# Wastewater Measurements for the RNA of SARS-CoV-2

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University of Miami

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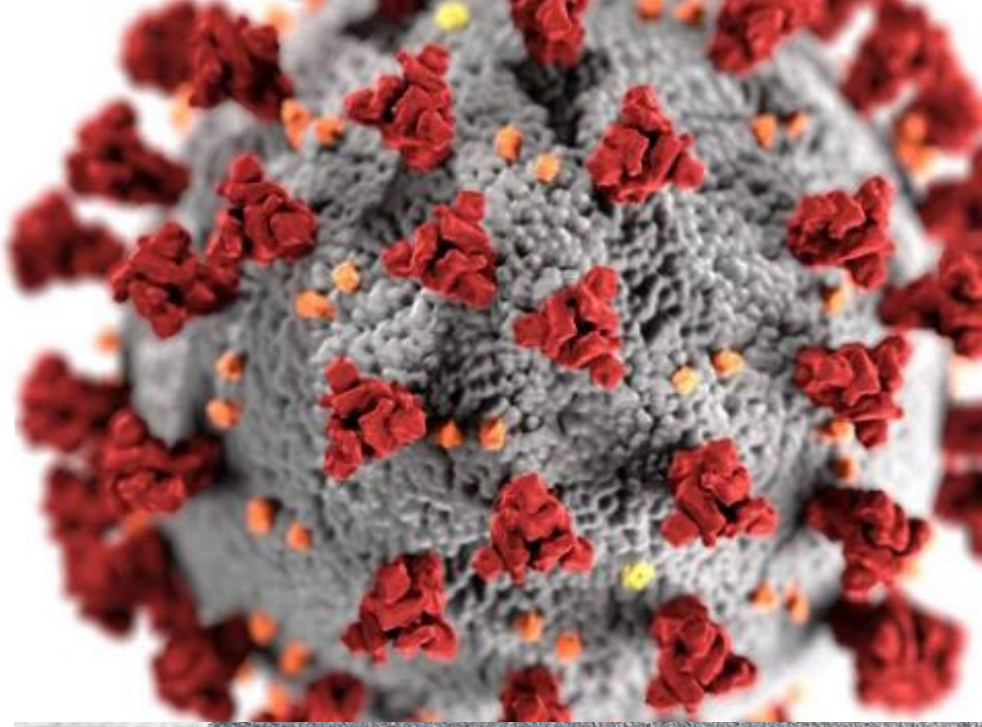
#AWWAvirtualsummit
awwa.org/feb-summit

virtual summit
AWWA
Sustainable Water | PFAS | Waterborne Pathogens

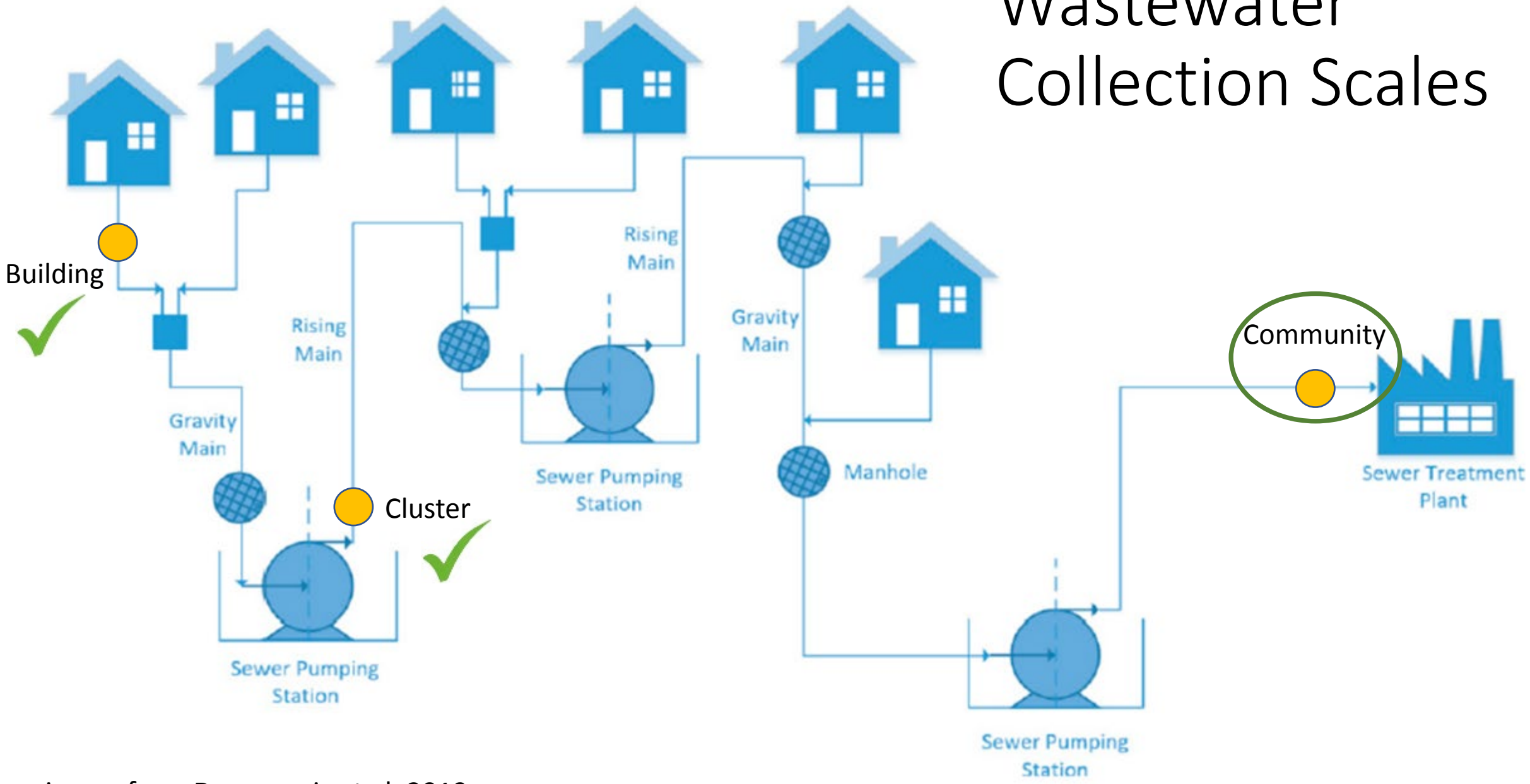
MIAMI

# Outline

- Design of sampling plan
- Water quality results
- Concentration and Detection for SARS-CoV-2
- SARS-CoV-2 results
- Lessons learned
- Acknowledgments



# Wastewater Collection Scales




Base image from Drenoyanis et al. 2019

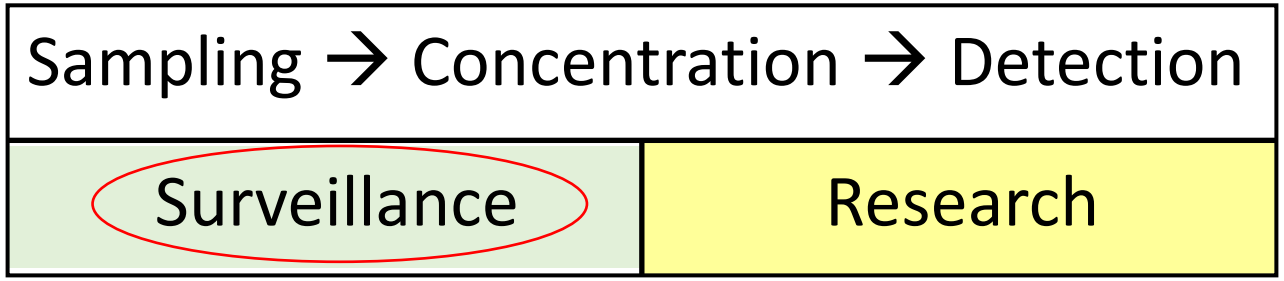
# Motivation & Objectives

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

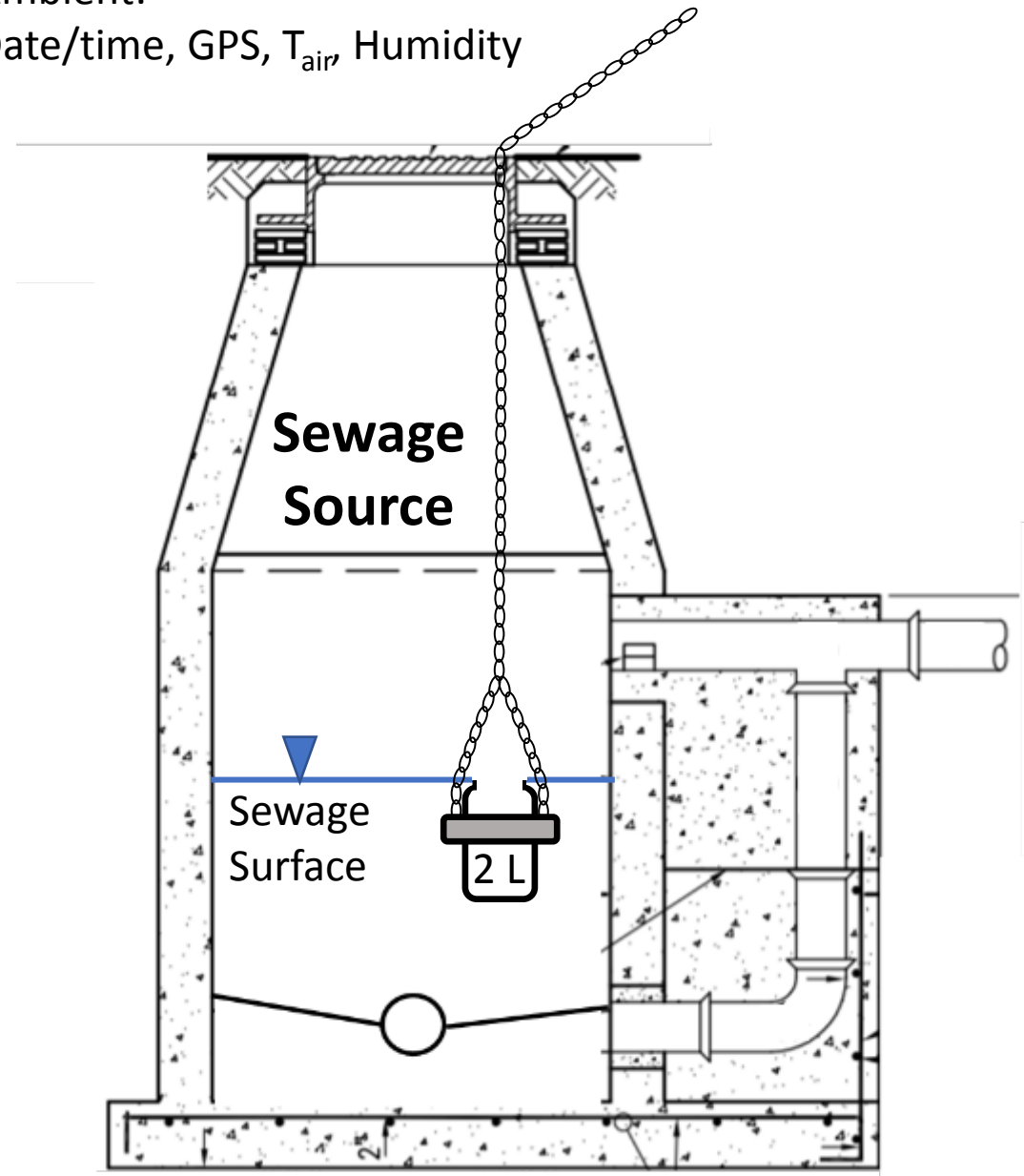
Ultimate objective: Can wastewater measurements be used to predict COVID-19 cases?

(building, cluster, and community scales)

Current objective:



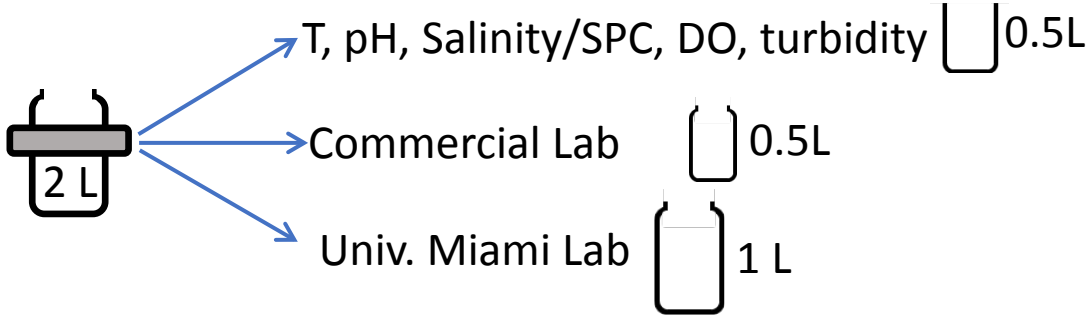
Ambient:
Date/time, GPS, $T_{air}$, Humidity




Collect Samples Weekly (Wednesdays)
Results available in 12 hours

Sampling Sites (6 to 12 per sampling day)

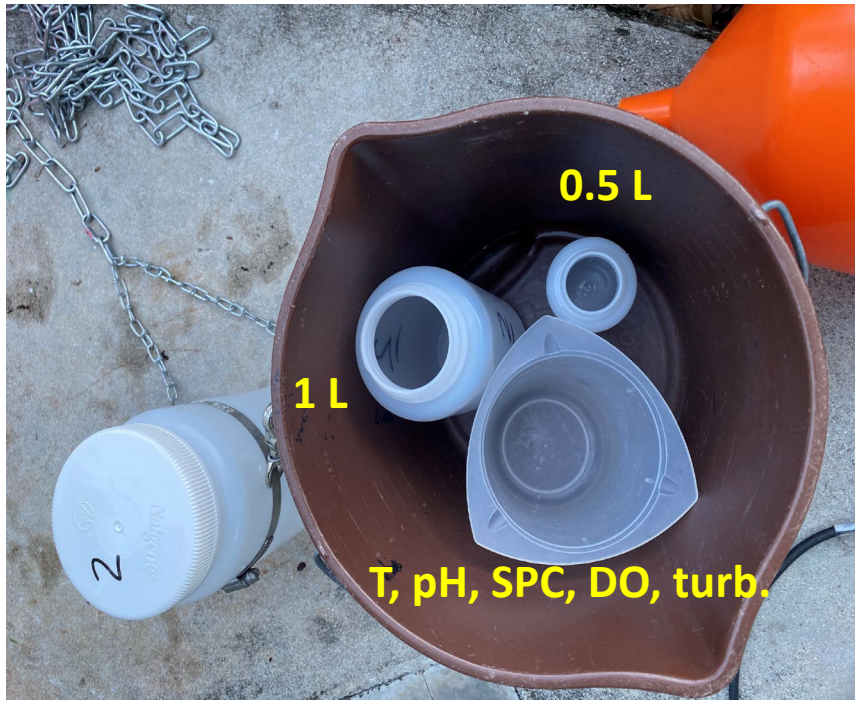
- Individual Buildings (B), includes hospitals
- Building Clusters (C), All 3 campuses



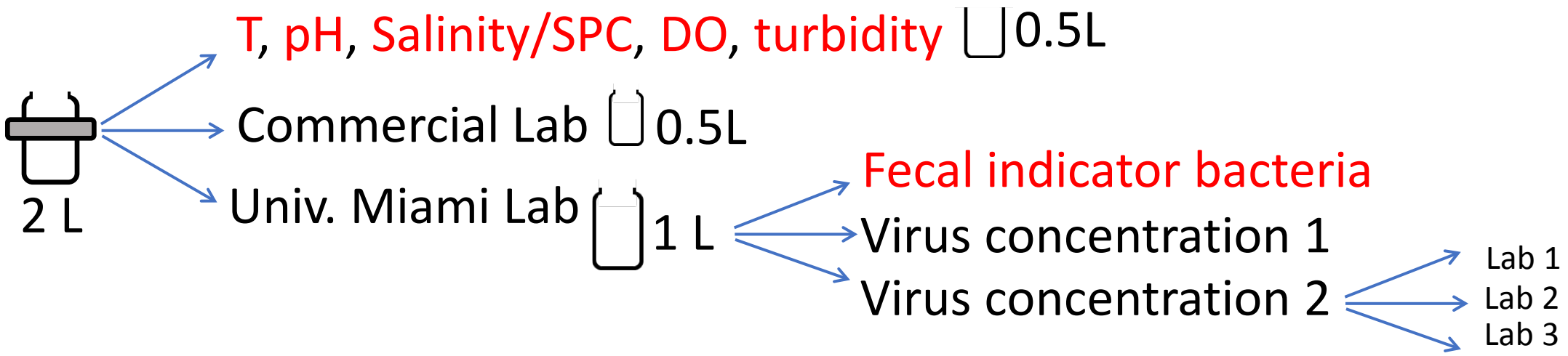

# SAMPLING

# Study Design

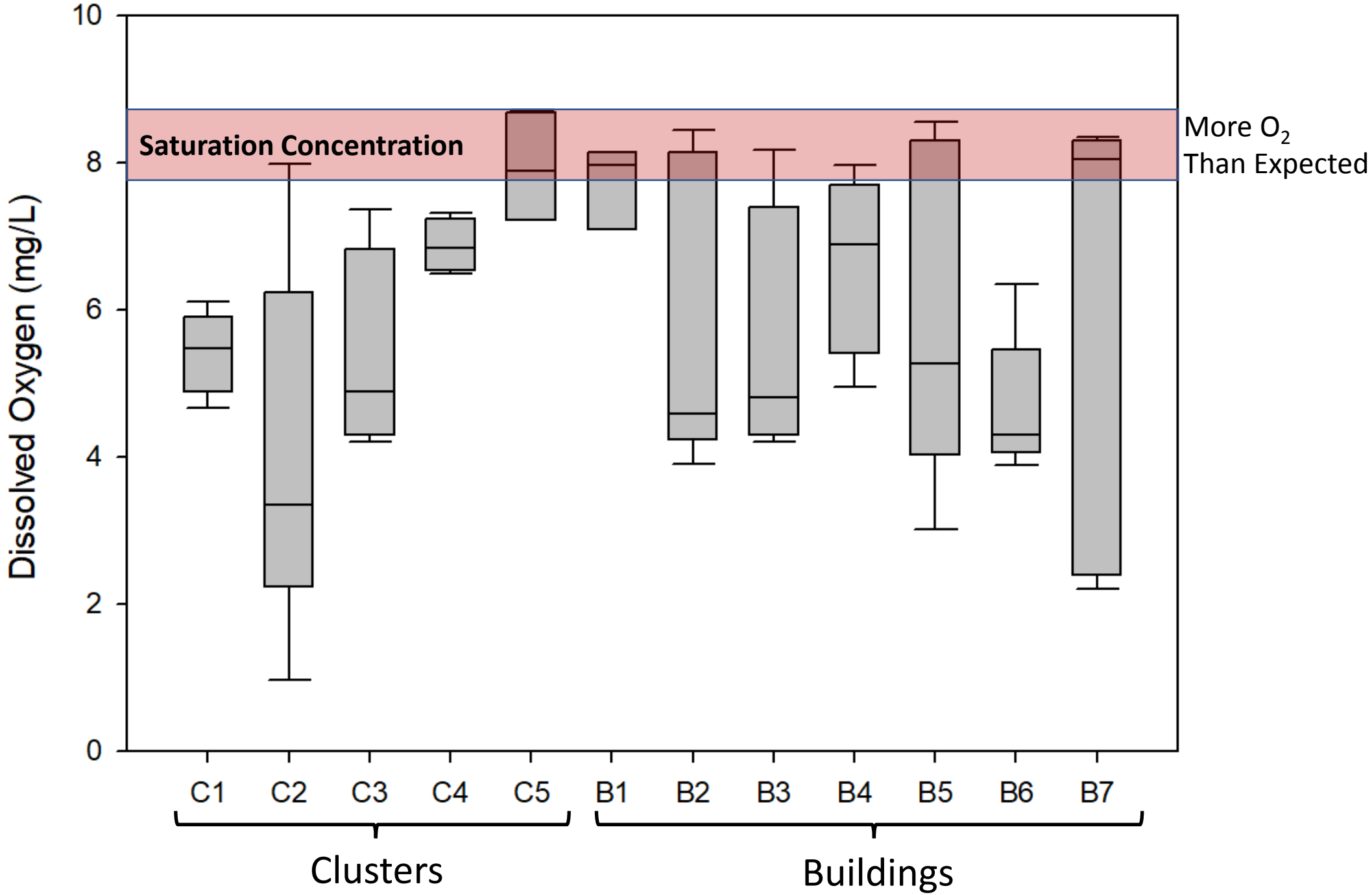
Sampling → Concentration → Detection

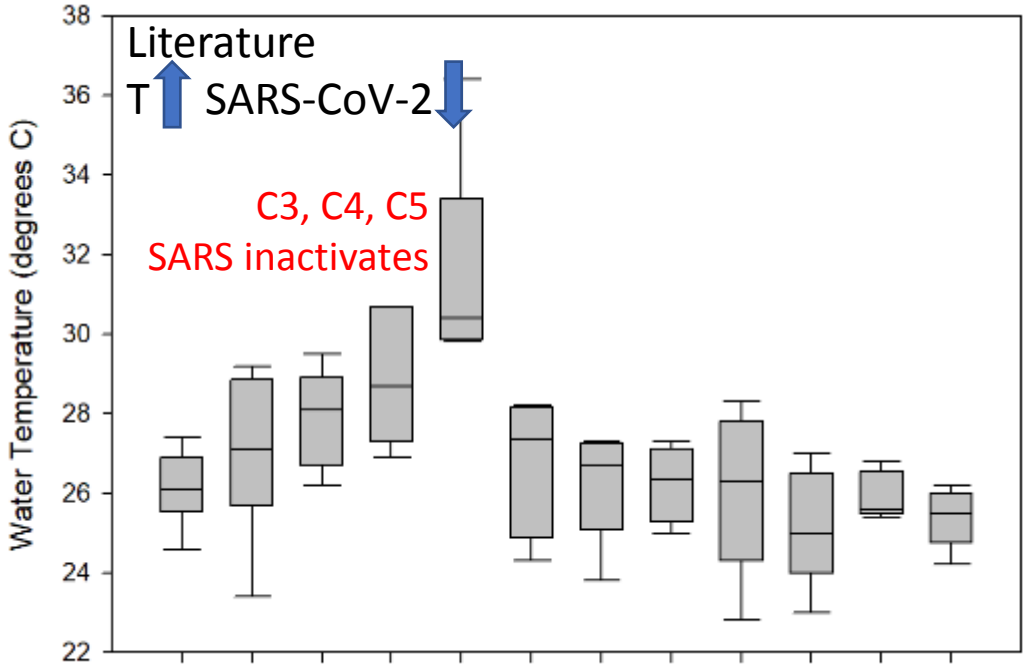


0.5 L
1 L
T, pH, SPC, DO, turb.

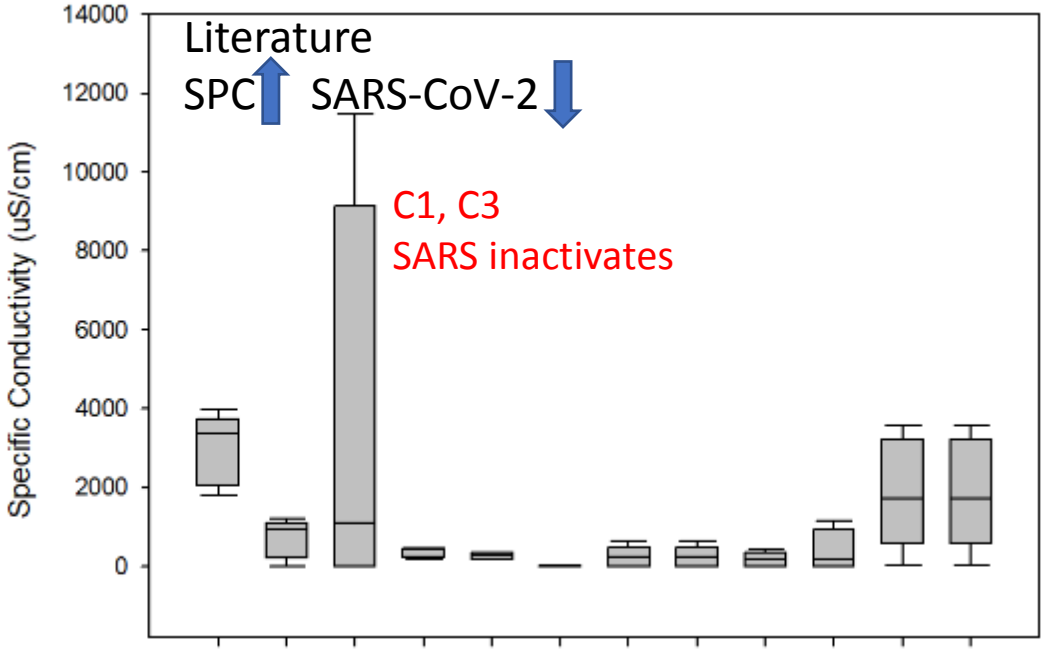

2 L

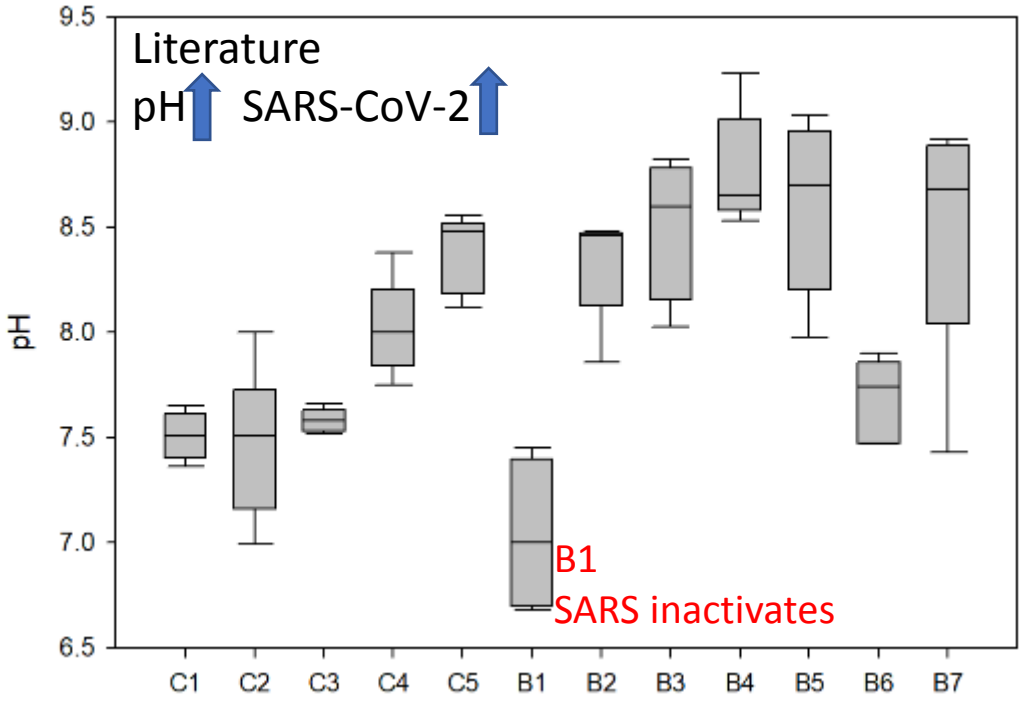

T, pH, Salinity/SPC, DO, turbidity
0.5L
Commercial Lab
0.5L
Univ. Miami Lab
1 L
2 L
Fecal indicator bacteria
Virus concentration 1
Virus concentration 2
Lab 1
Lab 2
Lab 3

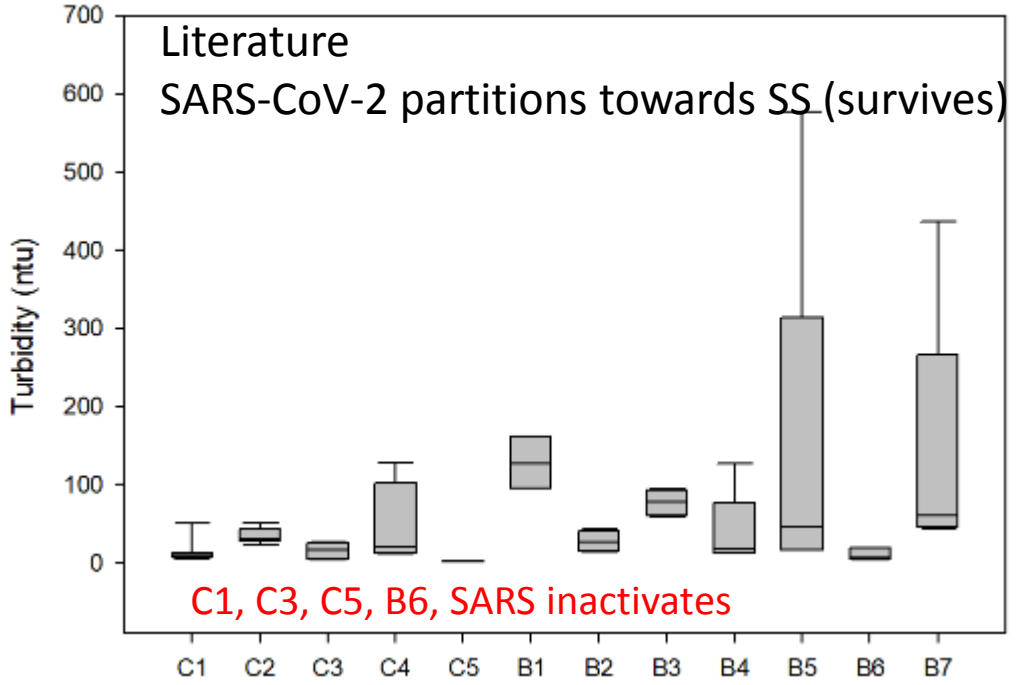
# Water Quality Results


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

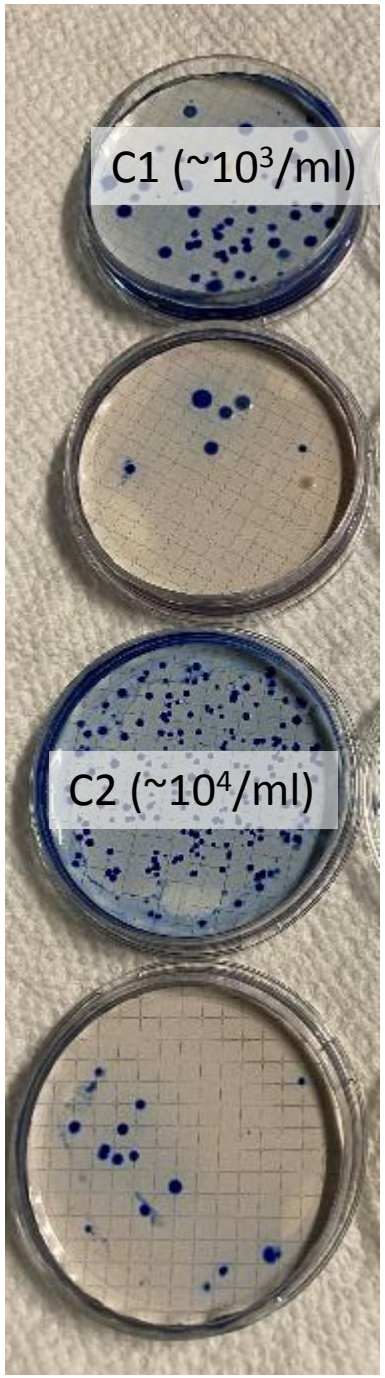

Literature
T SARS-CoV-2
C3, C4, C5
SARS inactivates
Water Temperature (degrees C)
38
36
34
32
30
28
26
24
22


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


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
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

**Clusters**

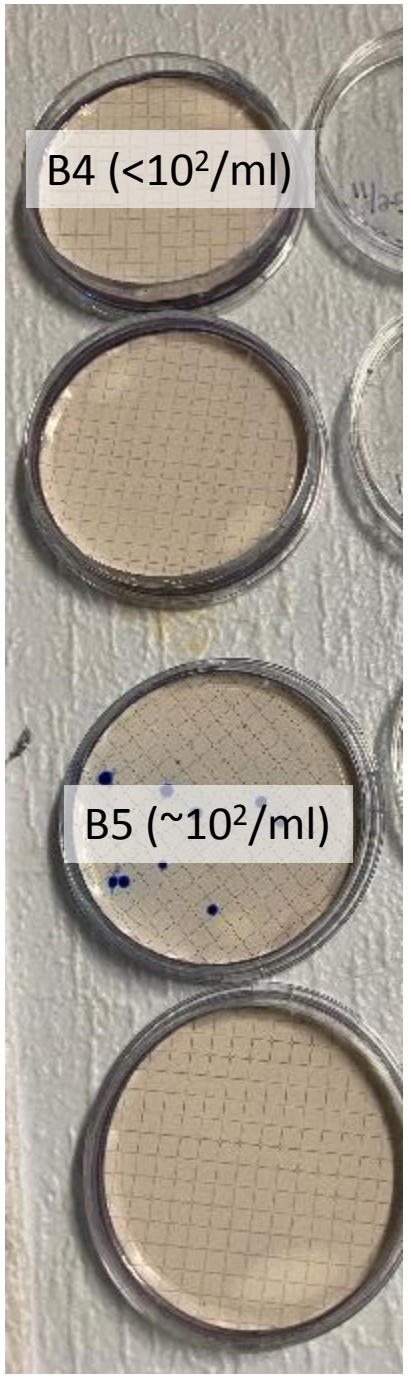


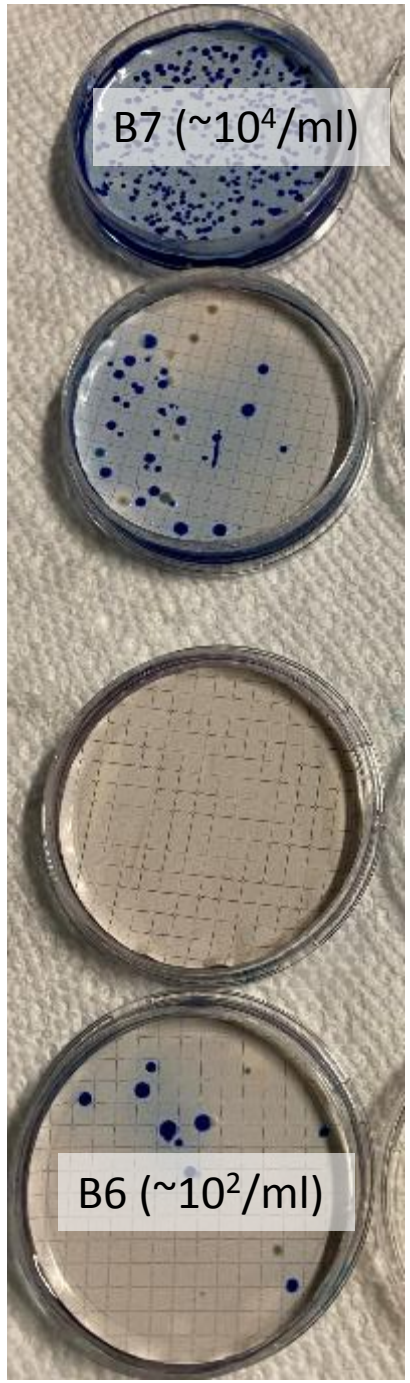

**Buildings**

Chlorine Residual?

↓

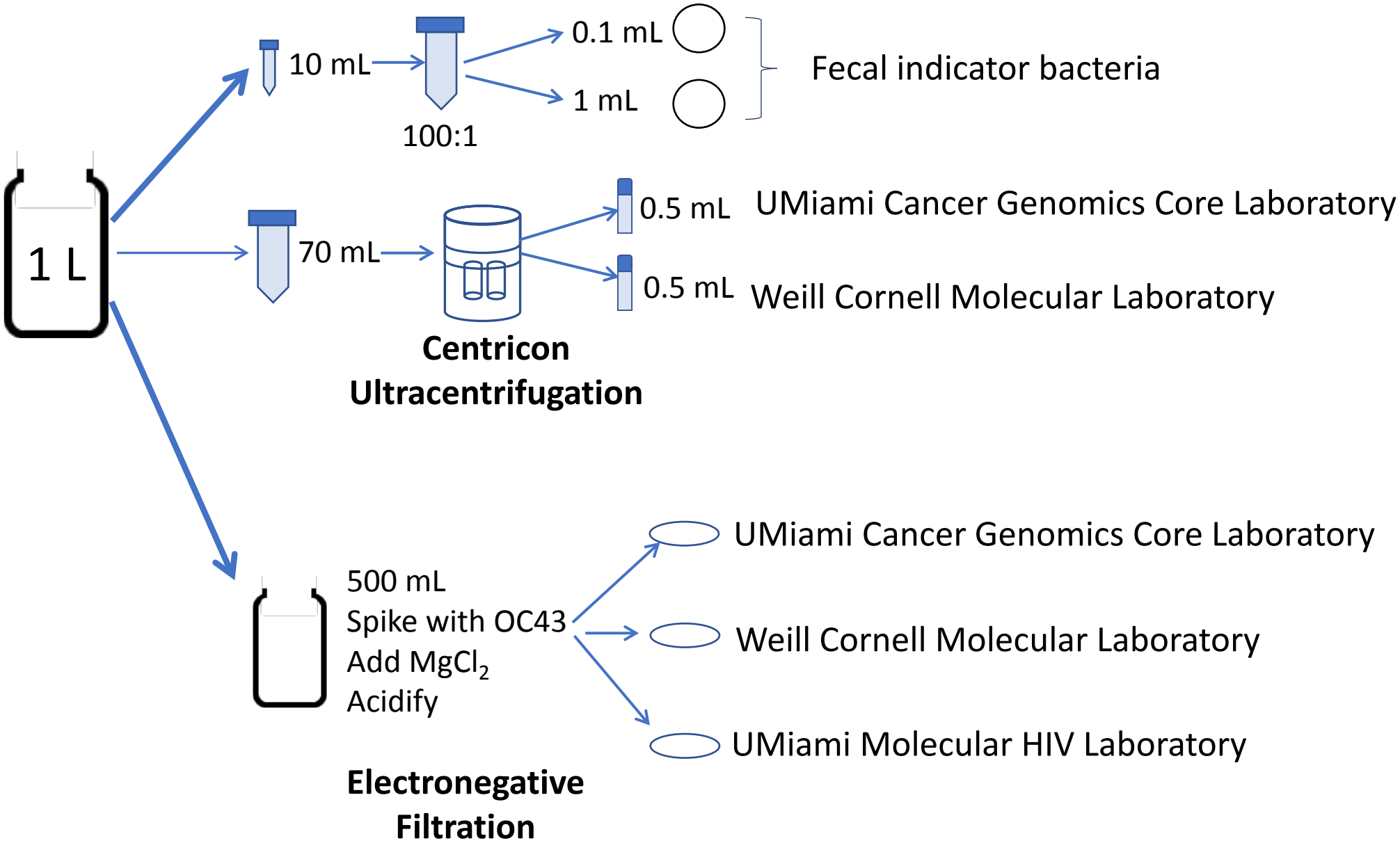
Neutralize Chlorine Upon Collection (sodium thiosulfate)

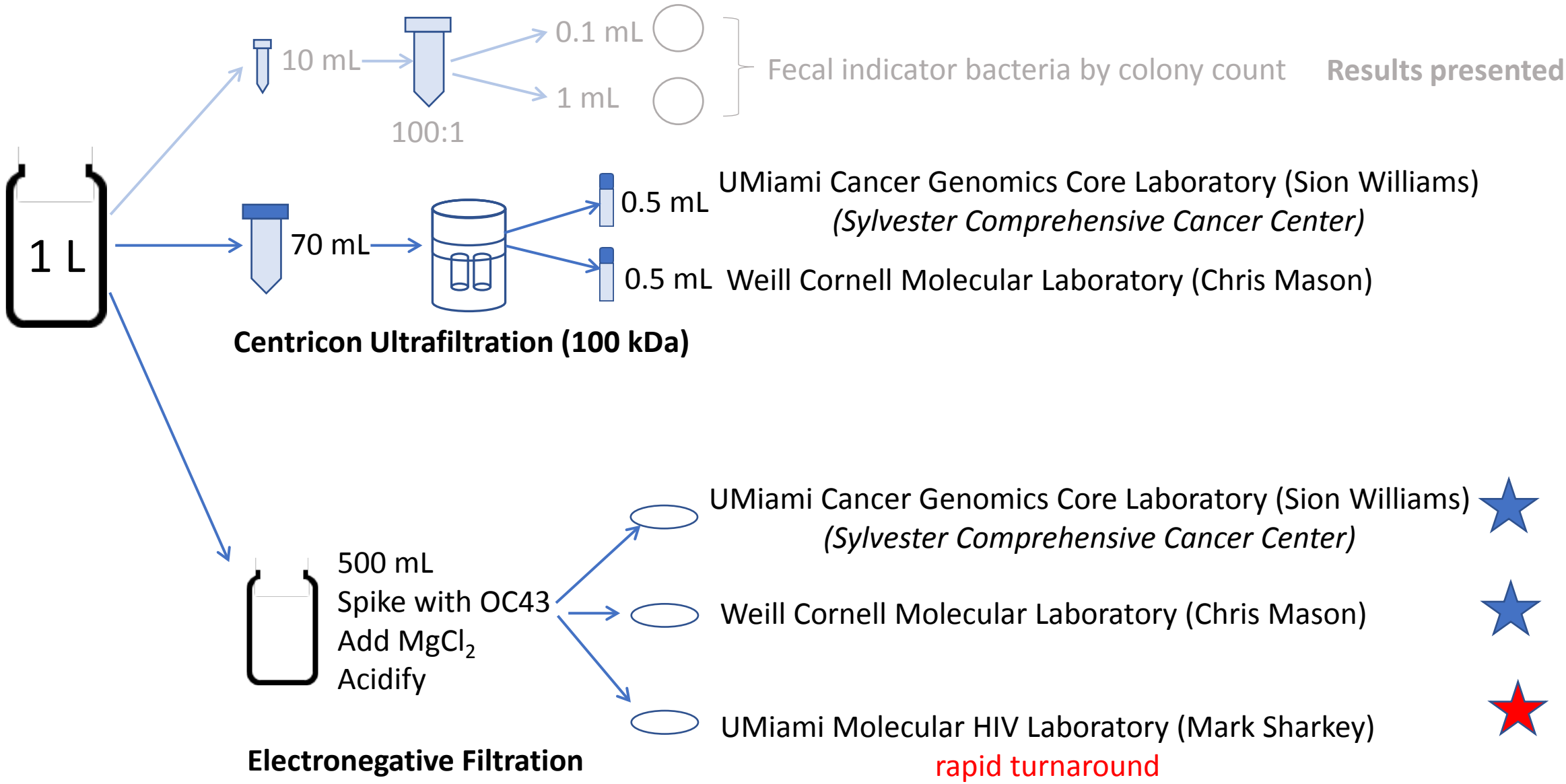


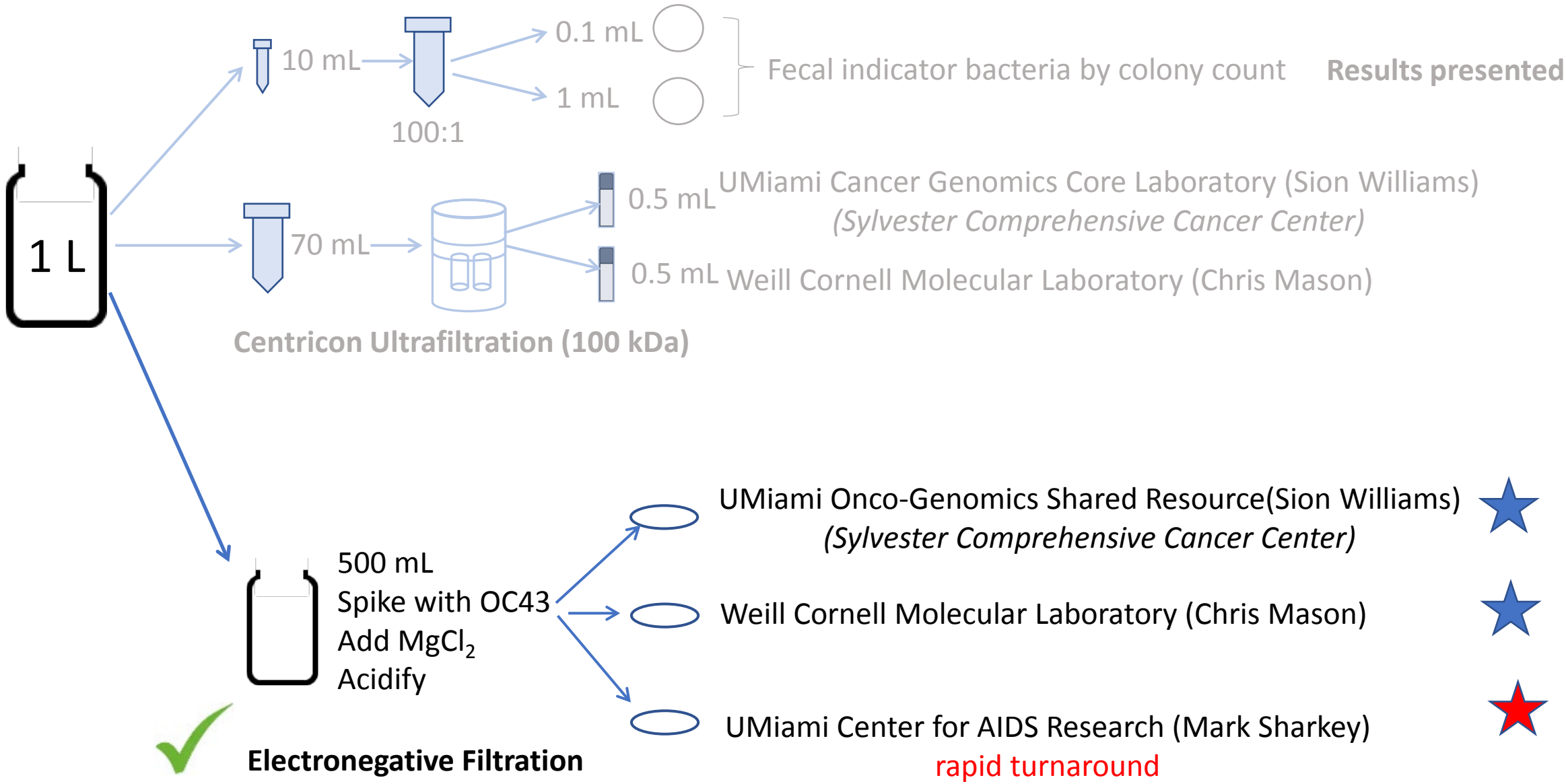




# Fecal coliform

# Concentration and Detection for SARS-CoV-2


10 mL
100:1
0.1 mL
1 mL
Fecal indicator bacteria
1 L
70 mL
0.5 mL
UMiami Cancer Genomics Core Laboratory
0.5 mL
Weill Cornell Molecular Laboratory
Centricon
Ultracentrifugation
500 mL
Spike with OC43
Add $MgCl_2$
Acidify
UMiami Cancer Genomics Core Laboratory
Weill Cornell Molecular Laboratory
UMiami Molecular HIV Laboratory
Electronegative
Filtration


10 mL
100:1
0.1 mL
1 mL
Fecal indicator bacteria by colony count
Results presented
1 L
70 mL
0.5 mL
0.5 mL
UMiami Cancer Genomics Core Laboratory (Sion Williams)
(Sylvester Comprehensive Cancer Center)
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

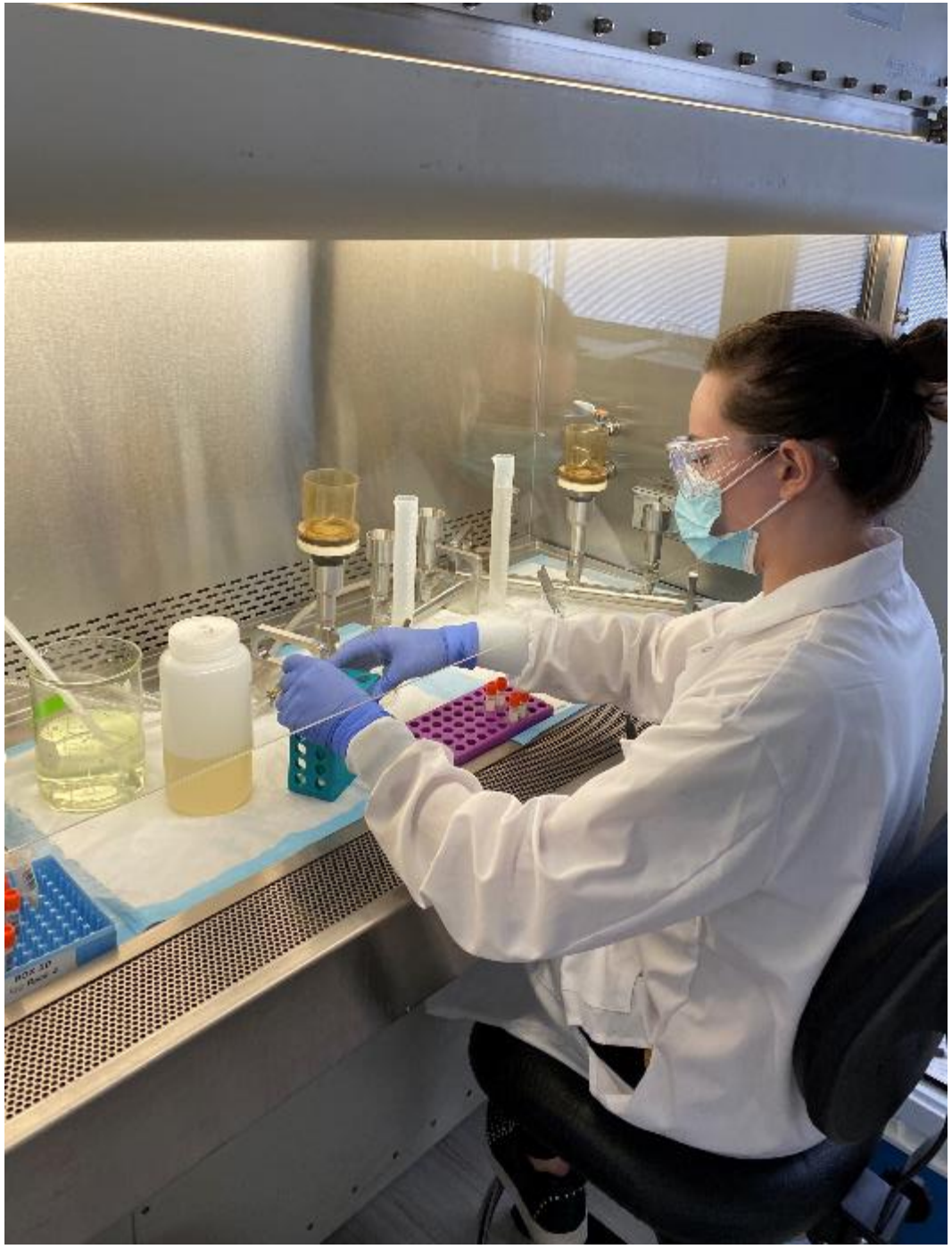



Sampling → Concentration → Detection

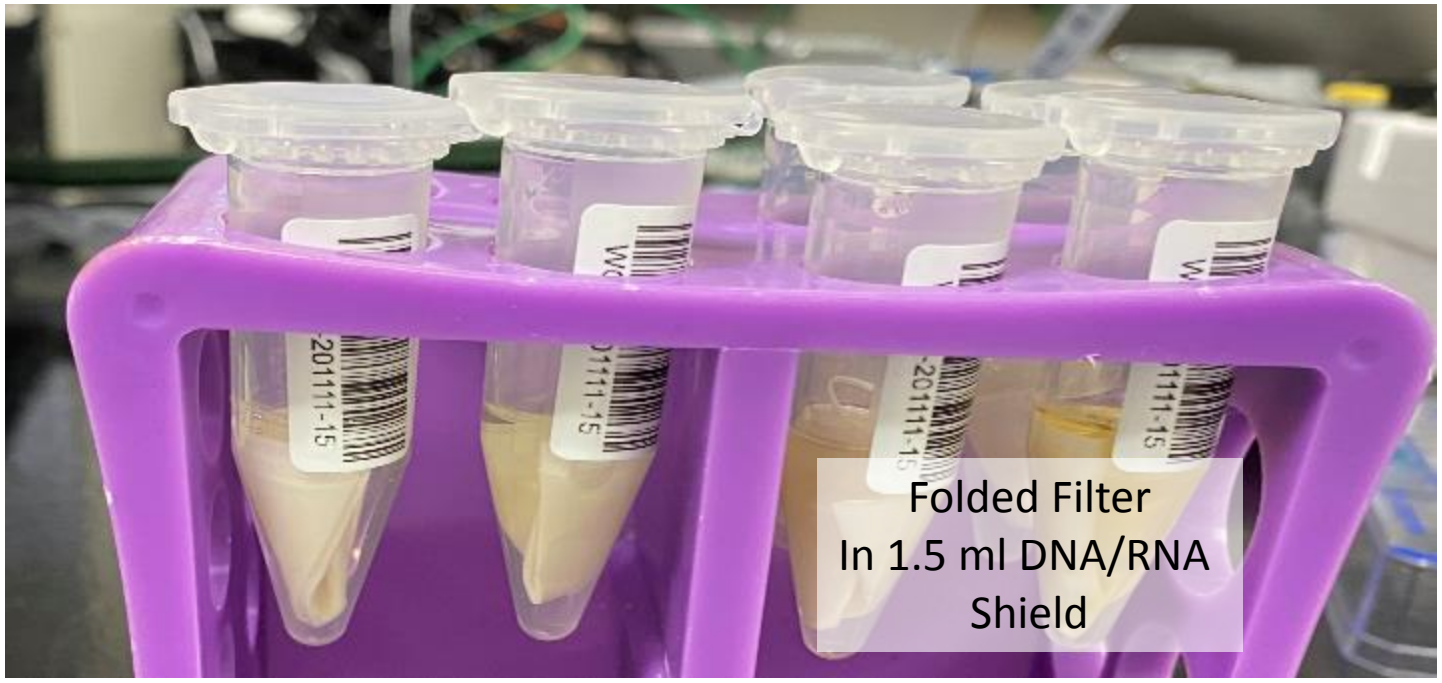
1. Add OC43 spike, recovery control
2. Add $MgCl_2$ (50 mM)
3. Acidify to pH 3.5-4.5

pH meter

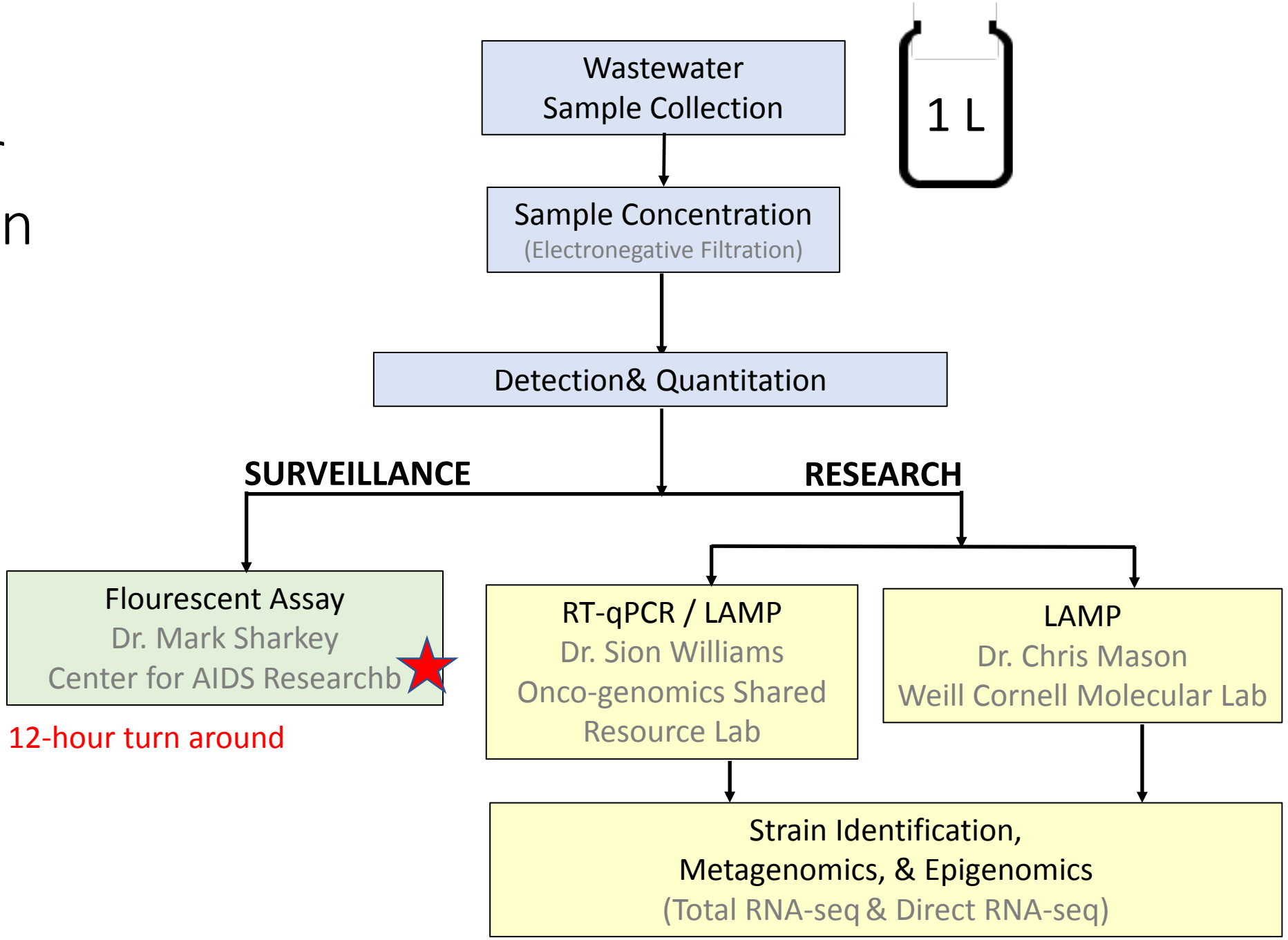
Continuous Stirring




Electronegative
Filter


Folded Filter
In 1.5 ml DNA/RNA
Shield

# Process Flow for Detection



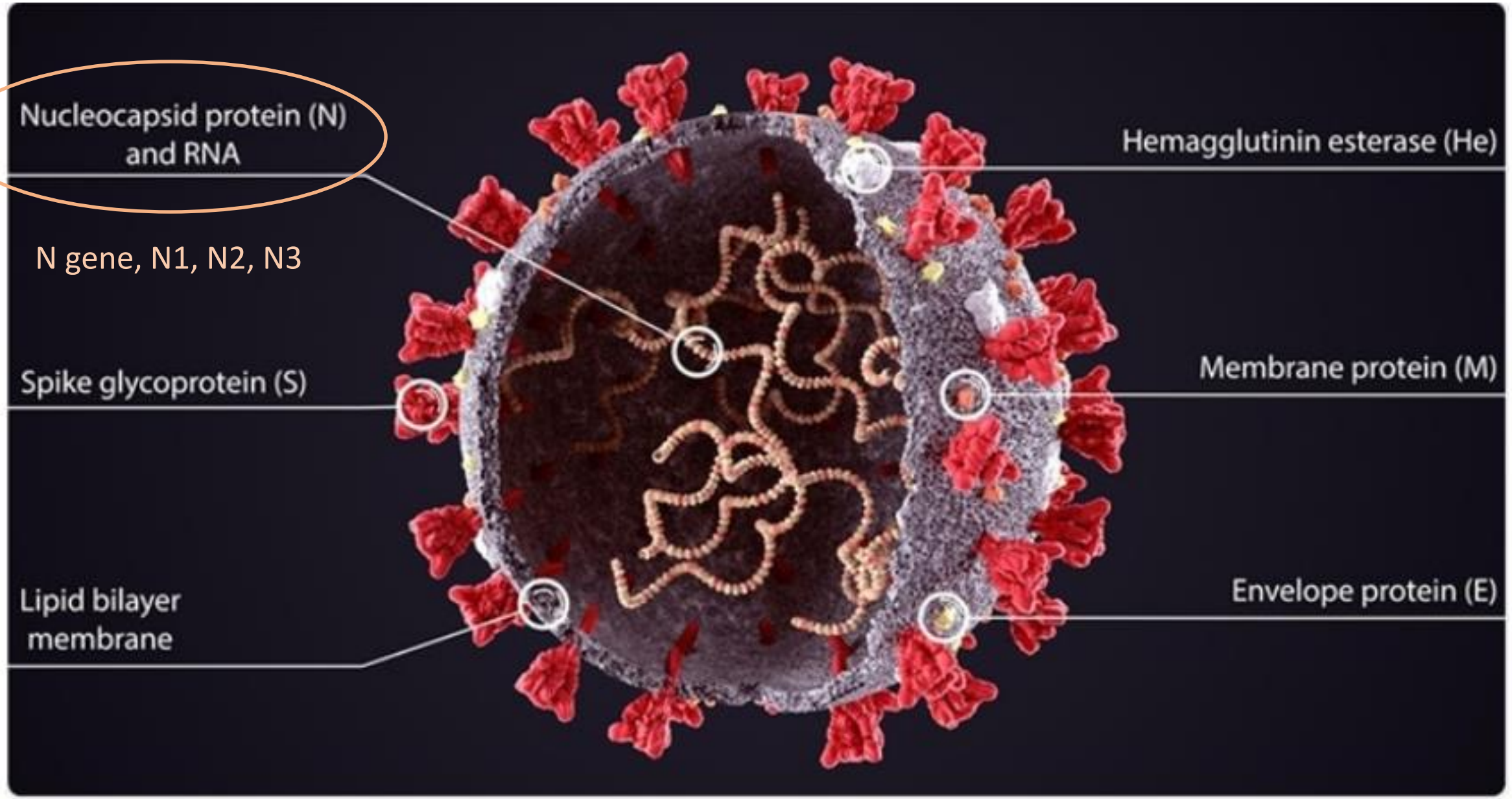
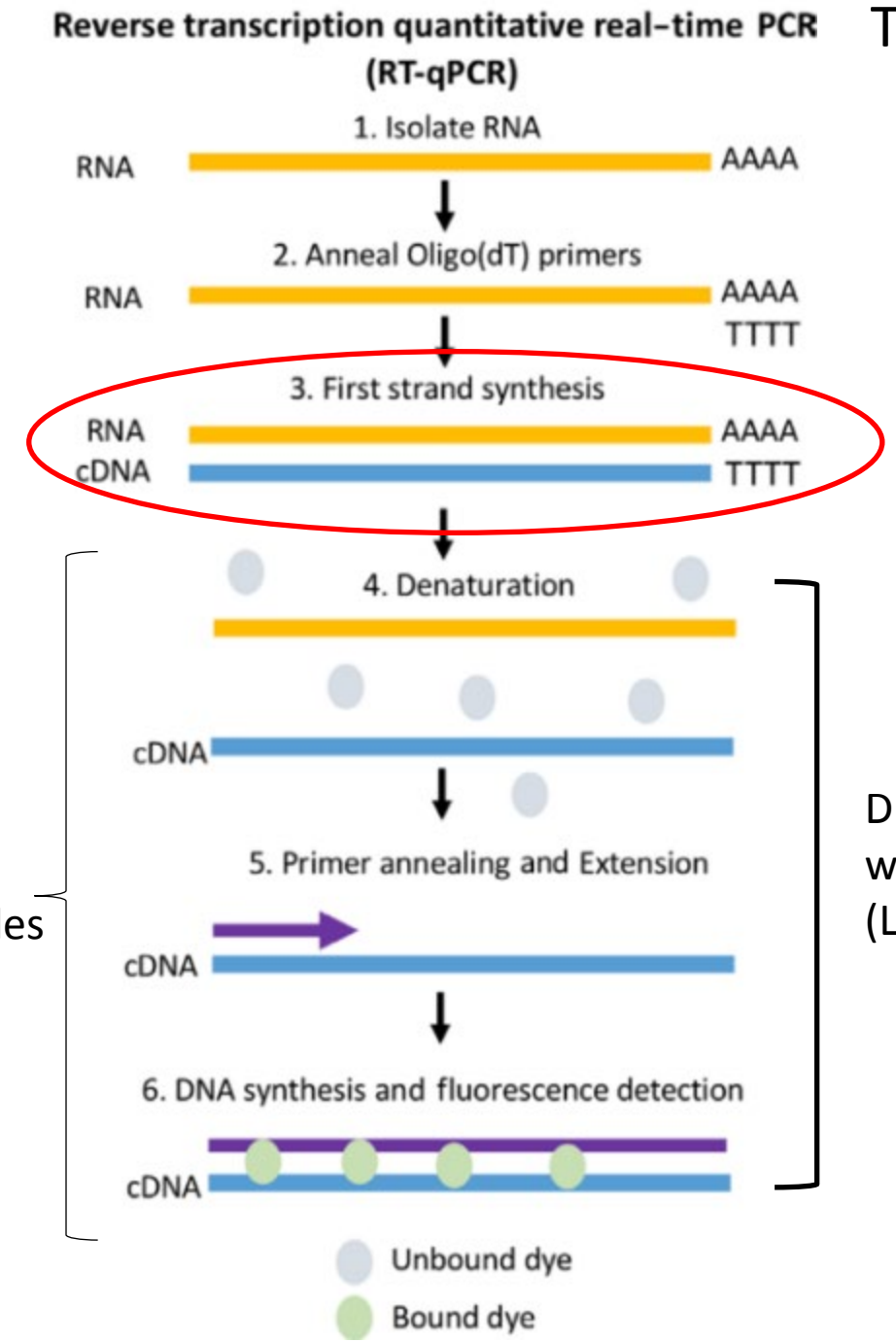



*Image Credit: Orpheus FX / Shutterstock*

Sampling → Concentration → Detection

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



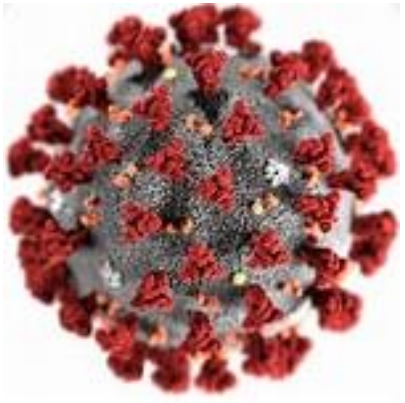

Dr. Sharkey's Method (FA) removes this step

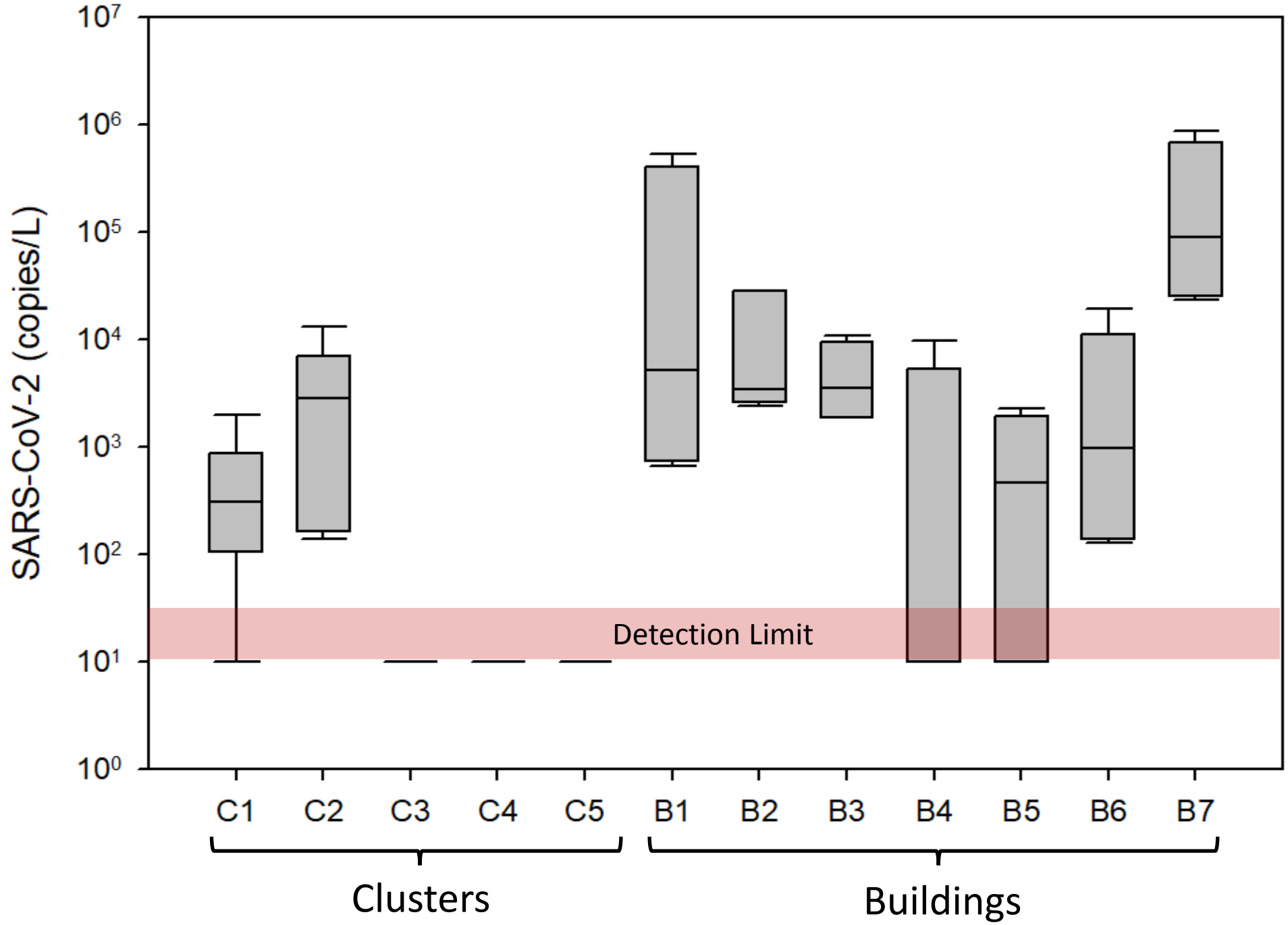
Heat & Cool Cycles

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

# SARS-CoV-2 Results

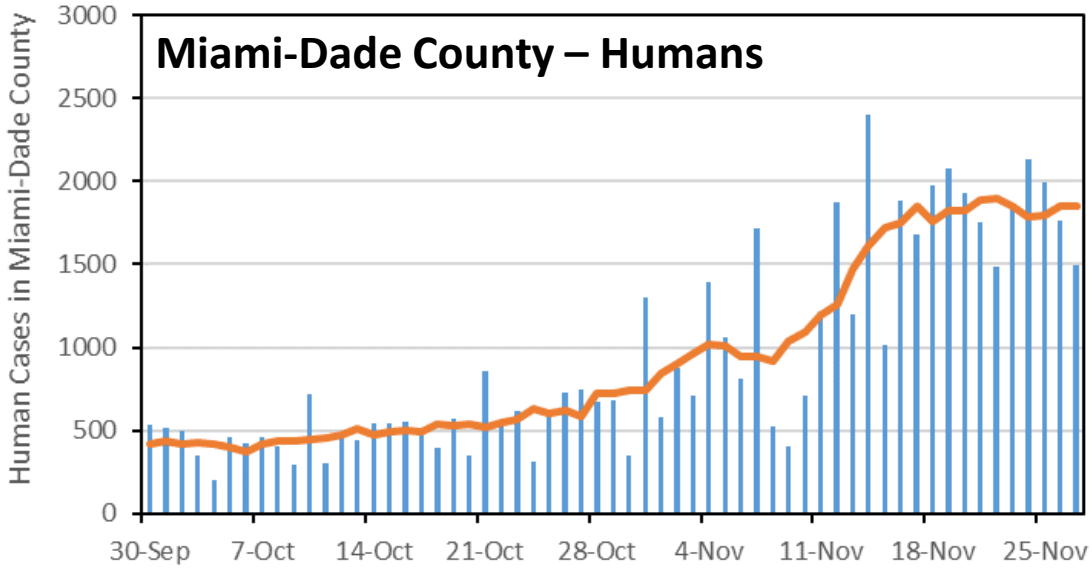
*(Surveillance only)*

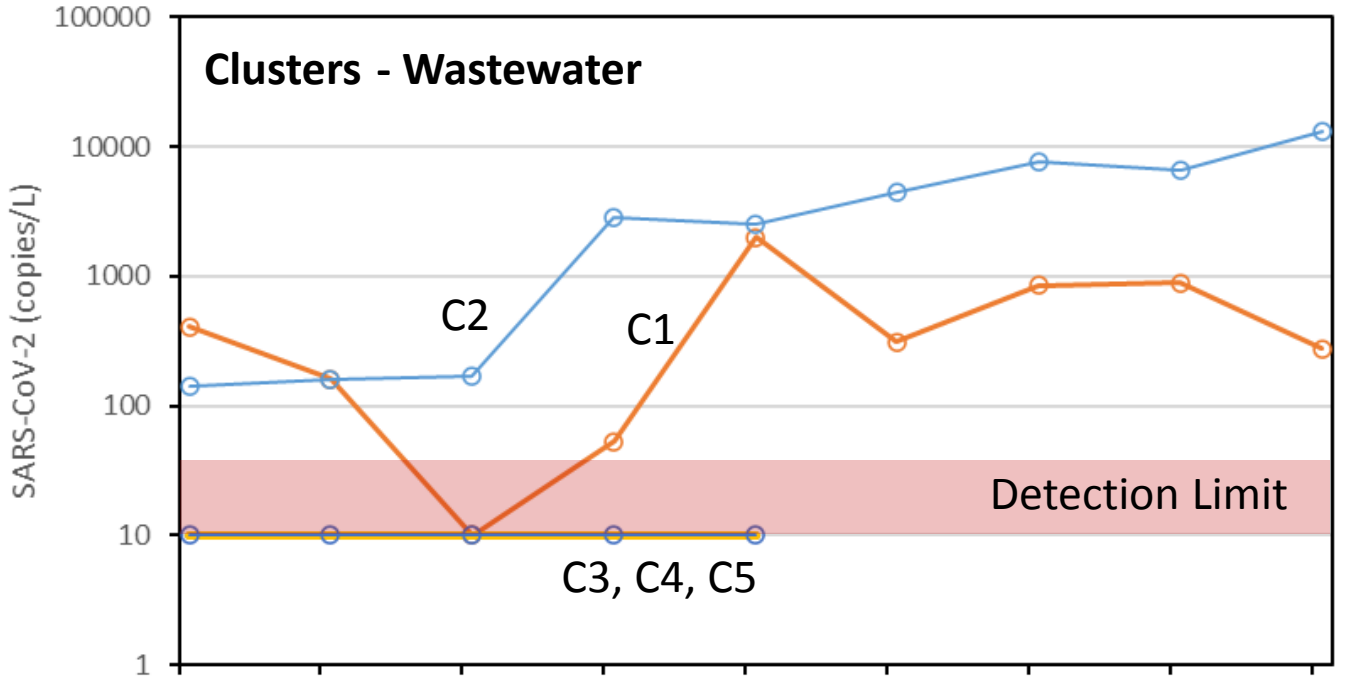


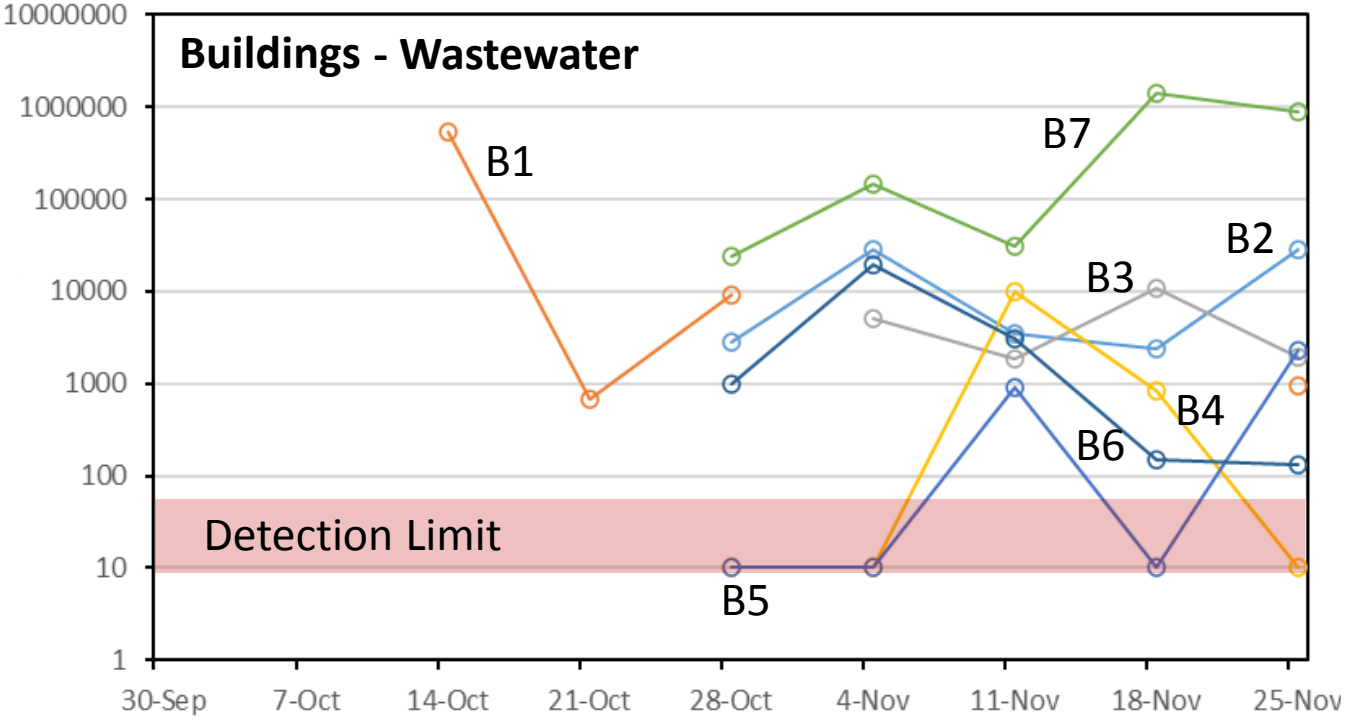

SARS-CoV-2 (copies/L)
$10^7$
$10^6$
$10^5$
$10^4$
$10^3$
$10^2$
$10^1$
$10^0$
Detection Limit
C1
C2
C3
C4
C5
B1
B2
B3
B4
B5
B6
B7
Clusters
Buildings

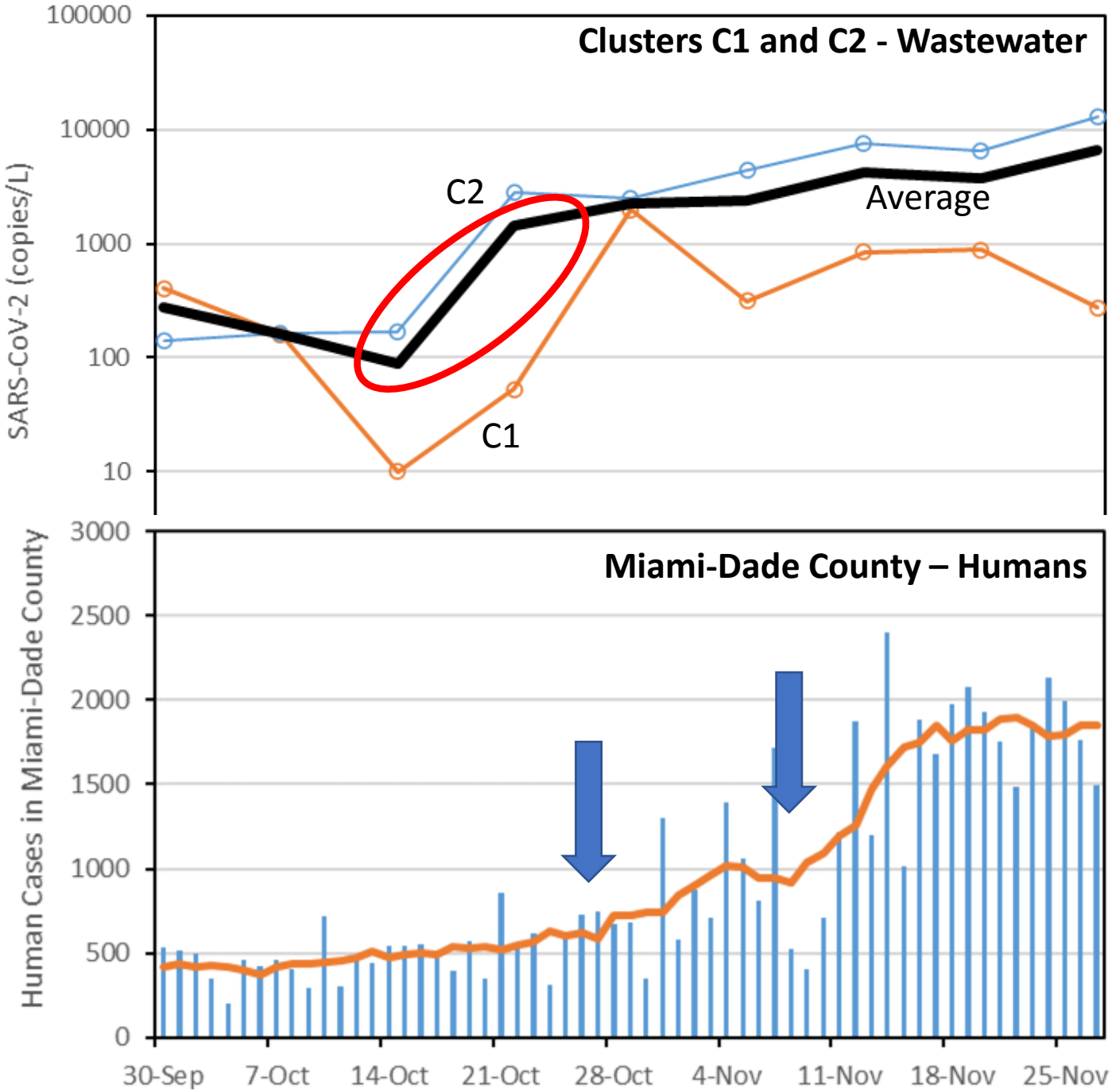
# Time Series

- 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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- Jessica Salinas

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