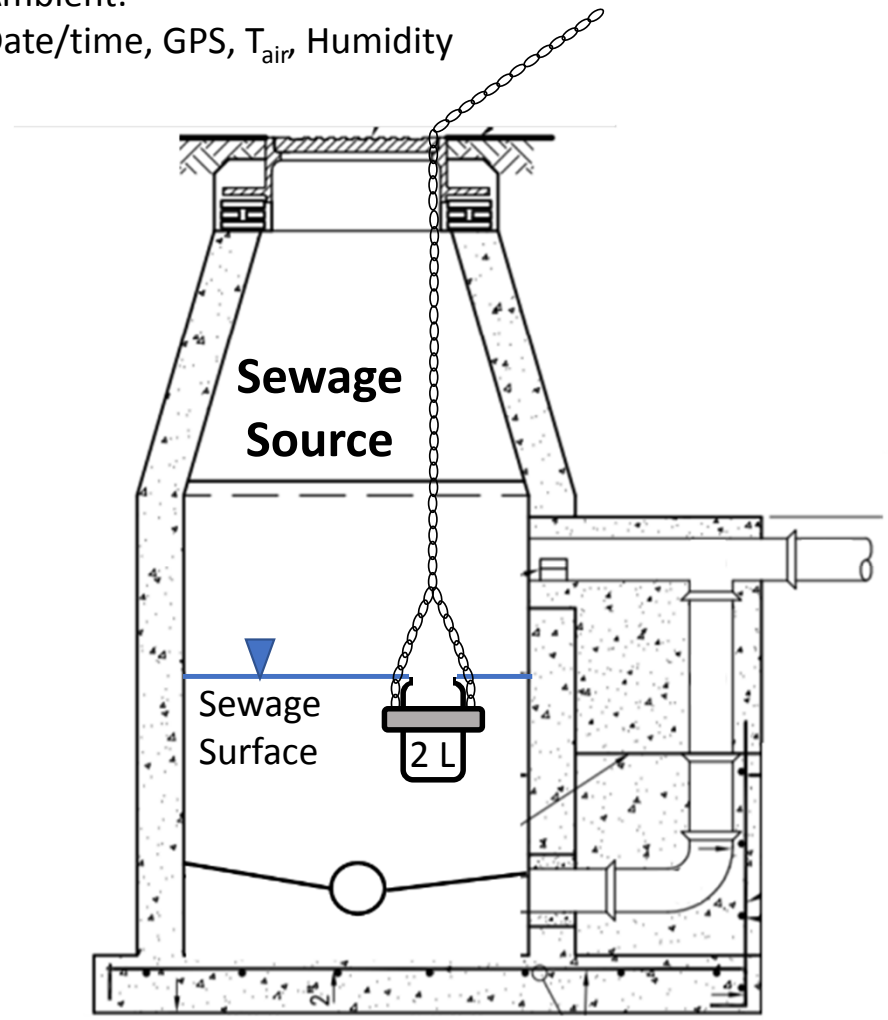
Ultimate objective: Can wastewater measurements be used to predict COVID-19 cases?

(building, cluster, and community scales)

Current objective:



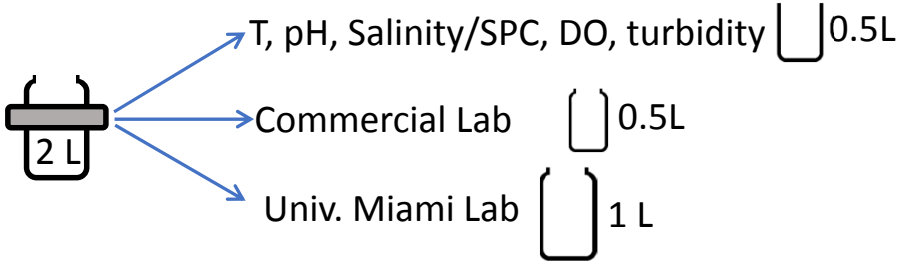
Ambient:
Date/time, GPS, T_{air}, Humidity



Collect Samples Weekly (Wednesdays)
Results available in 12 hours

Sampling Sites (6 to 12 per sampling day)

- Individual Buildings (B), includes hospitals
- Building Clusters (C), All 3 campuses

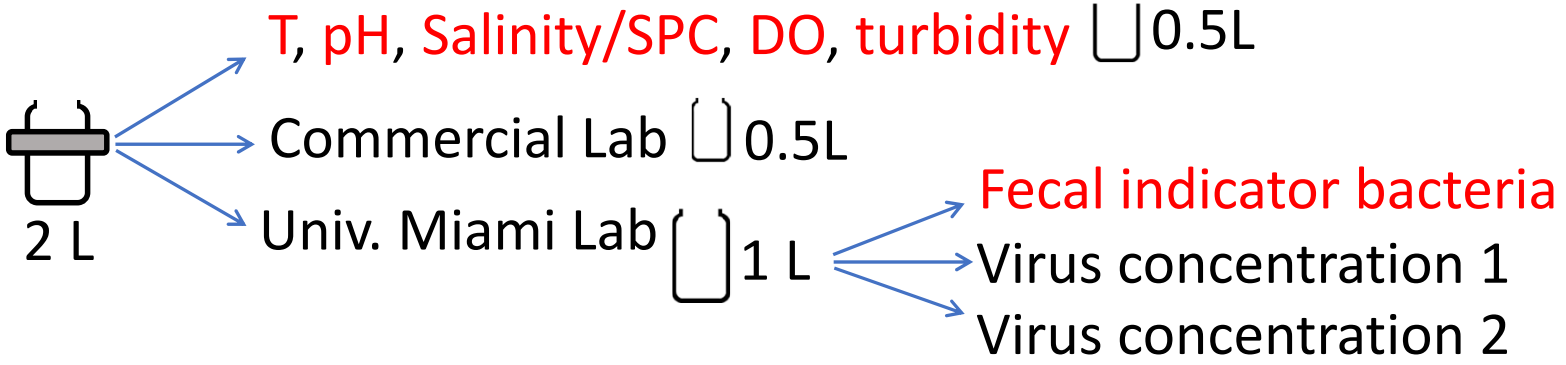


SAMPLING

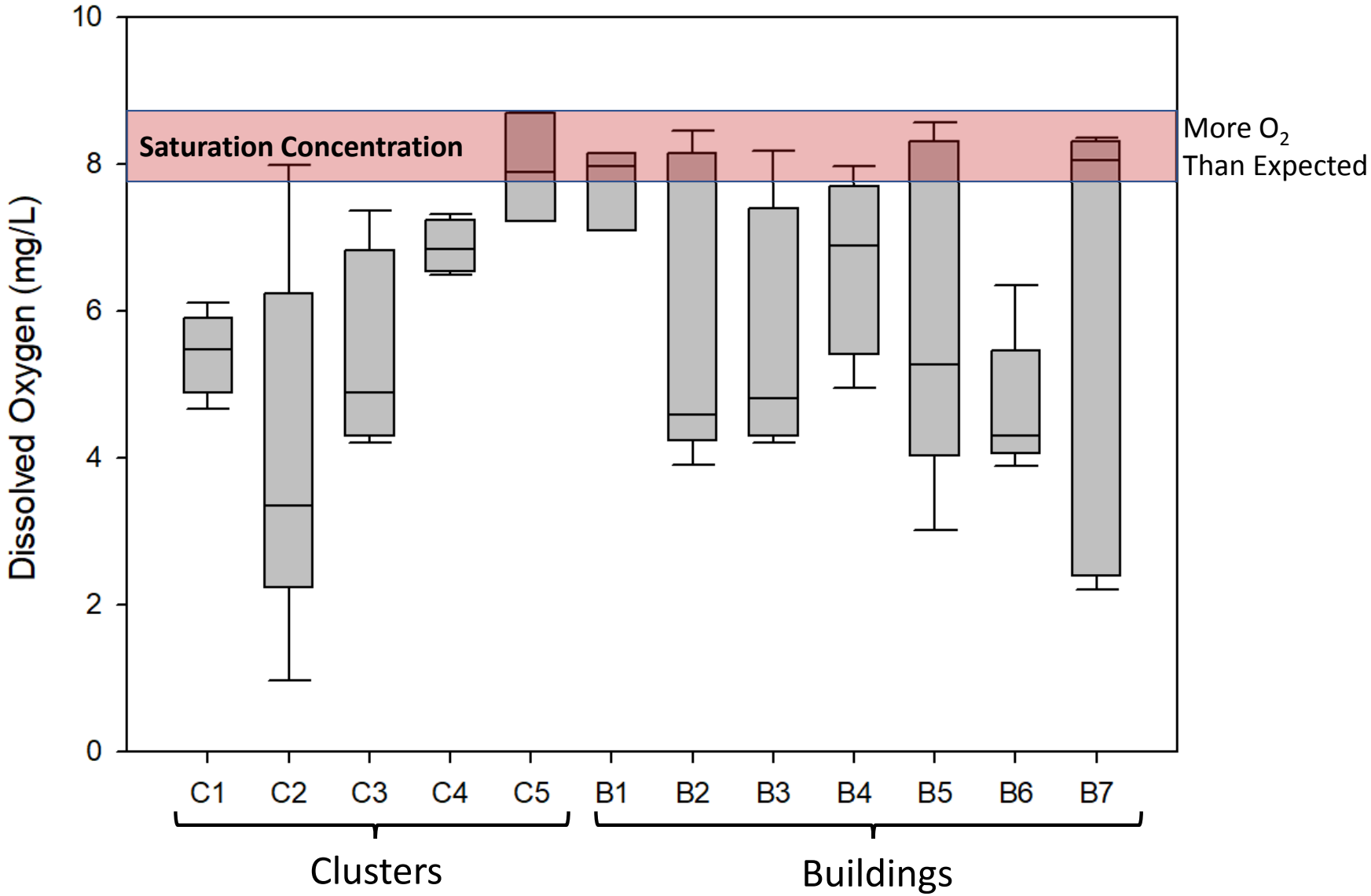
Study Design

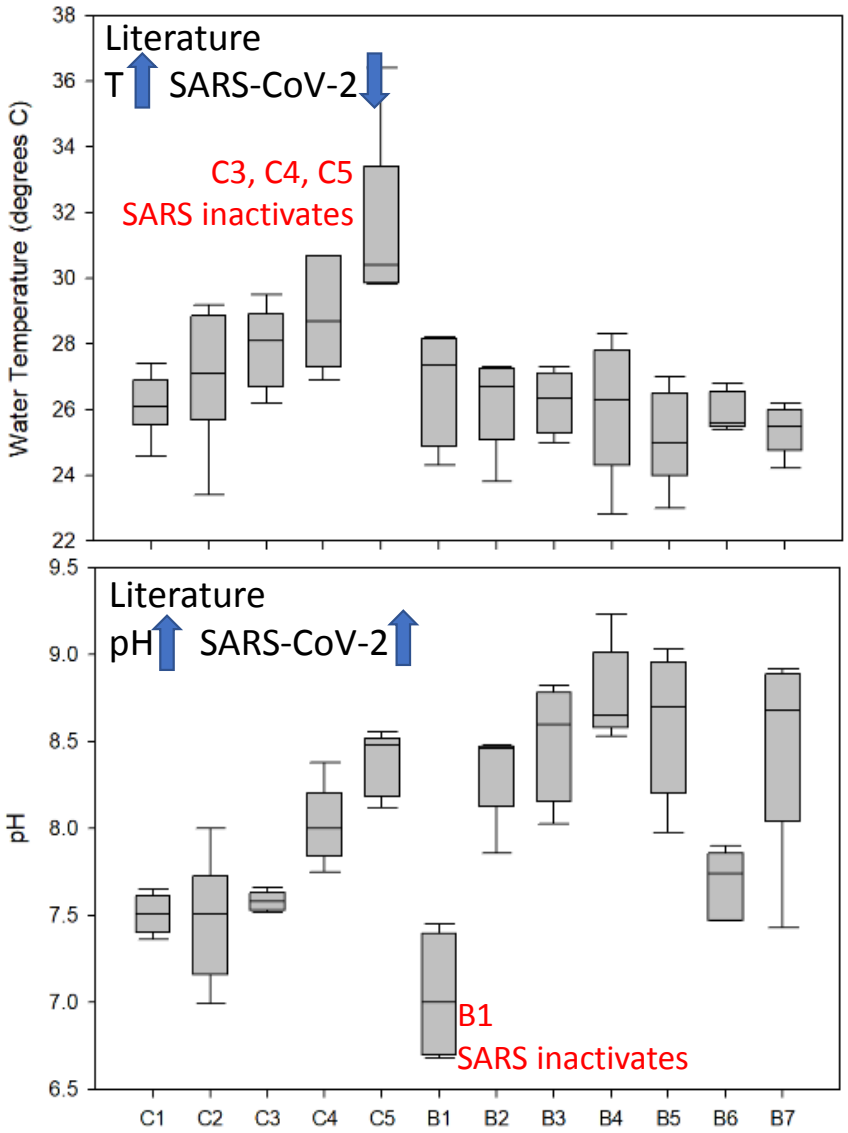
Sampling → Concentration → Detection


2 L

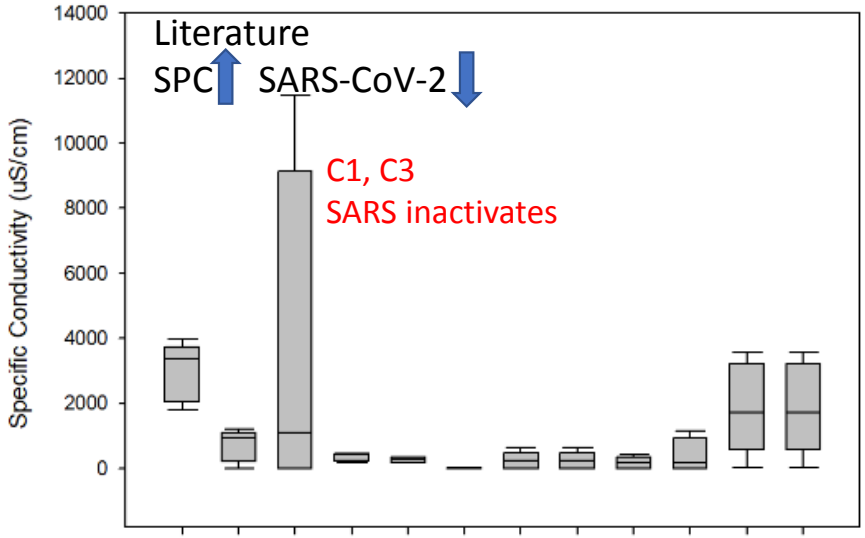

T, pH, Salinity/SPC, DO, turbidity 0.5L
Commercial Lab 0.5L
2 L
Univ. Miami Lab 1 L
Fecal indicator bacteria
Virus concentration 1
Virus concentration 2

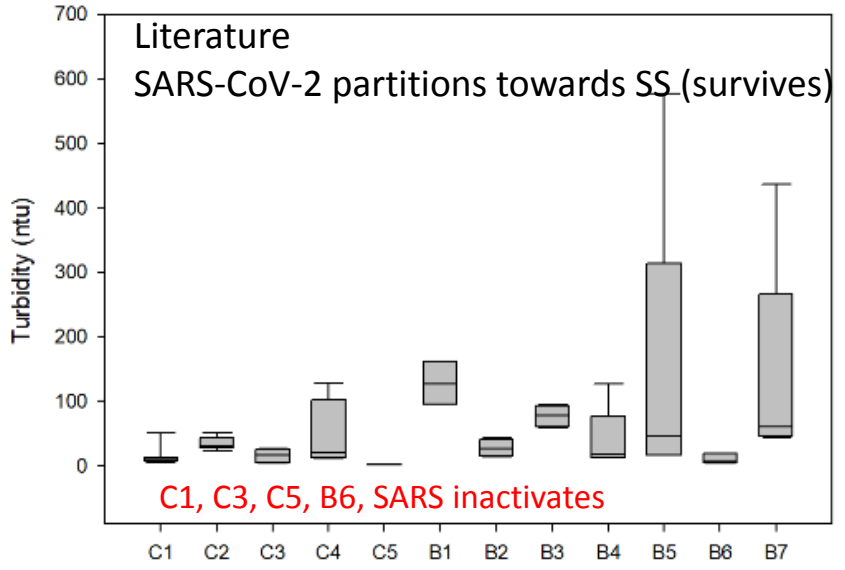
Water Quality Results


Dissolved Oxygen (mg/L)
10
8
6
4
2
0
Saturation Concentration
More O_2
Than Expected
C1
C2
C3
C4
C5
B1
B2
B3
B4
B5
B6
B7
Clusters
Buildings

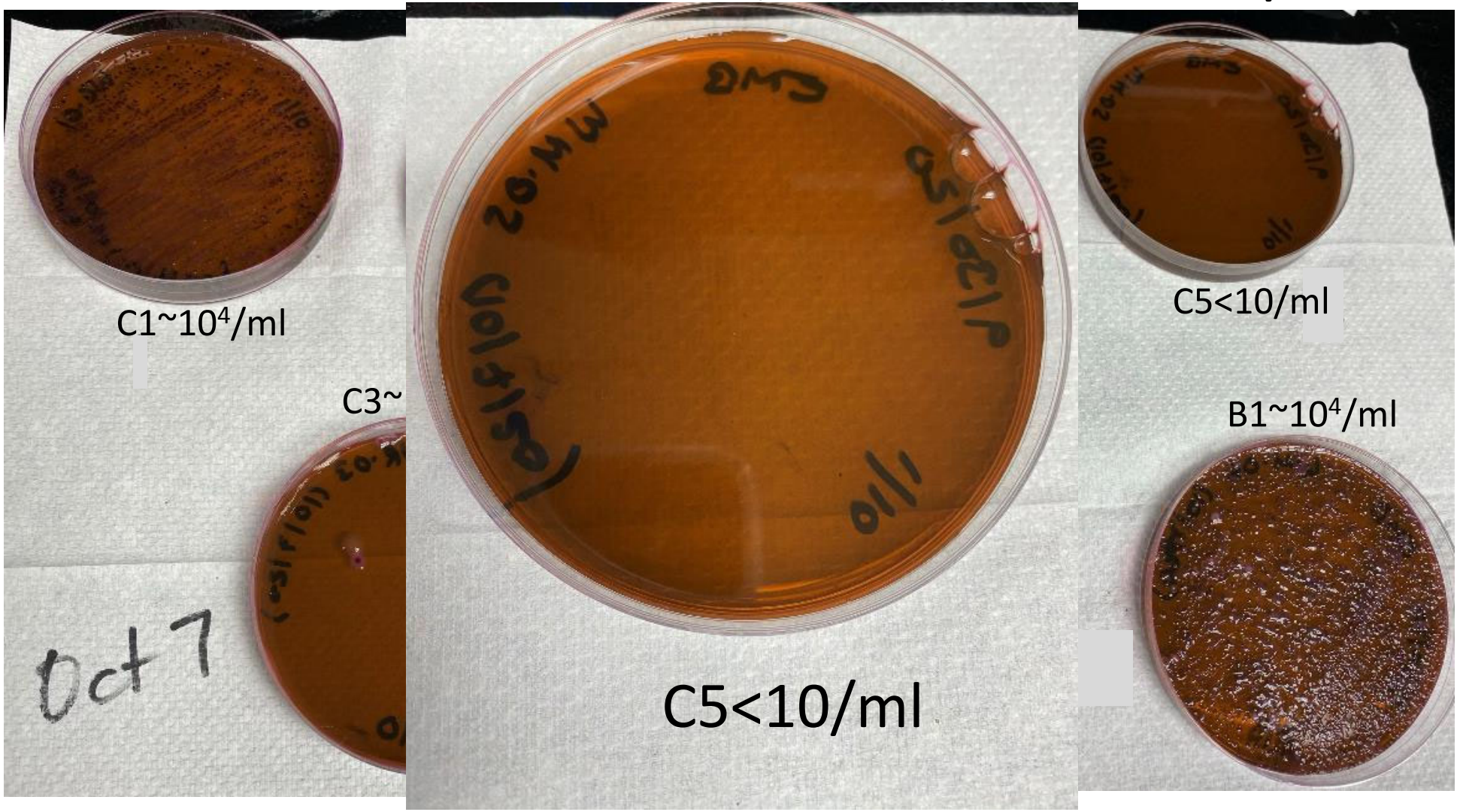

Literature
T SARS-CoV-2
C3, C4, C5
SARS inactivates
Water Temperature (degrees C)
38
36
34
32
30
28
26
24
22

Literature
pH SARS-CoV-2
B1
SARS inactivates
pH
9.5
9.0
8.5
8.0
7.5
7.0
6.5
C1 C2 C3 C4 C5 B1 B2 B3 B4 B5 B6 B7

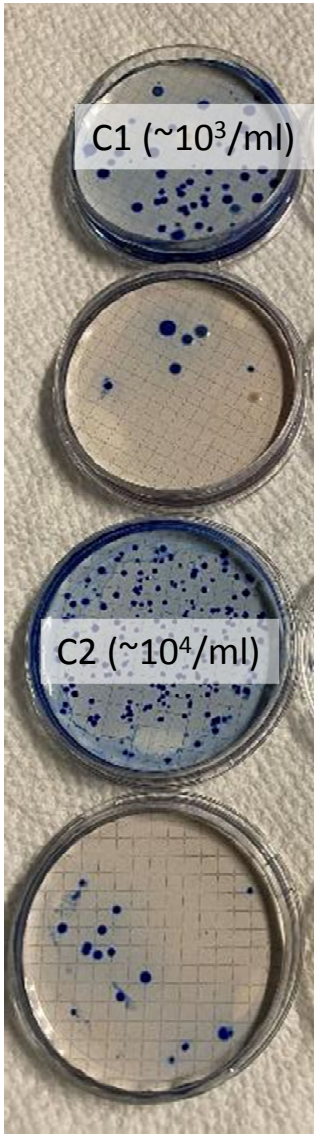

Literature
SPC SARS-CoV-2
C1, C3
SARS inactivates
Specific Conductivity (uS/cm)
14000
12000
10000
8000
6000
4000
2000
0


Literature
SARS-CoV-2 partitions towards SS (survives)
C1, C3, C5, B6, SARS inactivates
Turbidity (ntu)
700
600
500
400
300
200
100
0
C1 C2 C3 C4 C5 B1 B2 B3 B4 B5 B6 B7

Fecal Indicator Bacteria (*E. coli*) ~ 10^4 CFU/ml



Clusters

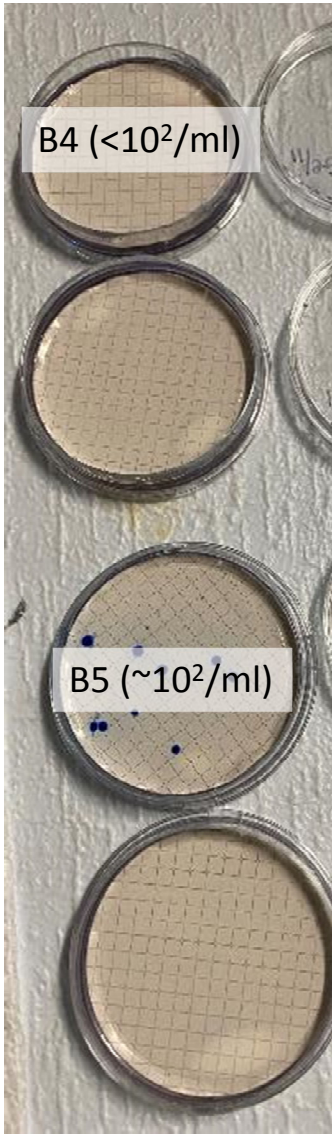


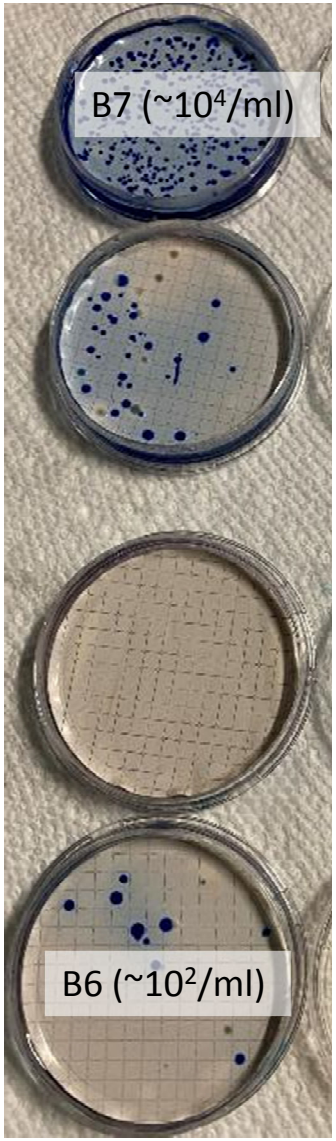
Buildings

Chlorine Residual?

↓

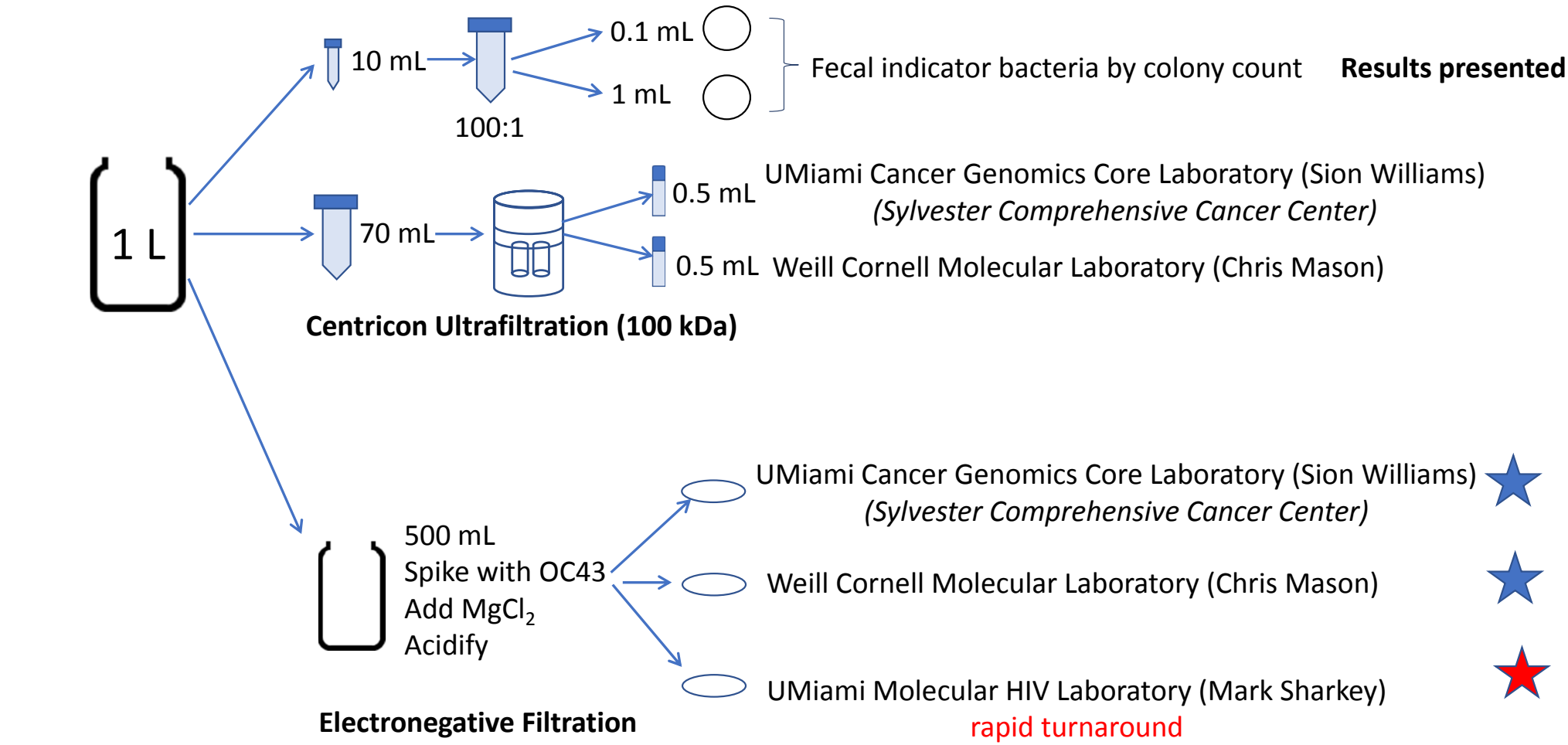
Neutralize Chlorine Upon Collection (sodium thiosulfate)

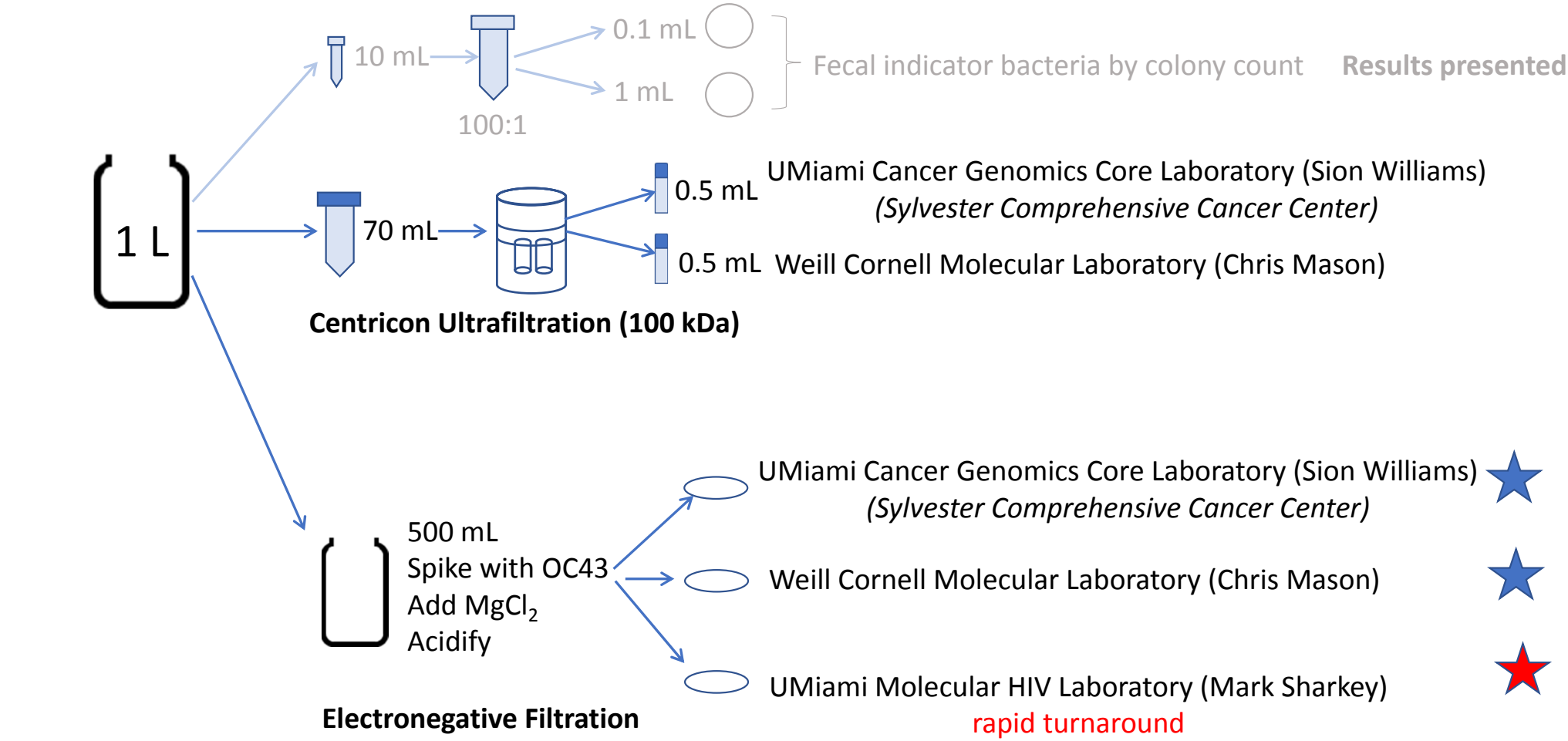




Fecal coliform

Concentration and Detection for SARS-CoV-2


10 mL
100:1
0.1 mL
1 mL
Fecal indicator bacteria by colony count
Results presented
1 L
70 mL
0.5 mL
UMiami Cancer Genomics Core Laboratory (Sion Williams)
(Sylvester Comprehensive Cancer Center)
0.5 mL
Weill Cornell Molecular Laboratory (Chris Mason)
Centricon Ultrafiltration (100 kDa)
500 mL
Spike with OC43
Add MgCl2
Acidify
UMiami Cancer Genomics Core Laboratory (Sion Williams)
(Sylvester Comprehensive Cancer Center)
Weill Cornell Molecular Laboratory (Chris Mason)
UMiami Molecular HIV Laboratory (Mark Sharkey)
rapid turnaround
Electronegative Filtration
Sampling → Concentration → Detection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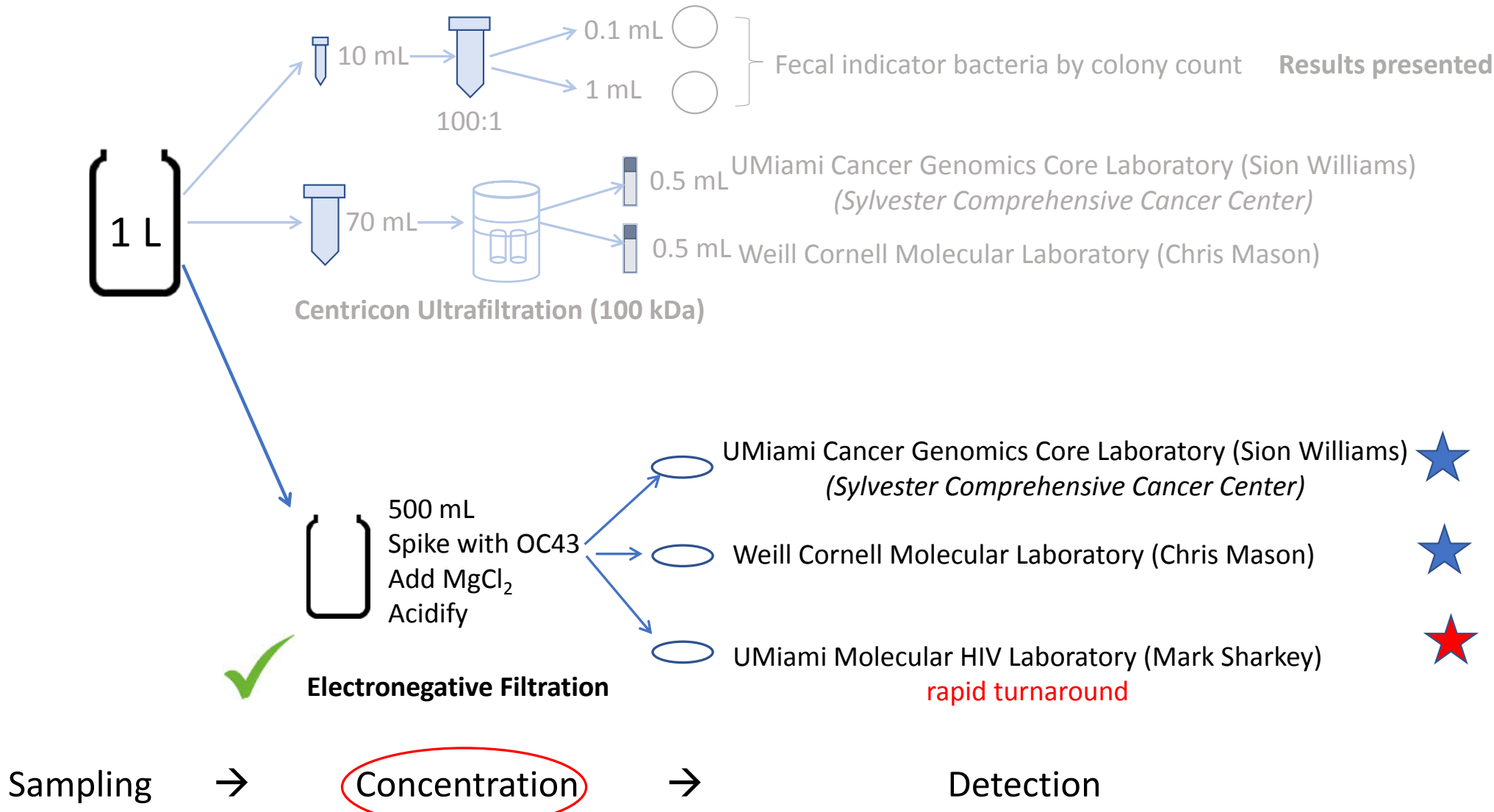
Sampling → Concentration → Detection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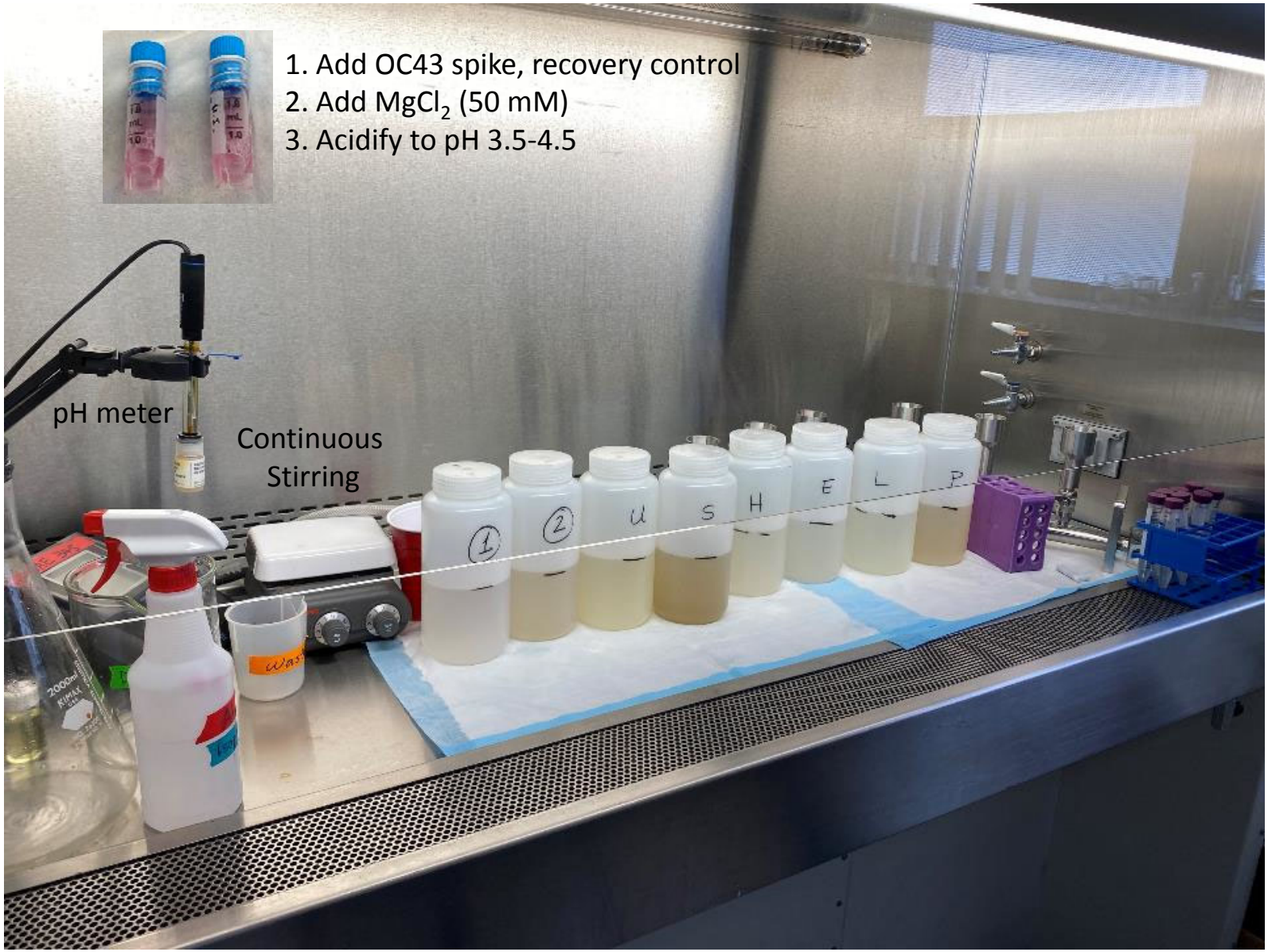
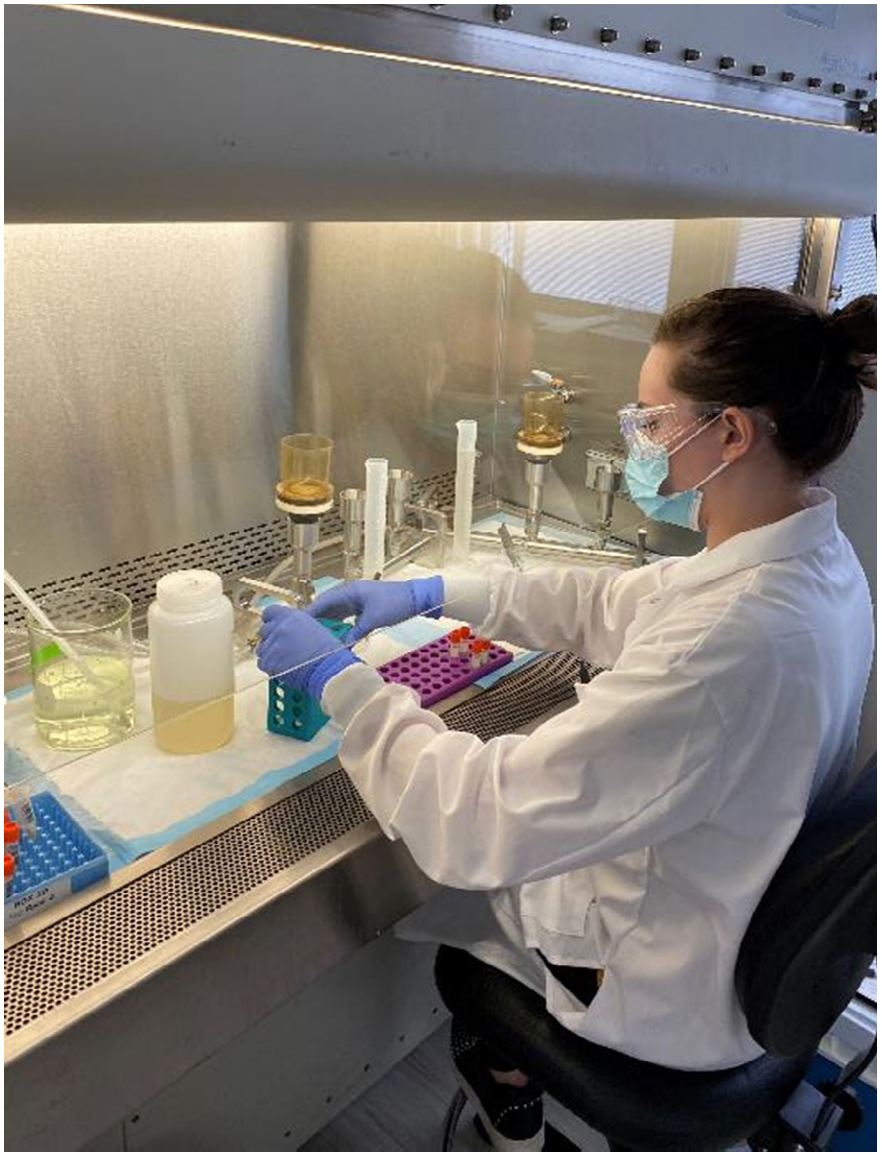
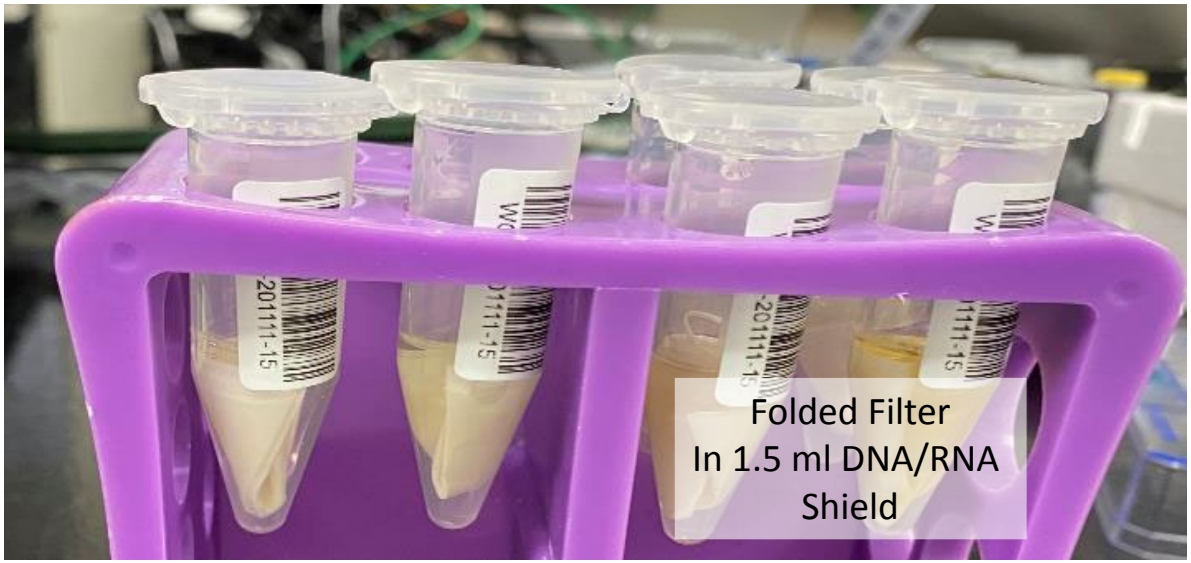
rapid turnaround
Electronegative Filtration
Sampling → Concentration → Detection

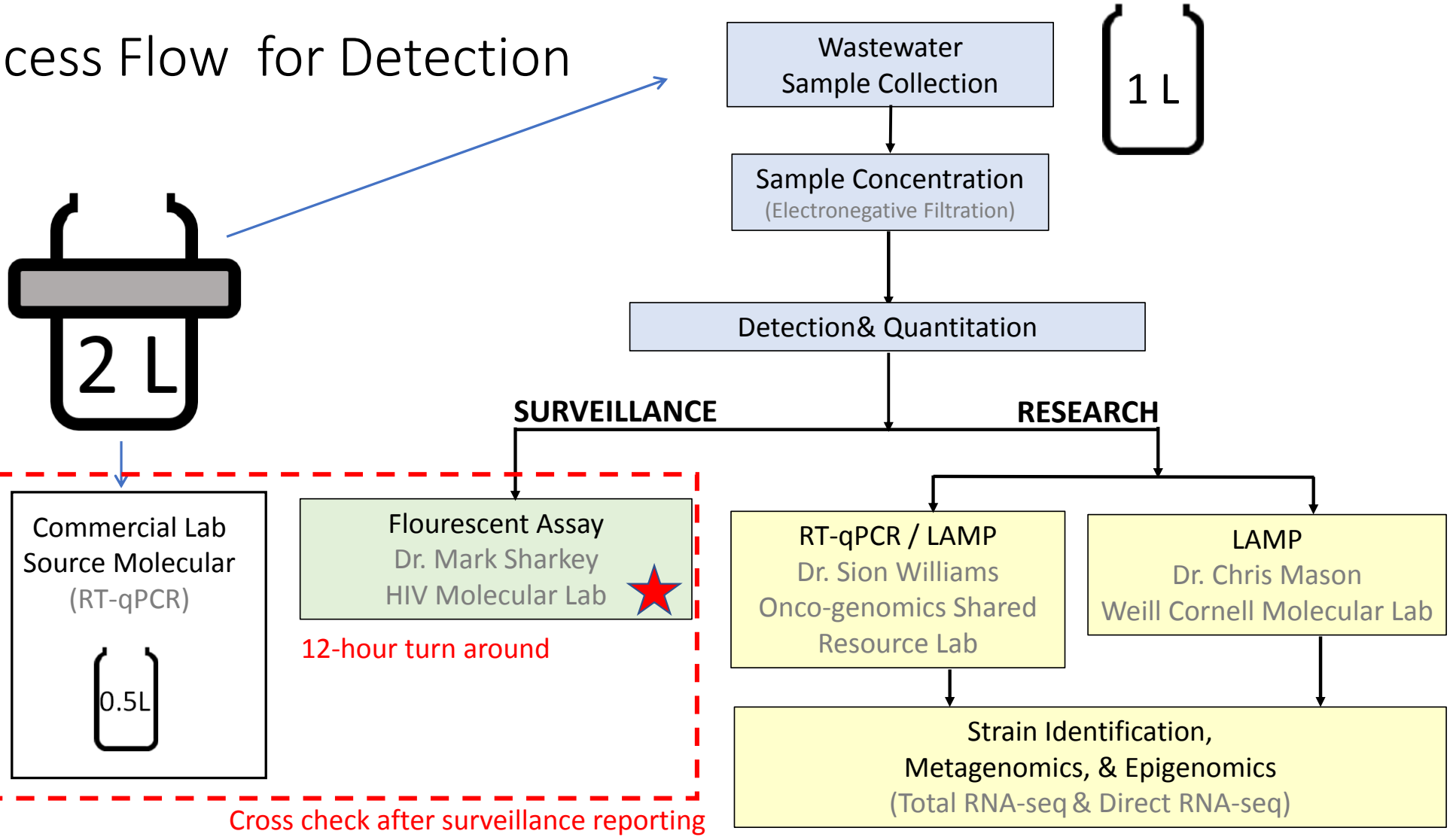

1. Add OC43 spike, recovery control
2. Add $MgCl_2$ (50 mM)
3. Acidify to pH 3.5-4.5
pH meter
Continuous Stirring




Electronegative
Filter


Folded Filter
In 1.5 ml DNA/RNA
Shield

Process Flow for Detection



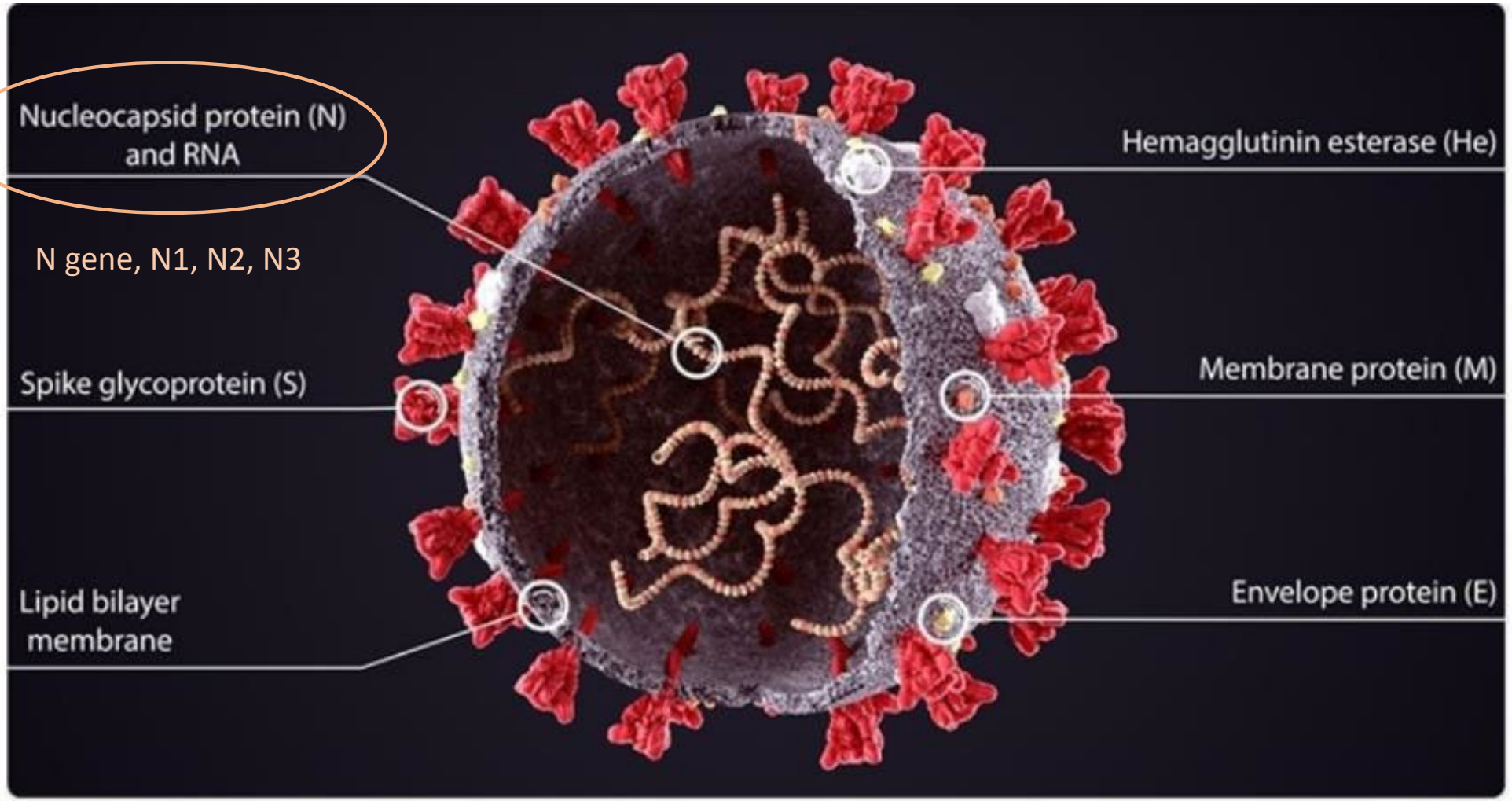
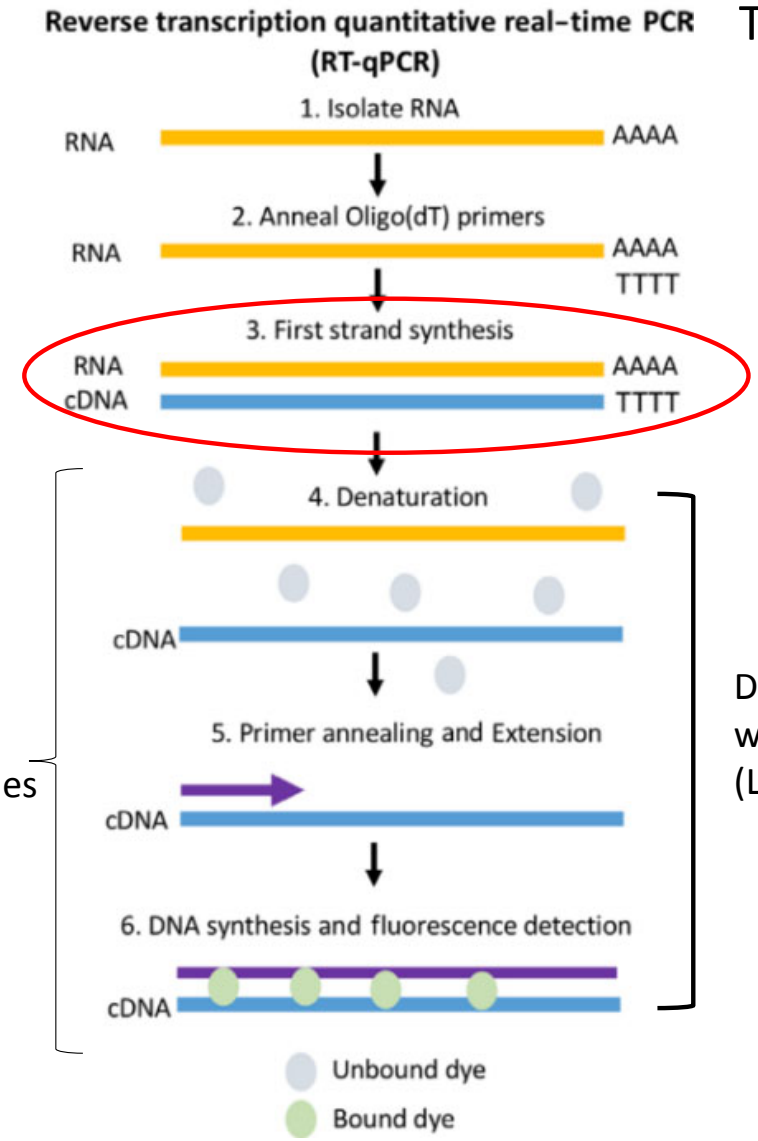


Image Credit: Orpheus FX / Shutterstock

Sampling → Concentration → Detection



Traditional Method Run by Commercial Lab and Dr. Williams Lab (RT-qPCR)

Dr. Sharkey's Method (FA) removes this step

Heat & Cool Cycles

Dr. Mason and Dr. Williams runs this step without need for heat and cool cycles (Loop-Mediated Isothermal Amplification, LAMP)

UMiami Molecular HIV Laboratory (Dr. Mark Sharkey)

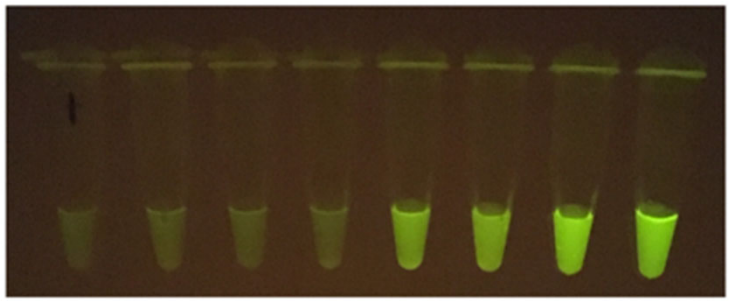
Fluorescent Assay (FA), Uses:

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 ★

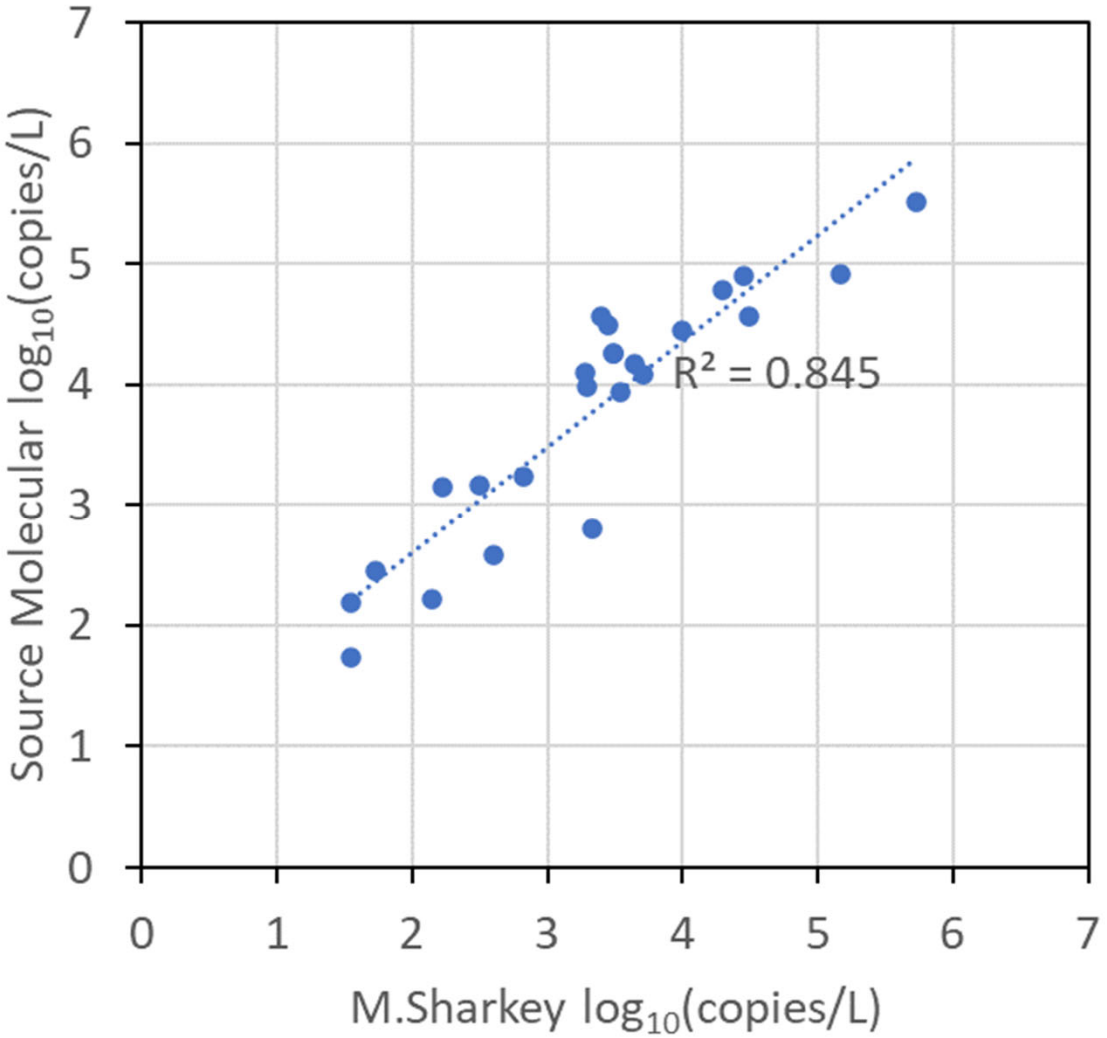
DETECTION

Comparison between

Commercial Lab (RT-qPCR)

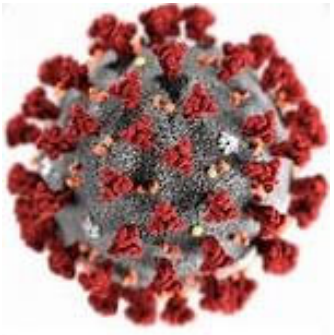
and

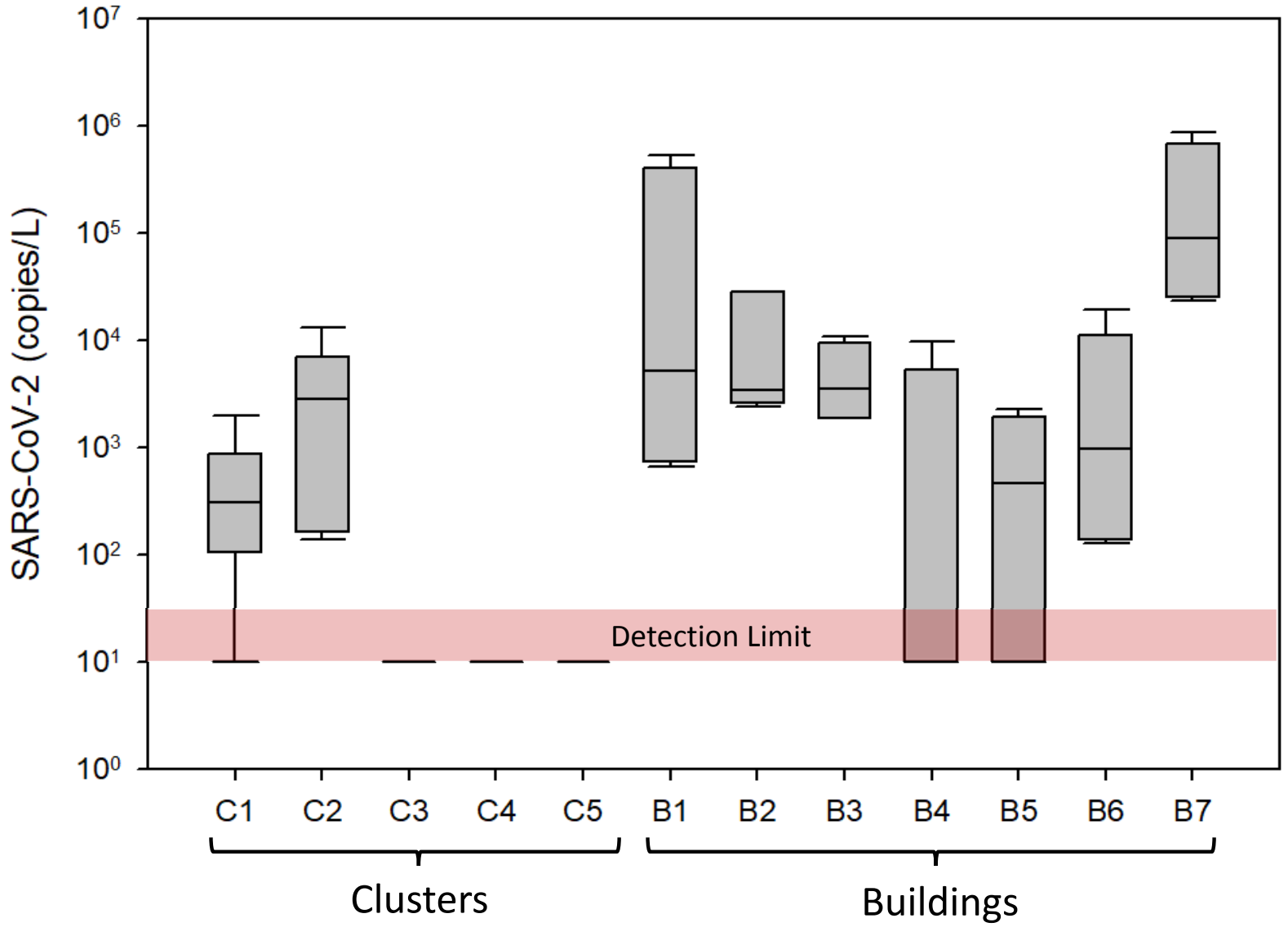
Dr. Sharkey's (FA) Results



SARS-CoV-2 Results

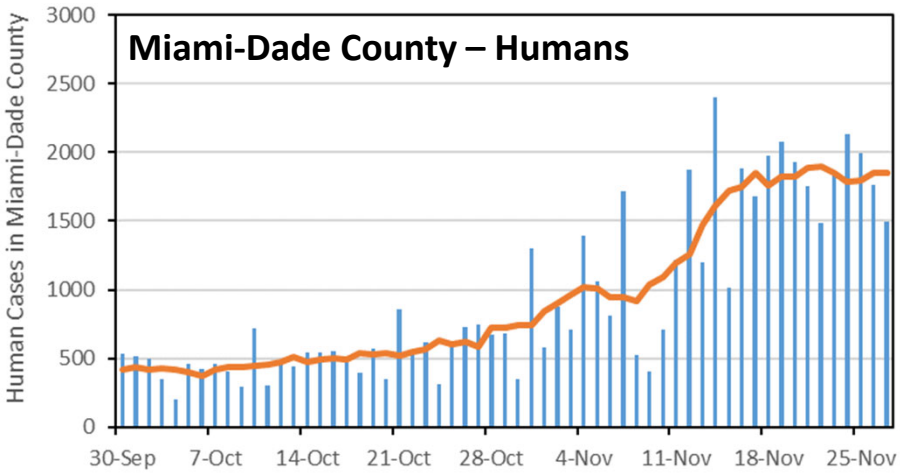
(Surveillance only)

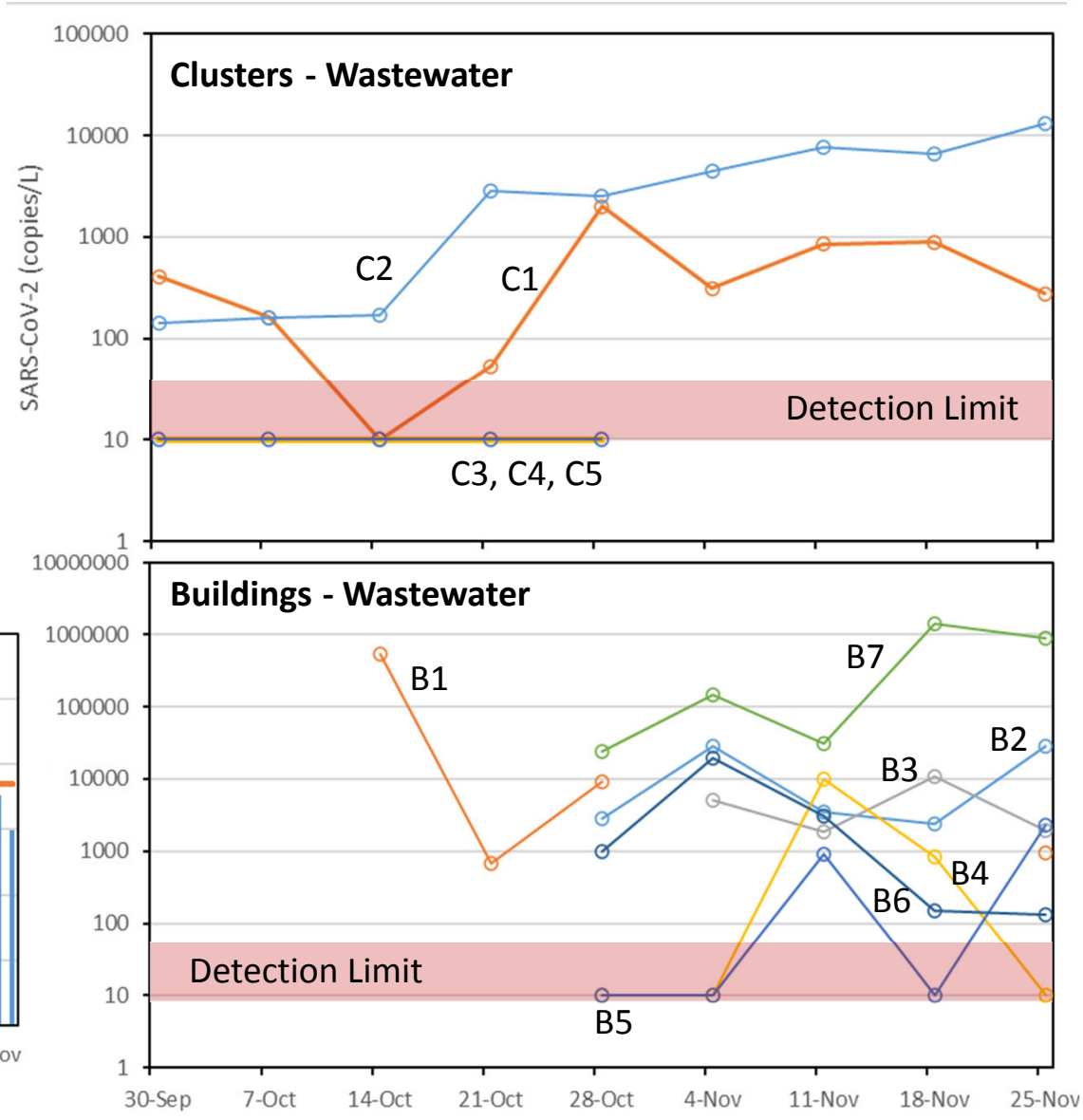


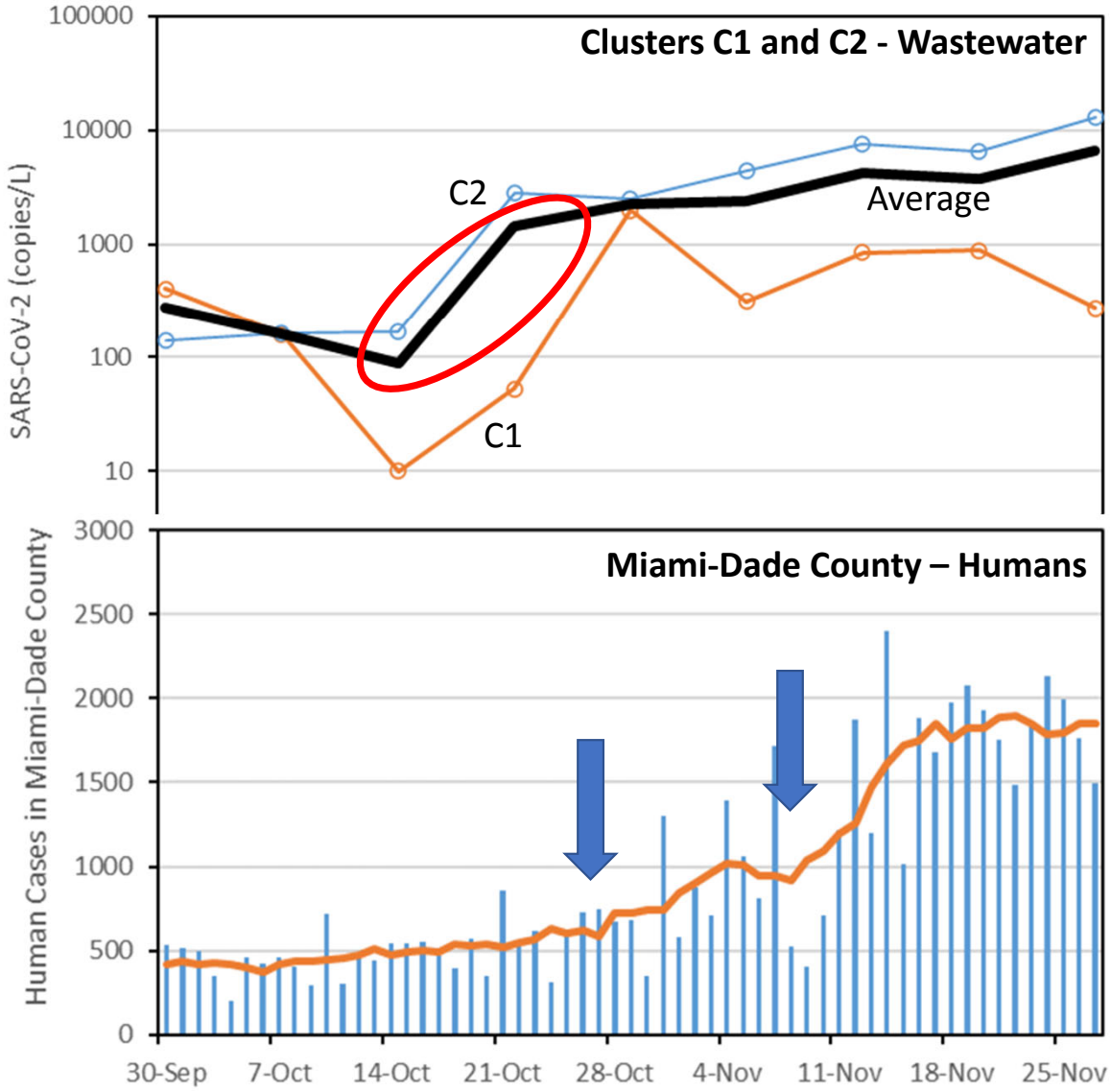

SARS-CoV-2 (copies/L)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Detection Limit
C1
C2
C3
C4
C5
B1
B2
B3
B4
B5
B6
B7
Clusters
Buildings

Time Series

- Clusters, trends more gradual
- Buildings, higher variability
- Buildings, strong + and –
- Scales in $\log_{10}$ units






Clusters C1 and C2 - Wastewater
SARS-CoV-2 (copies/L)
100000
10000
1000
100
10
C2
Average
C1
Miami-Dade County – Humans
Human Cases in Miami-Dade County
3000
2500
2000
1500
1000
500
0
30-Sep
7-Oct
14-Oct
21-Oct
28-Oct
4-Nov
11-Nov
18-Nov
25-Nov

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
- Measure basic physical-chemical parameters (T, pH, Spec Cond, Turbidity, DO)
- Consider normalizing data by a measure of fecal inputs
- Results possible within 12 hours

Acknowledgments

Questions (hmsolo@miami.edu)





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- Tom Stone
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- Elena Cortizas
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- Kristina Babler

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- Dr. Mark Sharkey
- Jessica Salinas

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- Dr. Jenny Kemper

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- Krysta Lyons

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Sylvester Comprehensive Cancer Center

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