Metropolitan Water Reclamation District of Greater Chicago

Welcome to the January Edition of the 2021 M&R Seminar Series

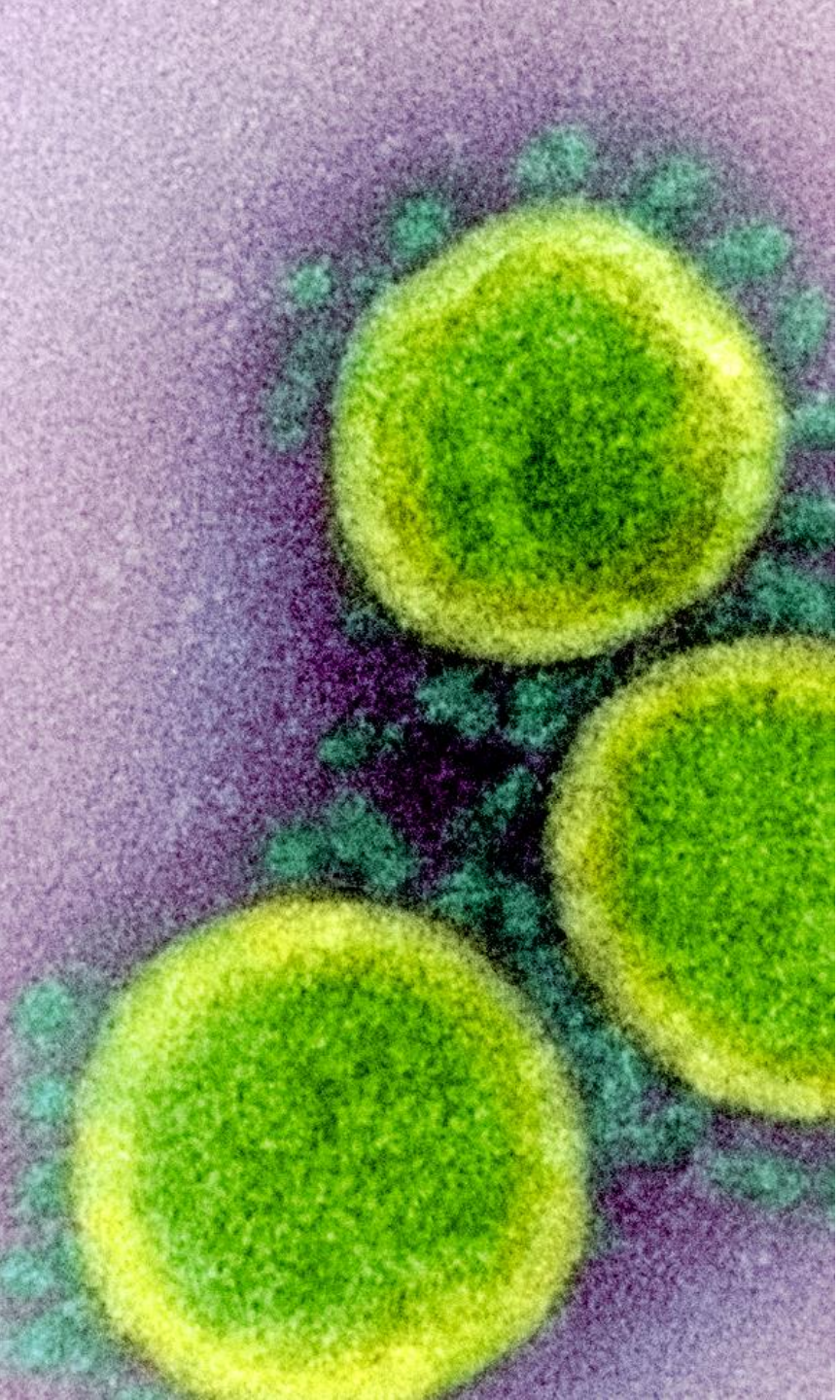
NOTES FOR SEMINAR ATTENDEES

- All attendees' audio lines have been muted to minimize background noise.
- A question and answer session will follow the presentation.
- Please use the Chat feature to ask a question via text to All Panelists.
- The presentation slides will be posted on the MWRD website after the seminar.
- ISPE has approved this seminar for one PDH. Certificates will only be issued to participants who attend the entire presentation.

Dr. Alexandria Boehm

Professor, Department of Civil and Environmental Engineering
Stanford University, Stanford, California

Dr. Alexandria Boehm is a professor at Stanford University, Department of Civil and Environmental Engineering. She received her bachelor of science from the California Institute of Technology in engineering and applied science, and her master's degree and doctorate in environmental engineering from the University of California Irvine. Her research focuses on pathogens in the environment including their sources, fate, and transport in natural and engineered systems. She is also interested broadly in coastal water quality where her work addresses the sources, transformation, transport, and ecology of biocolloids - specifically fecal indicator organisms, DNA, pathogens, and phytoplankton - as well as sources and fate of nitrogen. She presently serves on the State of California Ocean Acidification and Hypoxia science task force and is an associate editor at *Environmental Science & Technology* and *Environmental Science & Technology Letters*. She received the American Society of Civil Engineers Huber Prize in 2016 and a National Science Foundation CAREER award in 2007.



SCAN: Sewer Coronavirus Alert Network

Alexandria Boehm

Stanford University

UNIVERSITY OF MICHIGAN

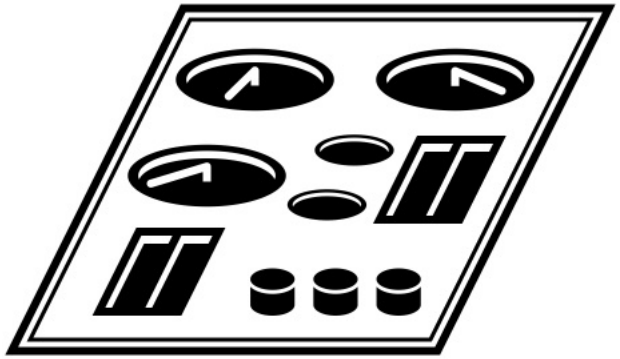
Krista Wiggington

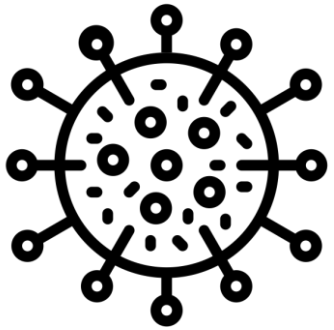
Team members: Katy Graham, Stephanie Loeb, Marlene Wolfe, Nasa Sinnott-Armstrong, Suzy Kim, Lorelay Mendoza, Laura Roldan-Hernandez, Kevan Yamahara, Lauren Sassoubre, David Catoe

How did we get here?!!

Outline

1. Background on wastewater-based epidemiology (WBE) and SARS-CoV-2 in feces
2. Sample acquisition and method development
3. Application to retrospective samples
4. Real time surveillance
5. Model to compare measurements across plants

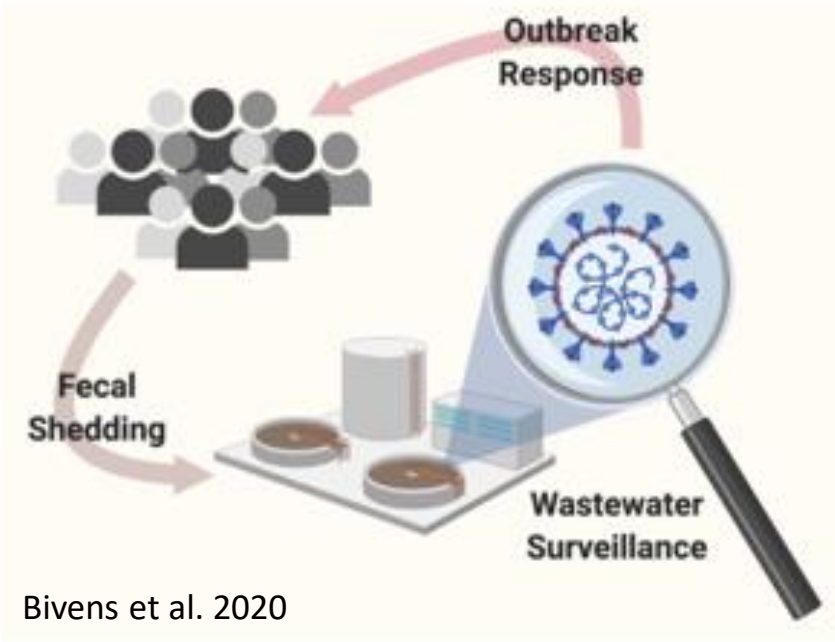




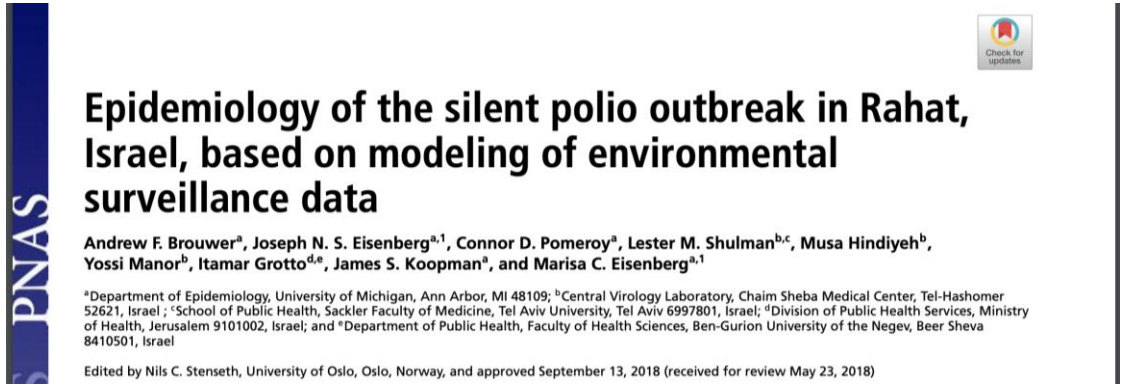
1. Background on wastewater-based epidemiology (WBE) and SARS-CoV-2 in feces

Wastewater Based Epidemiology

- Wastewater is a composite biological sample
- Everyone contributes
- Previous applications for polio, narcotics



Bivens et al. 2020



PNAS

Epidemiology of the silent polio outbreak in Rahat, Israel, based on modeling of environmental surveillance data

Andrew F. Brouwer[a], Joseph N. S. Eisenberg[a,1], Connor D. Pomeroy[a], Lester M. Shulman[b,c], Musa Hindiyeh[b], Yossi Manor[b], Itamar Grotto[d,e], James S. Koopman[a], and Marisa C. Eisenberg[a,1]

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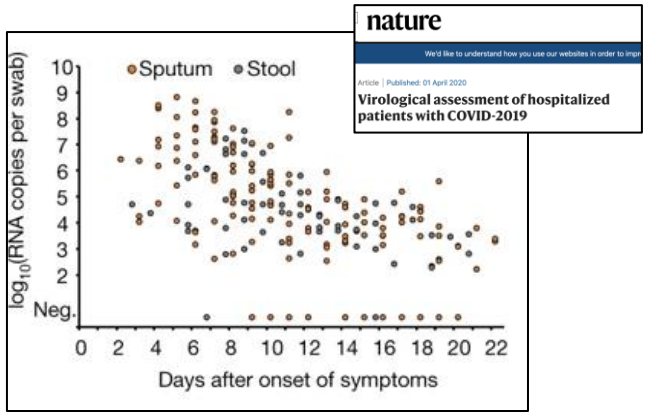
Edited by Nils C. Stenseth, University of Oslo, Oslo, Norway, and approved September 13, 2018 (received for review May 23, 2018)

COVID-19 Surveillance

Clinical Testing

- Lack of test-seeking - asymptomatic and mild cases
- Diagnostic test shortages/delays
- Lag times for reporting



Wastewater Testing

- SARS-CoV-2 RNA shed in feces
- No behavior change required
- Potential as an early indicator

~50% of patients have fecal samples that tested positive for SARS-CoV-2 RNA and shedding of RNA persists for up to several weeks

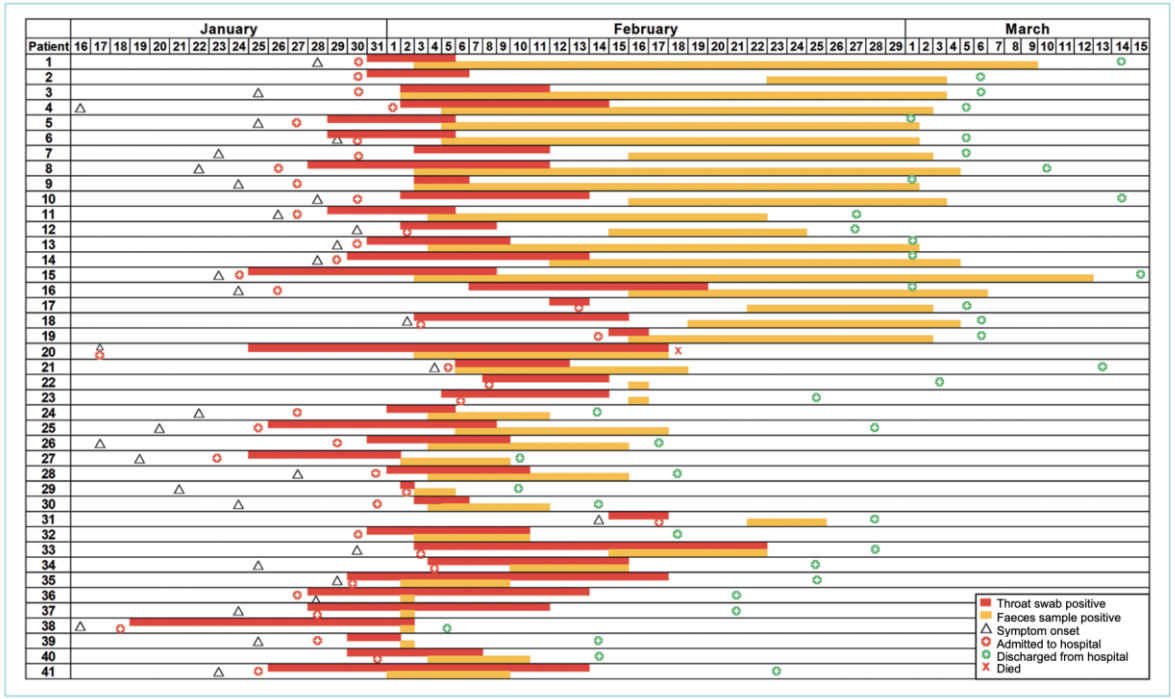


Figure: Timeline of results from throat swabs and faecal samples through the course of disease for 41 patients with SARS-CoV-2 RNA positive faecal samples,

THE LANCET
Gastroenterology & Hepatology

Access provided by UNIVERSITY OF MICHIGAN

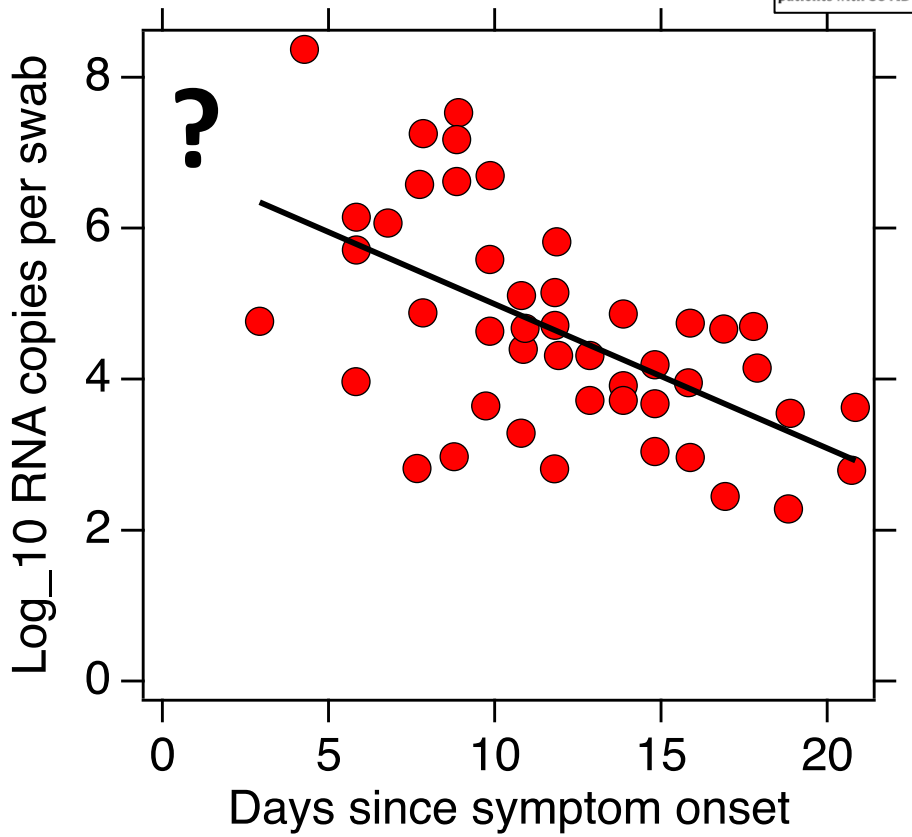
CORRESPONDENCE | VOLUME 5, ISSUE 5, P434-435, MAY 01, 2020

Prolonged presence of SARS-CoV-2 viral RNA in faecal samples

nature

Article | Published: 01 April 2020

Virological assessment of hospitalized patients with COVID-2019



Wastewater vs Clinical Data Timeline

Clinical Results (to patient)

Clinical Results (to county)

Clinical

Tests Reported

4 days (range 1-10)

Test Turnaround

2 days (range 1-5)

Symptomatic

1-21 days

Incubation

5 days (range 1-14)

Days

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Latent

5 days (range 1-14)

Wastewater

Fecal Shedding

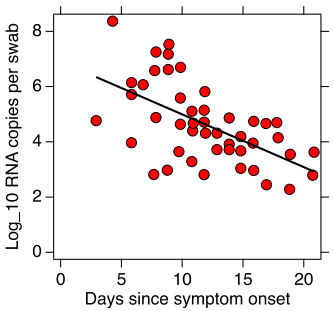
1-? days

Test Turnaround

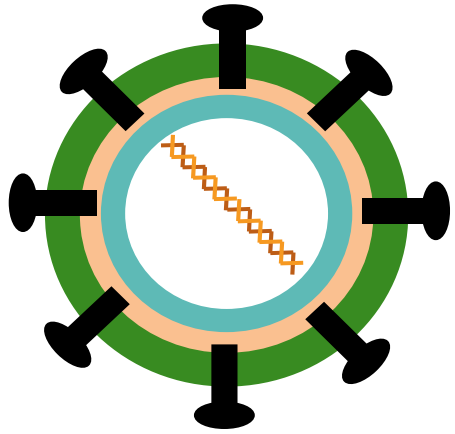
1 day

Wastewater Results

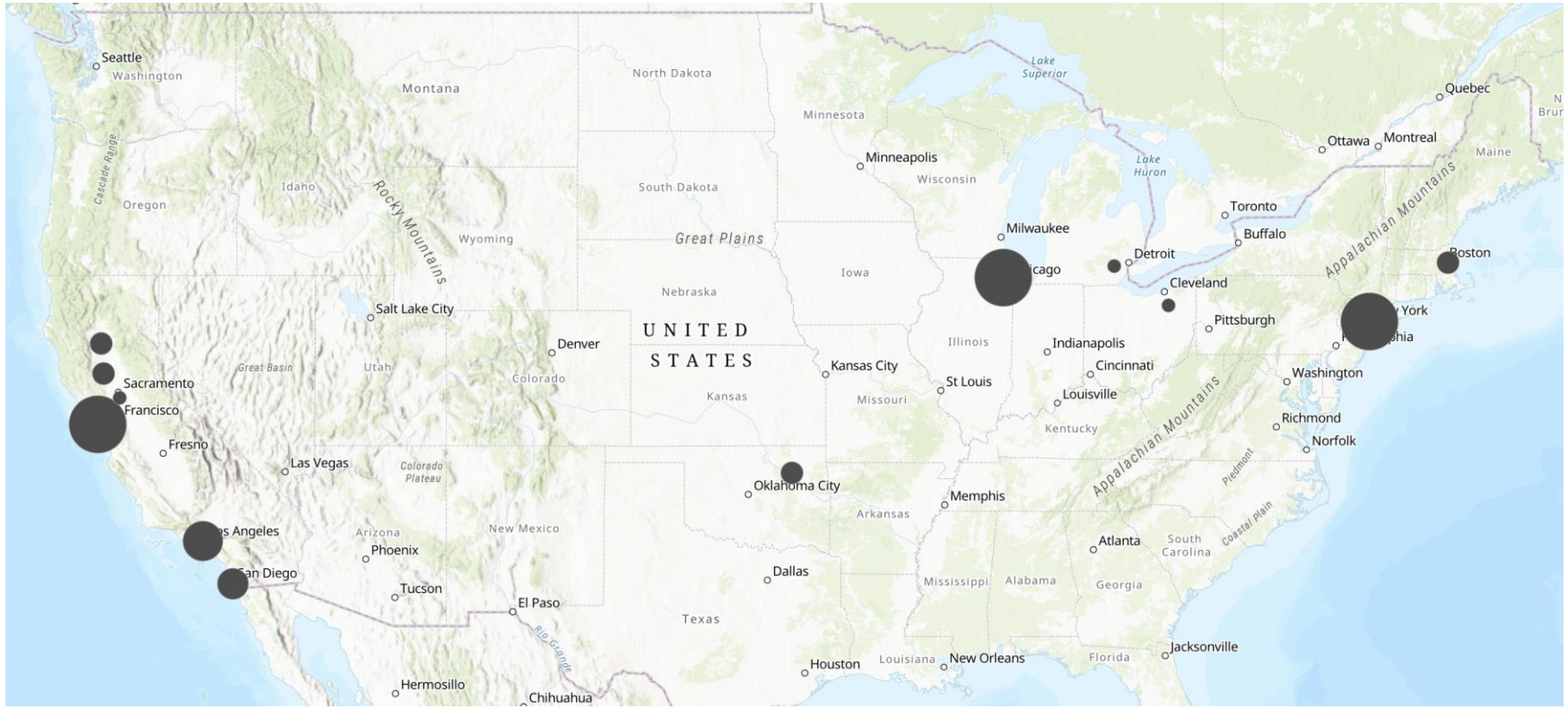
data from Wölfel paper on fecal shedding



2. Sample acquisition and method development



Sampling

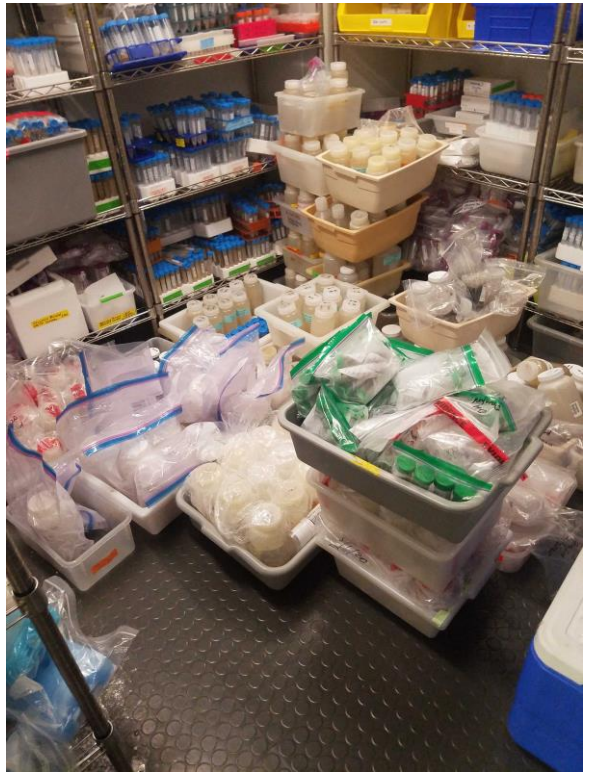


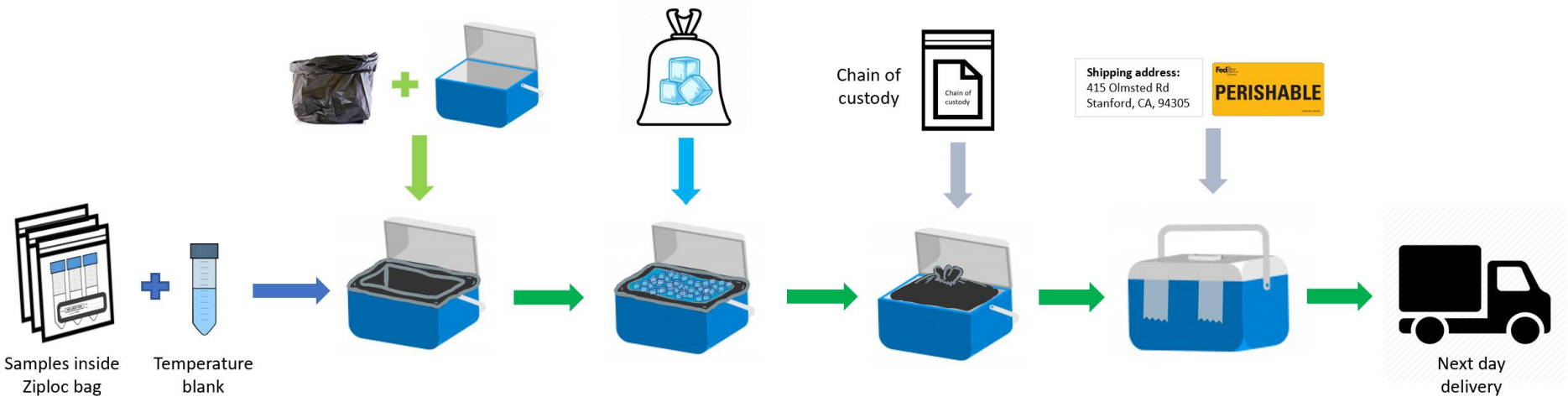
Regular sampling of wastewater took place at a total of 50 WWTPS nationwide

Influent and primary settled solids

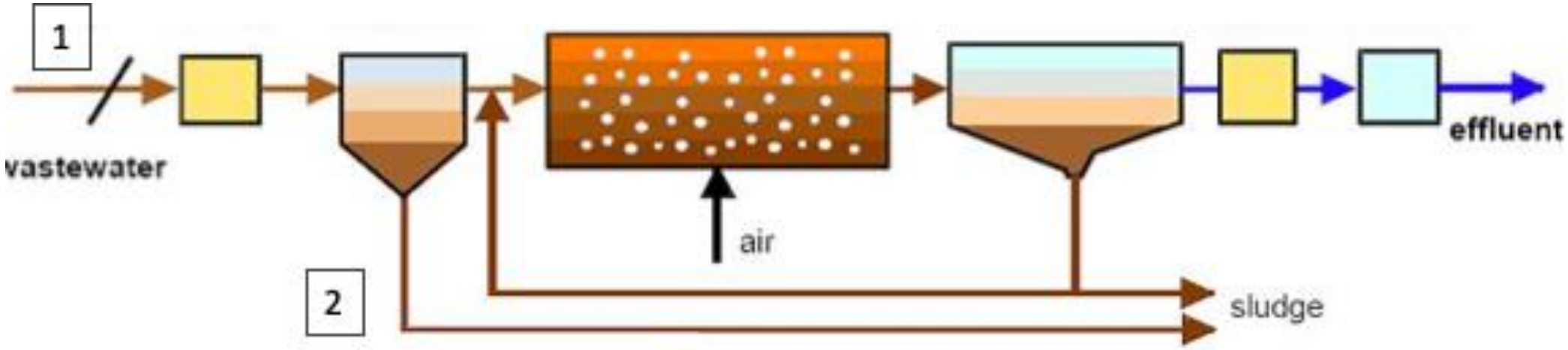
Sample aquisition

- We spent a significant amount of time in Feb and March 2020 organizing sample collection, shipping, pick-up and storage
- Influent? Solids? How to store?
- We collected ~ 1500 samples





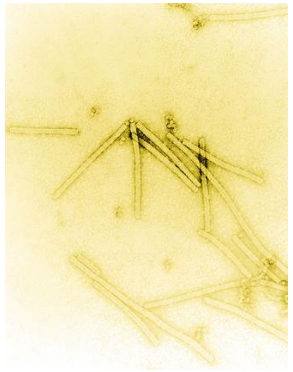
Method optimization and testing



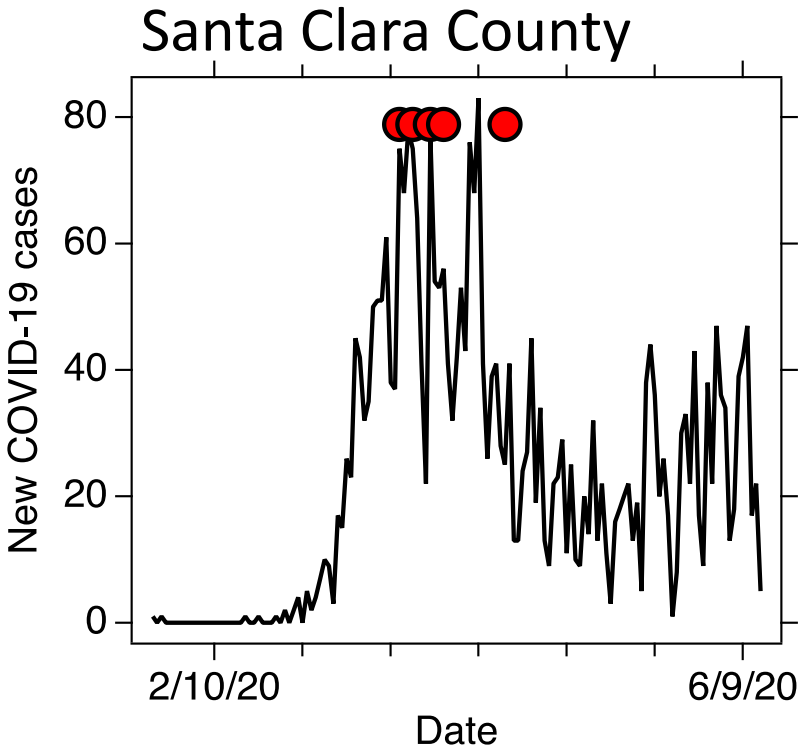
Previous work out of the Wigginton group showed enveloped viruses like SARS-CoV-2 have an affinity for wastewater solids, so we wanted to sample settle solids in addition to influent.

Method testing study design

- Compare SARS-CoV-2 RNA levels in influent and solids at 2 wastewater plants on 5-7 days
- Compare results from QPCR analyses to ddPCR analyses
- Measure viral RNA recovery (BCoV)
- Measure “fecal strength” of waste stream using pepper mild mottle virus (PMMoV), also serves as a recovery control

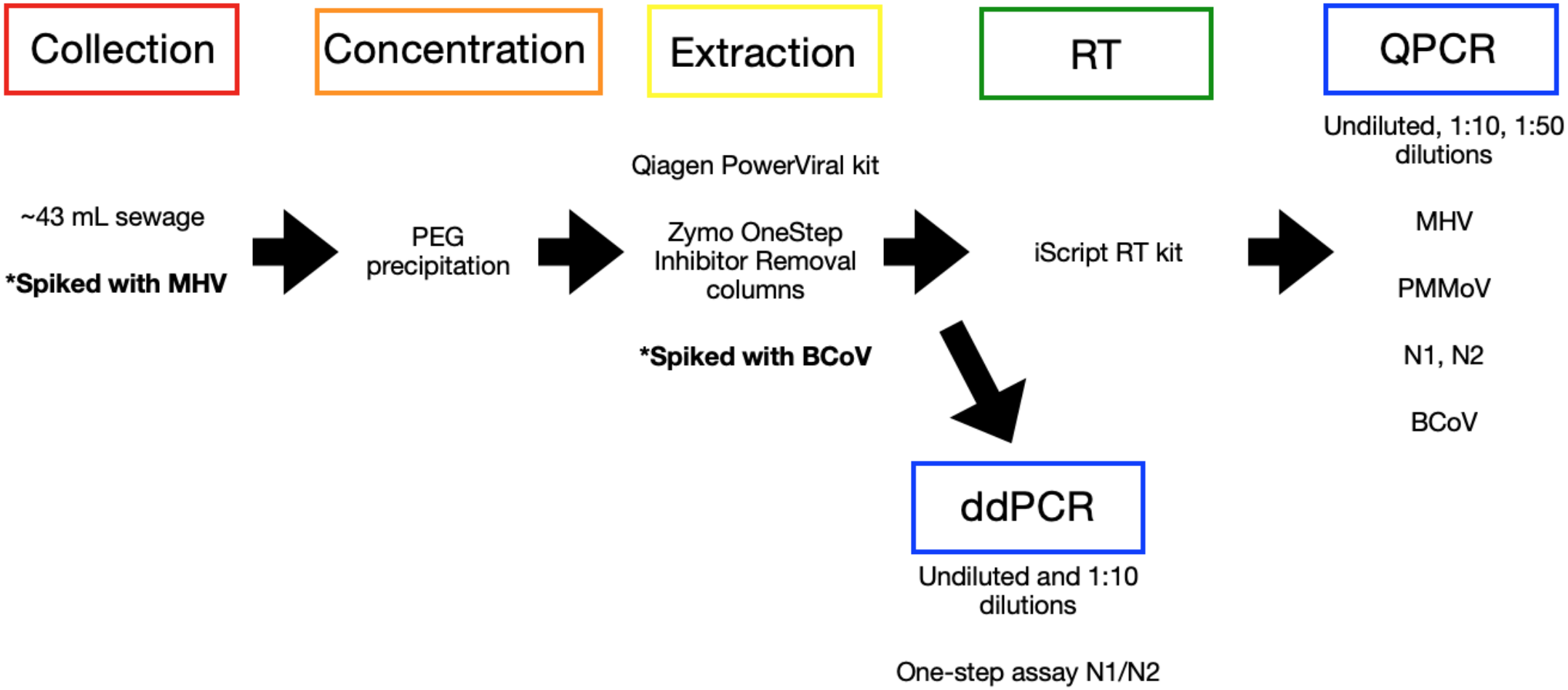


PMMoV is a rod shaped, non-enveloped, RNA virus

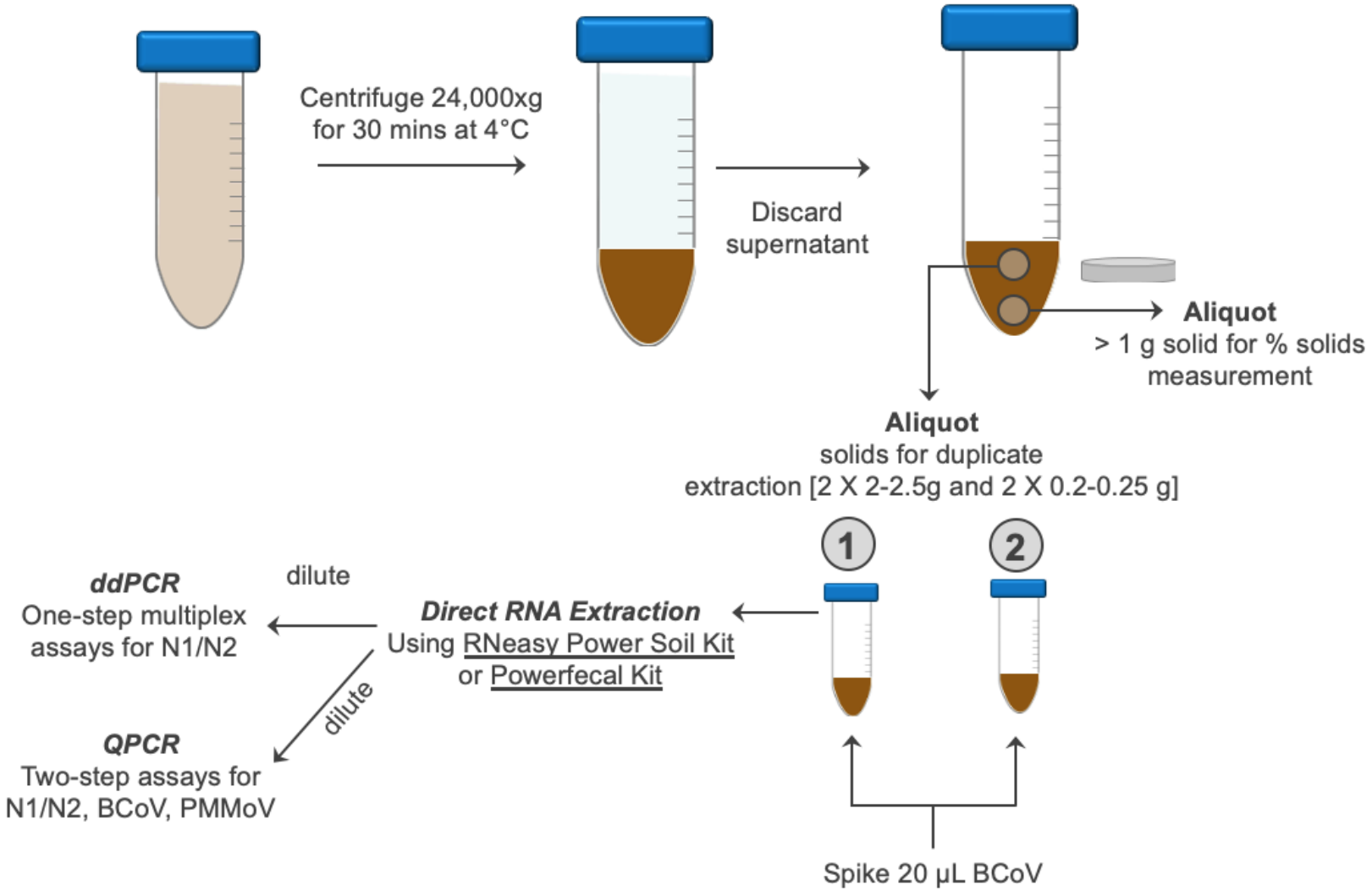


Samples labeled by date

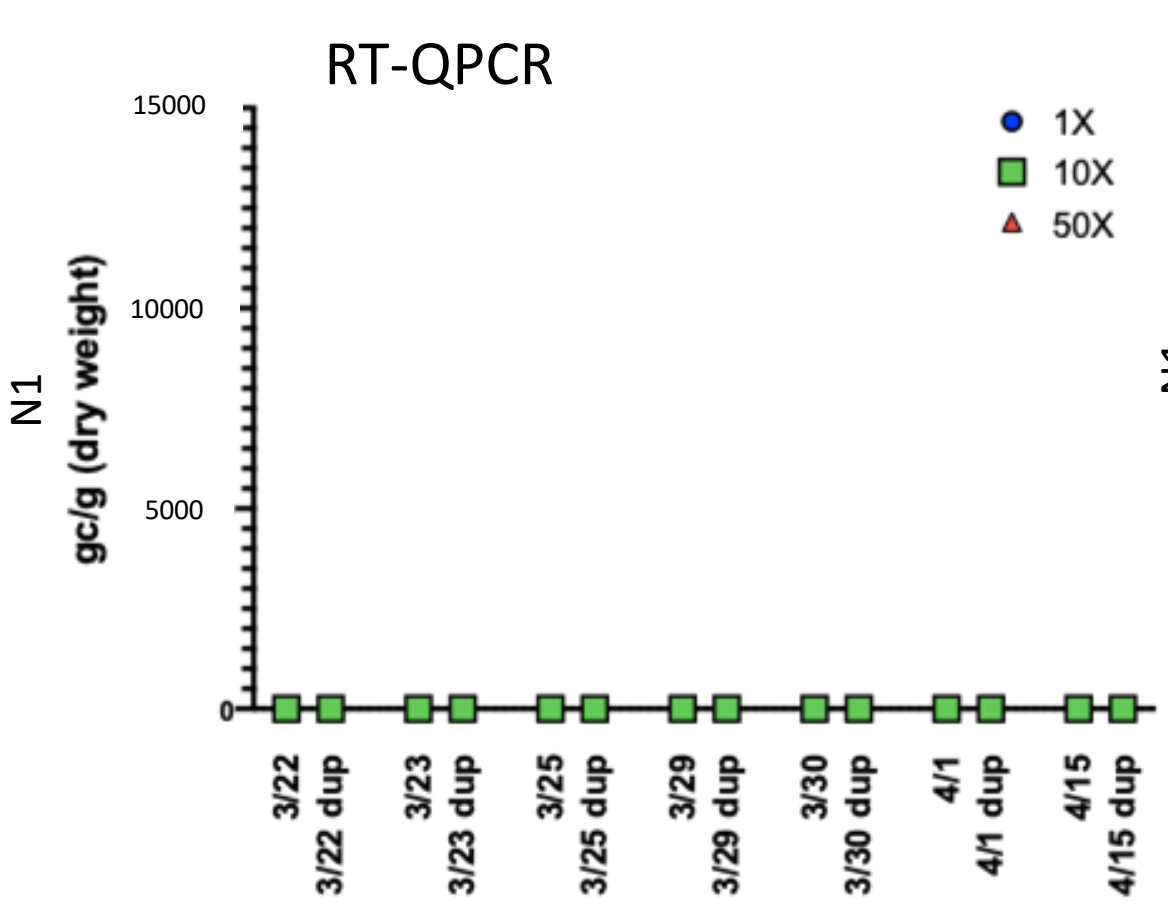
Pre-analytical & analytical workflow for influent

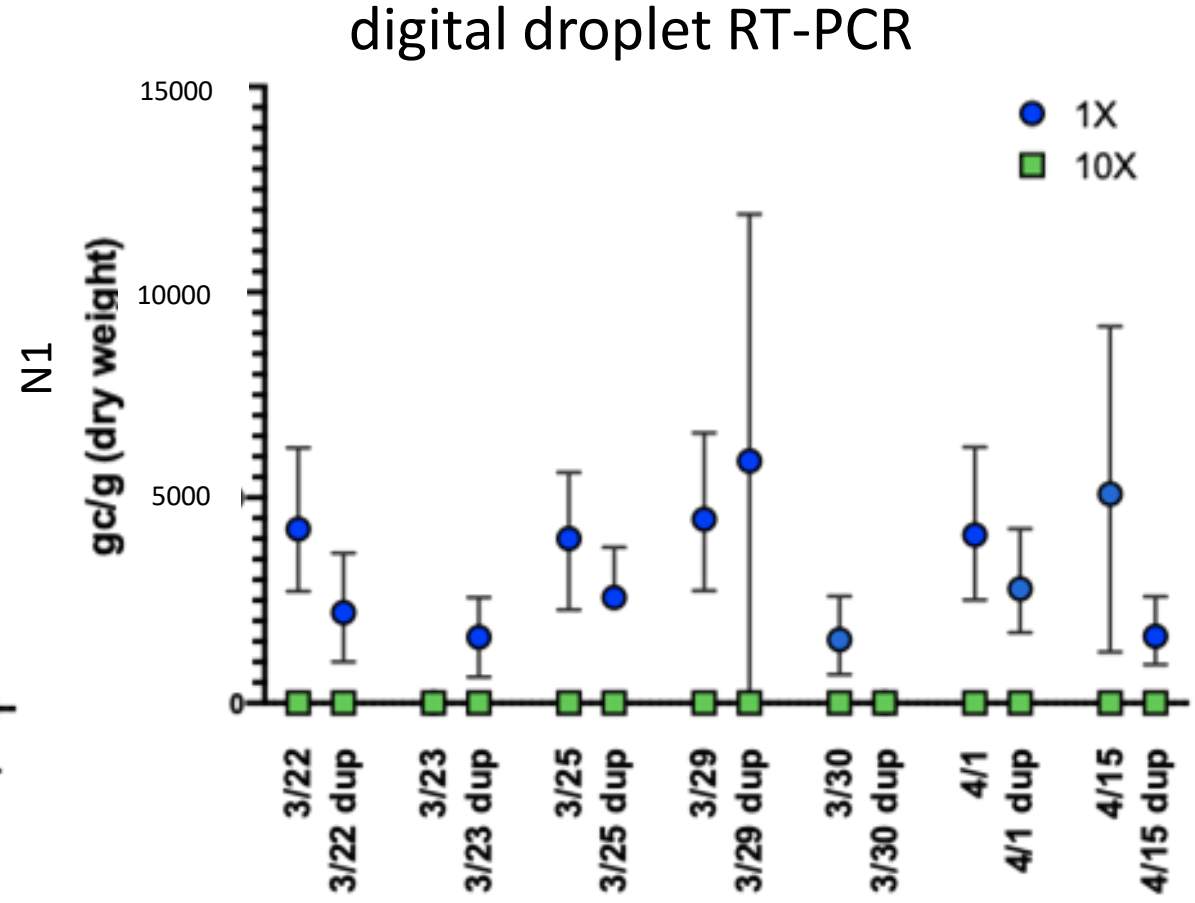


Pre-analytical & analytical workflow for solids



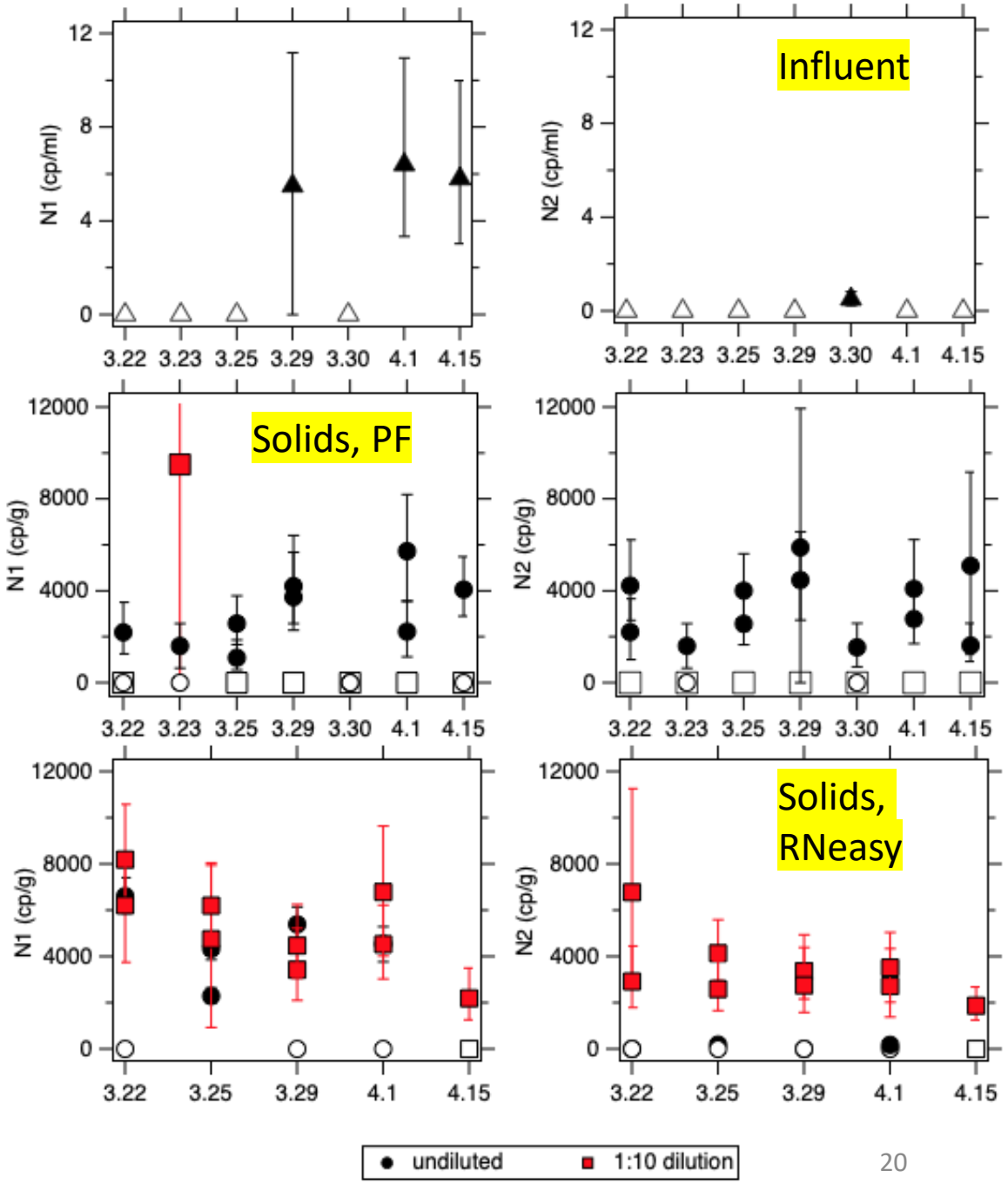
Two-step RT-QPCR vs one-step digital PCR for solid sample





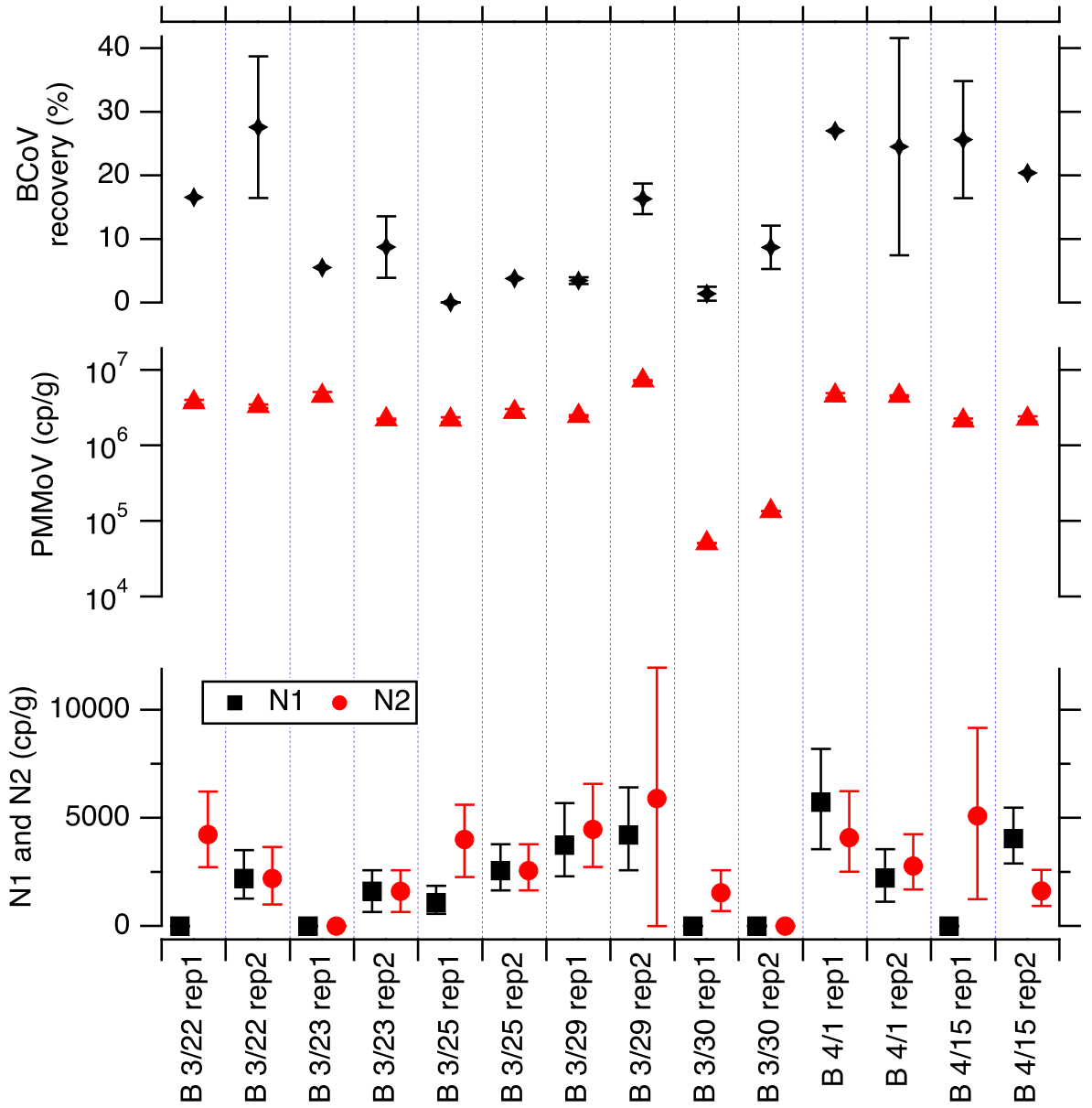
Solids outperform influent

- Limited variation among replicates
- Inhibition an issue for some samples
- Concentrations of N1 and N2 1000 x higher in solids than influent on a per mass basis

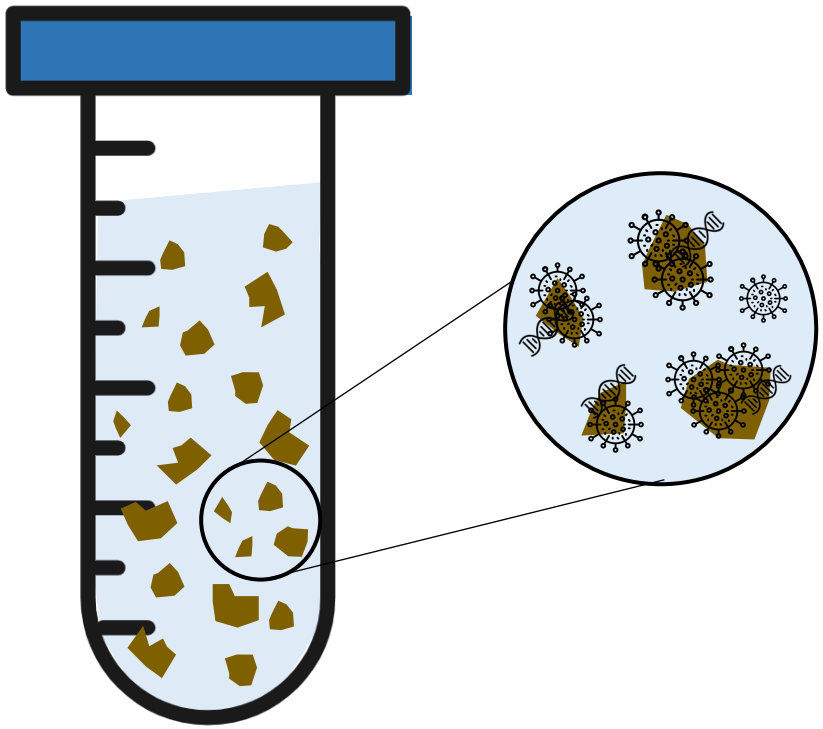


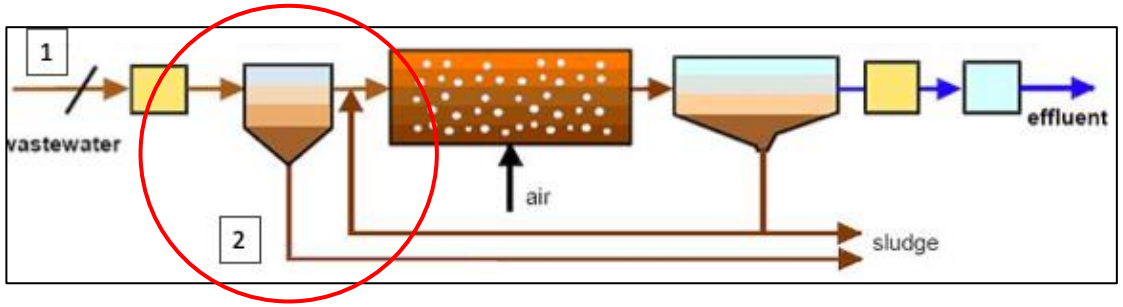
Example BCoV recoveries and PMMoV for Powerfecal extraction kit

- Recoveries varied between 0 and 25%.
- PMMoV was fairly constant (except one day)
- No correlation between recovery and PMMoV, N1, and N2 concentration



Method evaluation conclusion

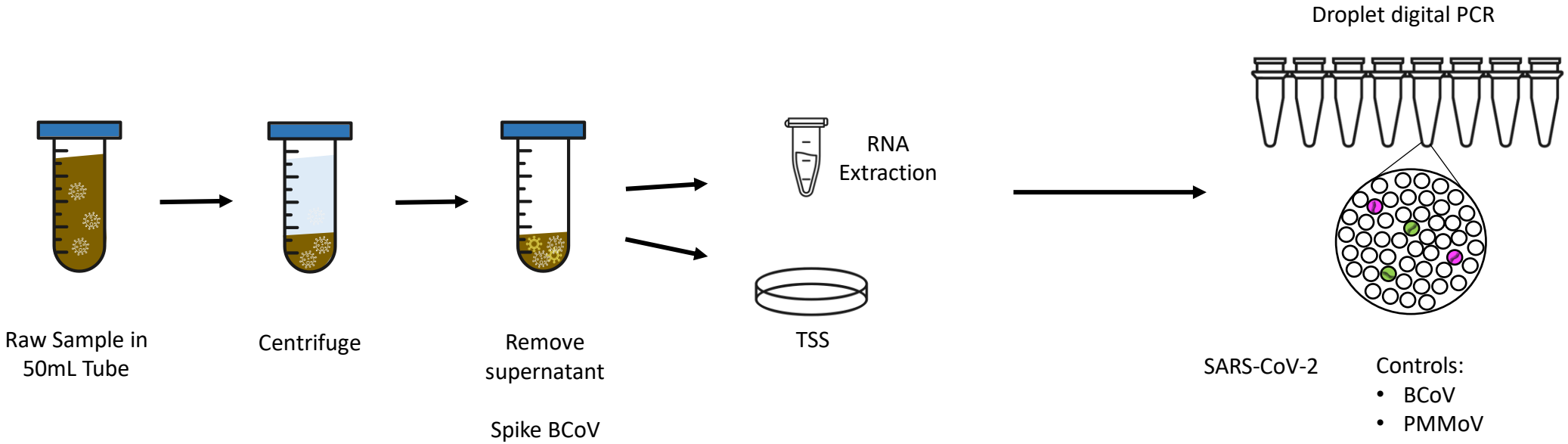




- Higher rate of detection in **solids**
- SARS-CoV-2 genes 1000x higher in **solids** (per mass)
- Limited variation among replicates
- Move forward with N1 and N2 in settled solids for SARS-CoV-2 surveillance at wastewater treatment plants
- Protocols available at protocols.io

Laboratory Methods

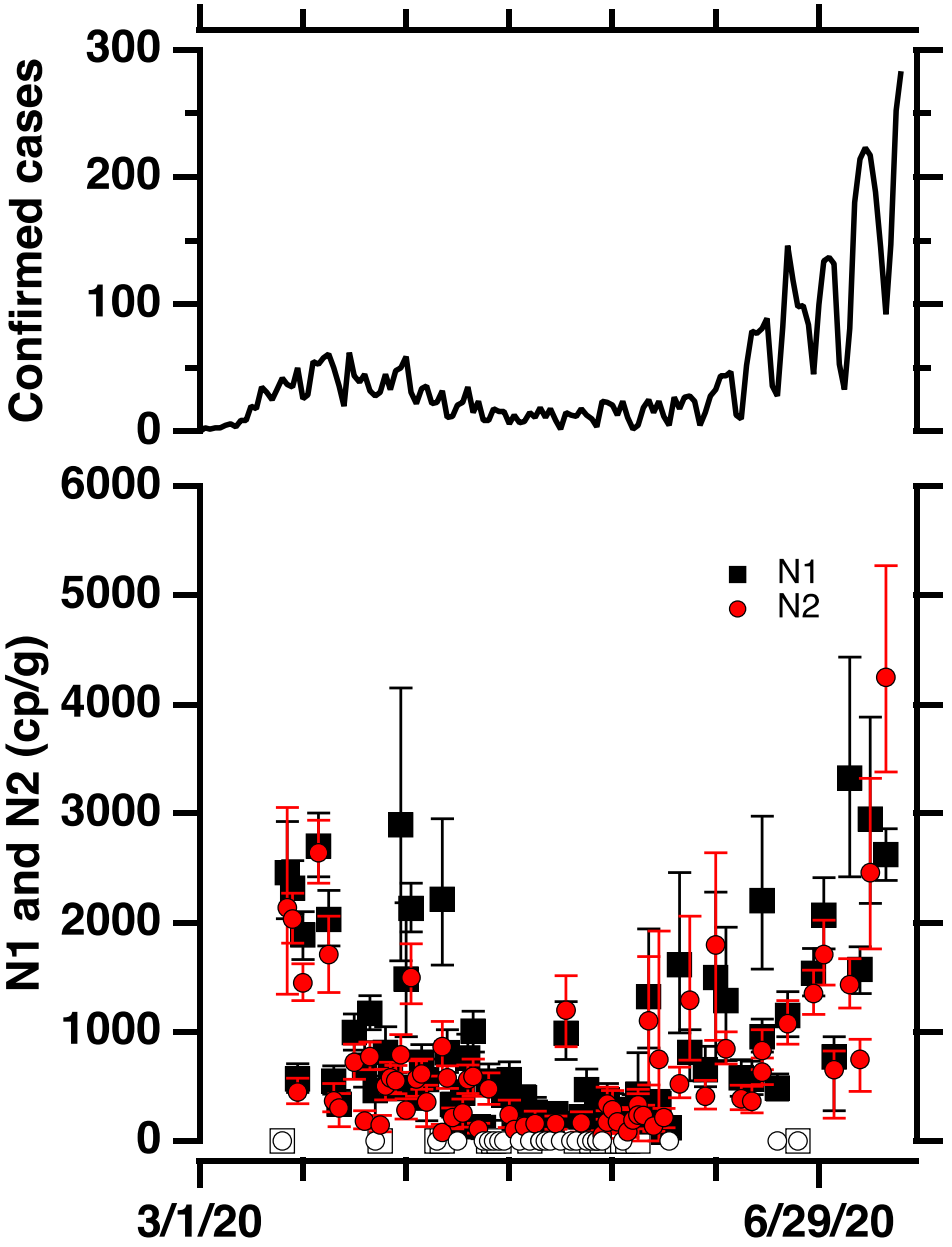
- Direct RNA extraction from dewatered solids
- Droplet digital PCR for SARS-CoV-2 genes and analytical controls
- Controls:
  - Pepper Mild Mottle Virus as an endogenous control and measure of fecal strength
  - Bovine coronavirus (BCoV) as a process control, to estimate recovery

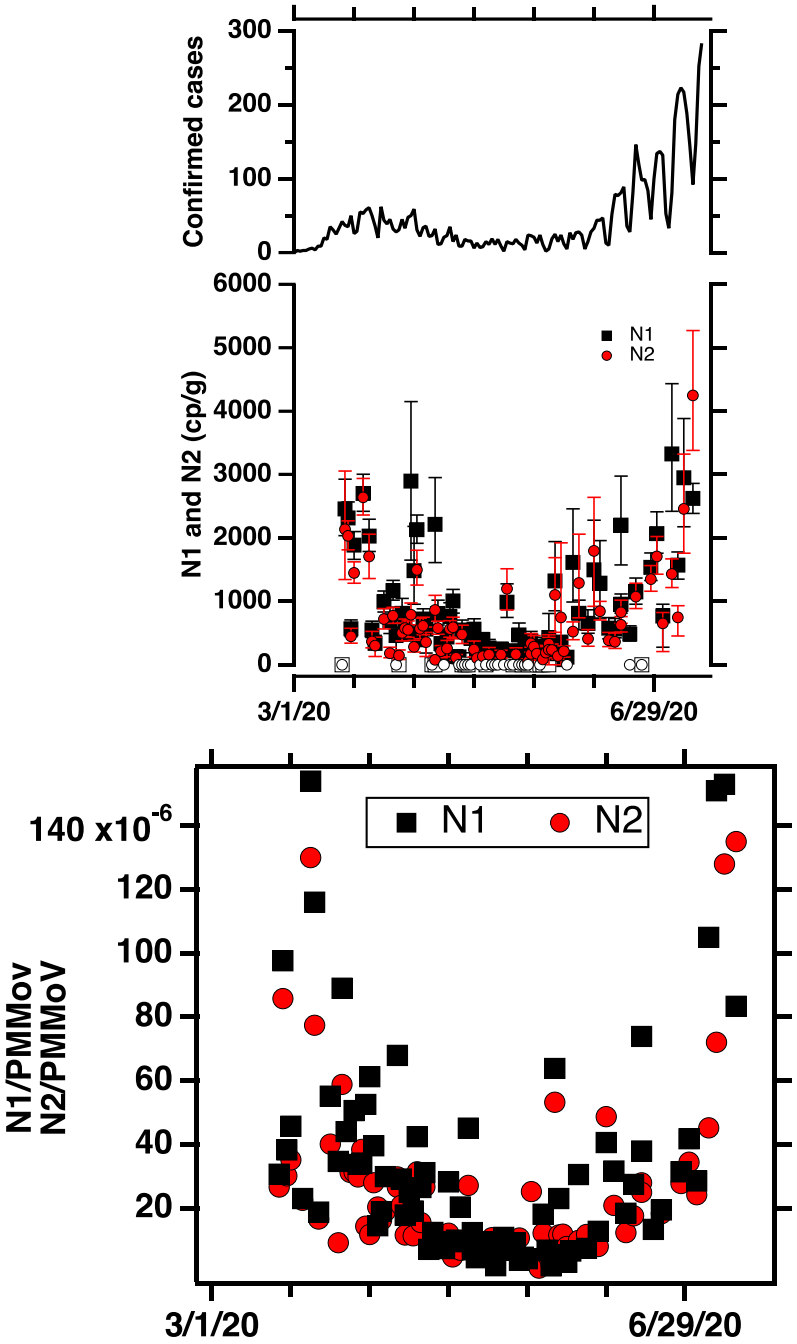


3. Application to retrospective samples

Longitudinal data from San Jose WWTP

- Serves 1.5 million people in Silicon Valley
- Add $FeCl_2$ as pre-treatment step
- Daily to 3 times per week, 24 h composite samples
- Followed solids protocol with Rneasy extraction kit
- Measured N1 and N2 SARS-CoV-2 gene targets
- Measured PMMoV and BCoV recovery
- Confirmed clinical cases in sewershed from county epidemiologist





Association between N1 and N2 and case data?

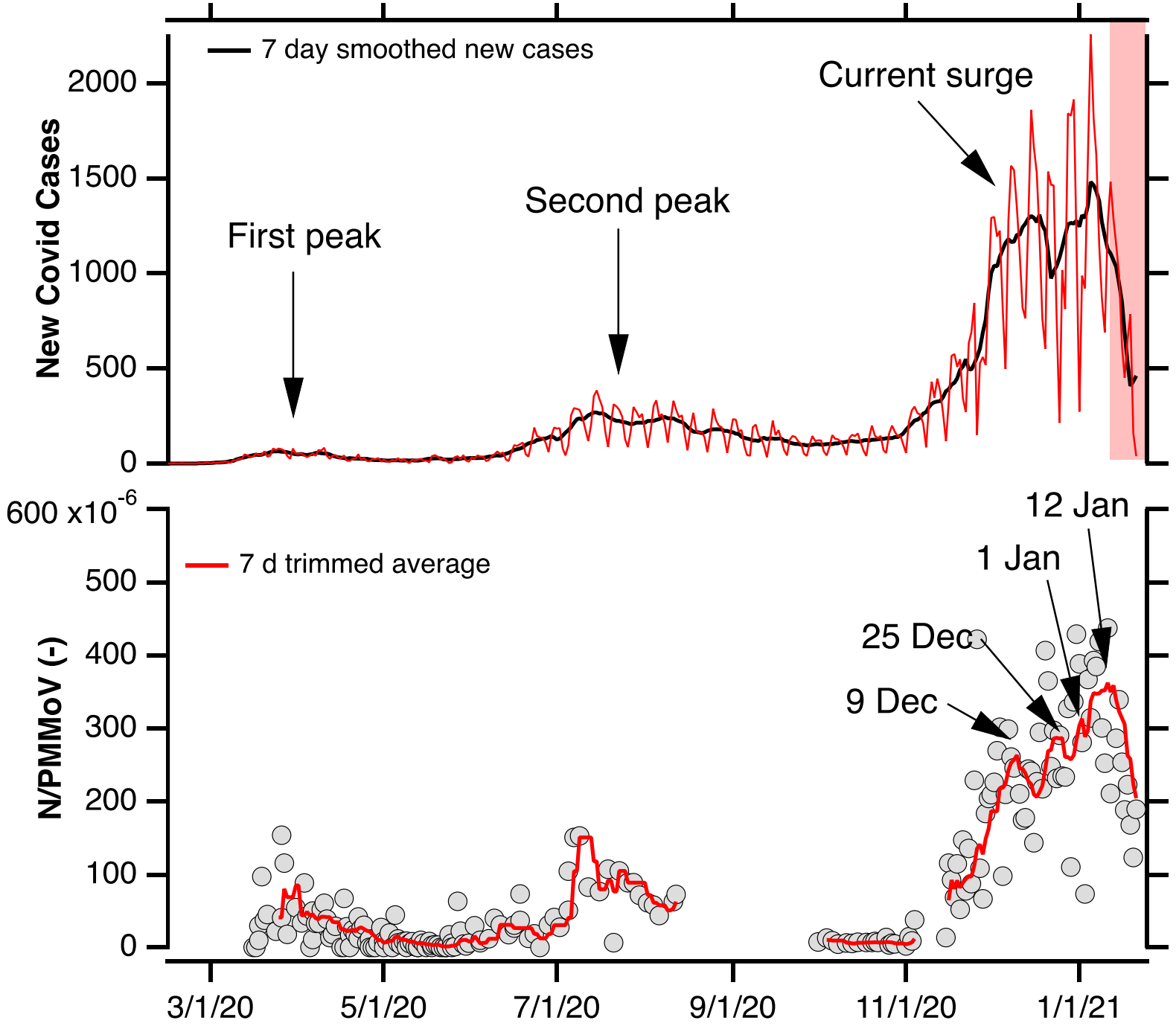
- Positive correlation (first difference approach used to account for autocorrelation)
- Downsampling analysis → sampling less than 2 X per week – correlation not significant
- Normalizing by PMMoV – correlation still strong

How could these data have been used retrospectively?

- In early June during apparent rise in new cases, POTW data could have confirmed not a testing artifact
- Could have been used to confirm disappearance of cases during late April / early May

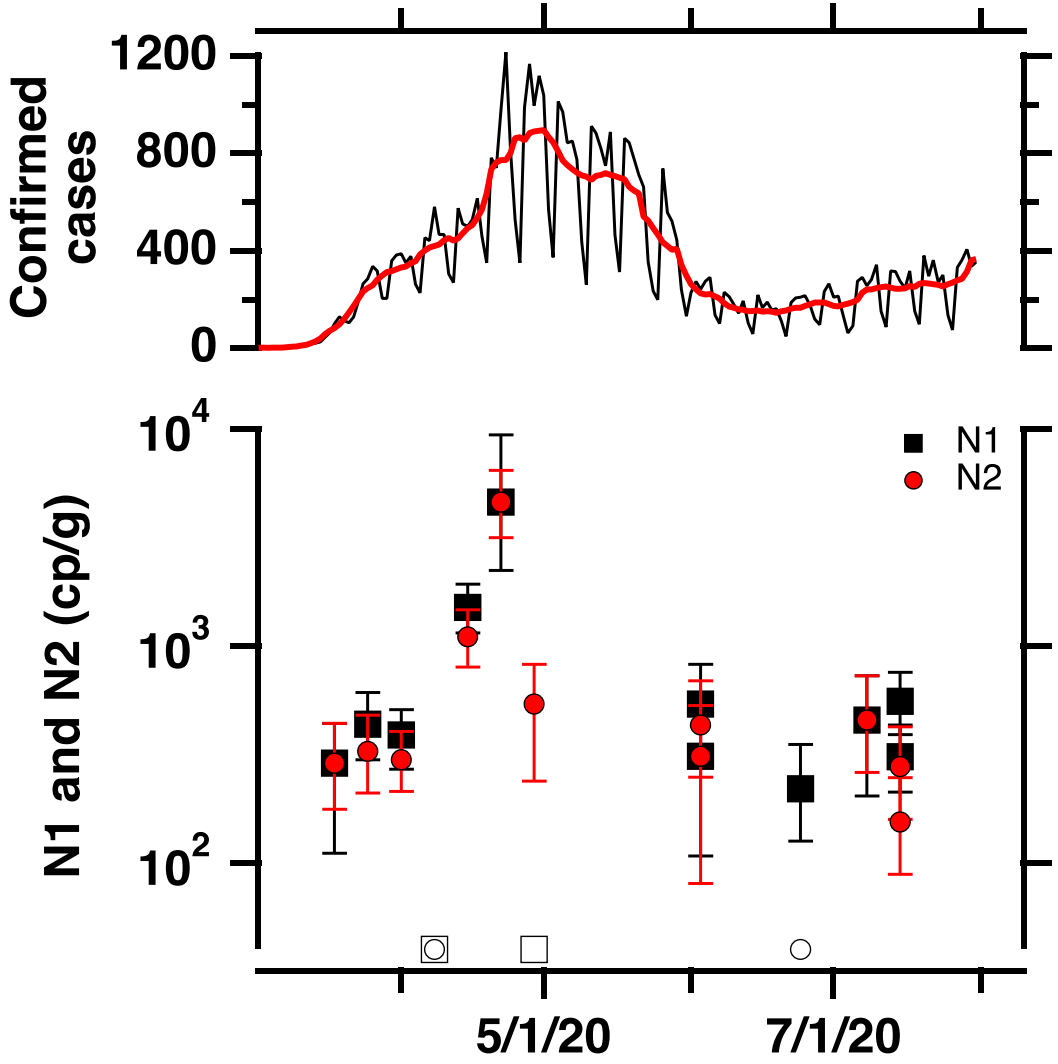
How can results be used to learn about infectious prevalence?

- First peak actually much higher than reflected in case data (owing to limitation in testing).
- Can these data provide insight into time course of fecal shedding or infection prevalence?


7 day smoothed new cases
New Covid Cases
2000
1500
1000
500
0
First peak
Second peak
Current surge
600 x10-6
500
400
300
200
100
0
7 d trimmed average
N/PMMoV (-)
12 Jan
1 Jan
25 Dec
9 Dec
3/1/20
5/1/20
7/1/20
9/1/20
11/1/20
1/1/21

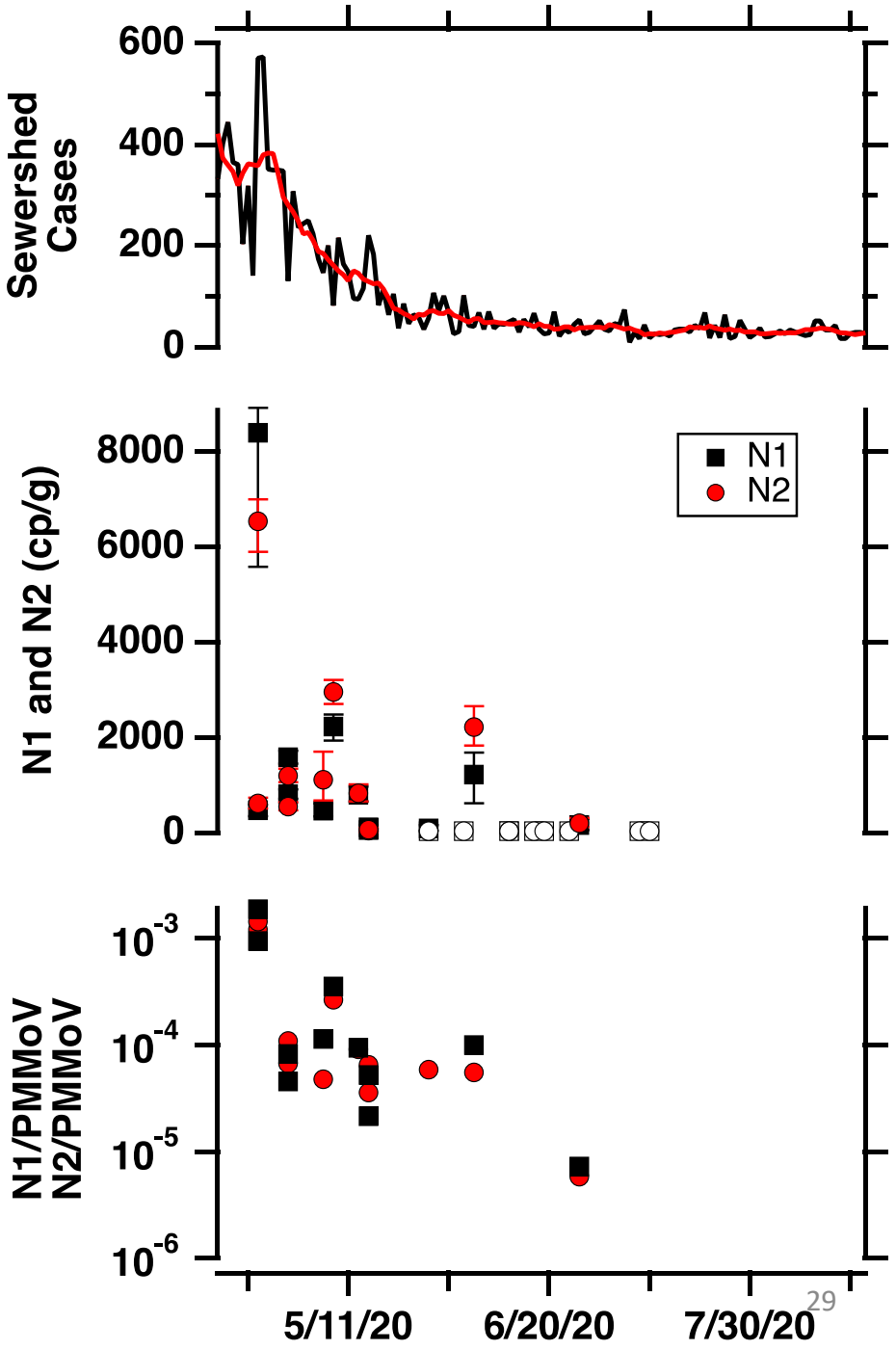
Longitudinal data from Stickney, Chicago

- Serves 2.5 million people
- Weekly data, 24 h composite samples
- Followed solids protocol with Rneasy extraction kit
- Measured N1 and N2 SARS-CoV-2 gene targets
- Measured PMMoV and BCoV recovery

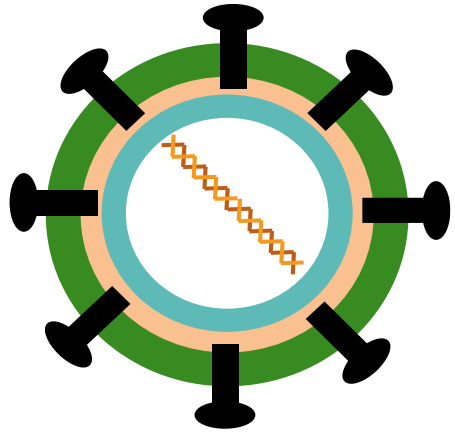


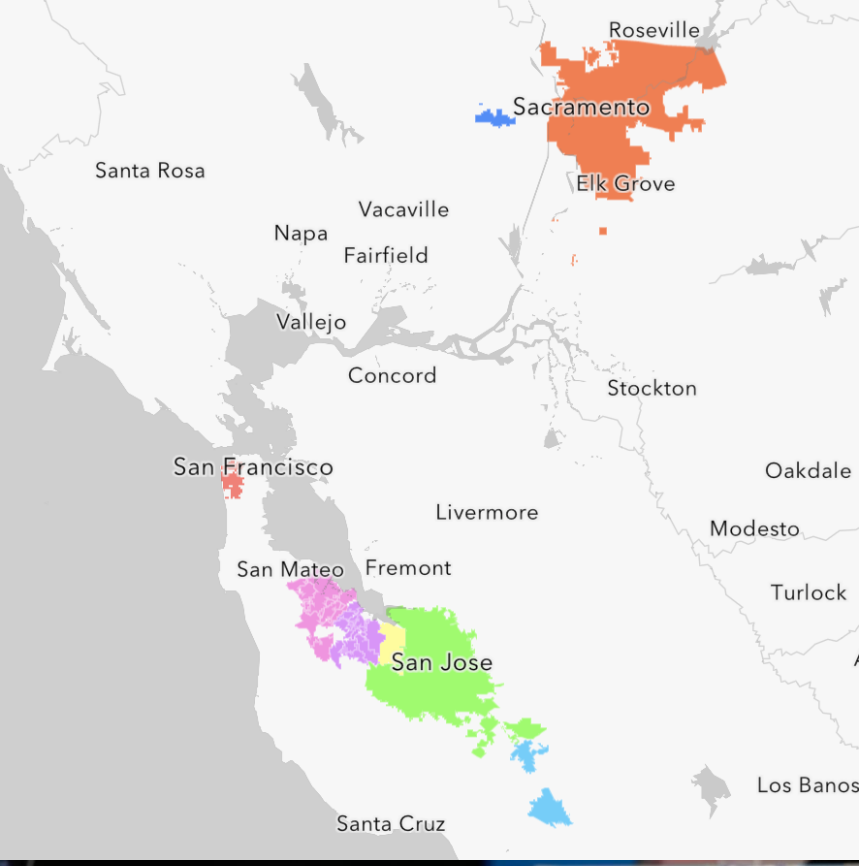
Longitudinal data from New York

- Serves 848,328 people
- Weekly data, grab samples
- Followed solids protocol with Rneasy extraction kit
- Measured N1 and N2 SARS-CoV-2 gene targets
- Measured PMMoV and BCoV recovery
- Sewer-shed level clinical data



4. Real time surveillance



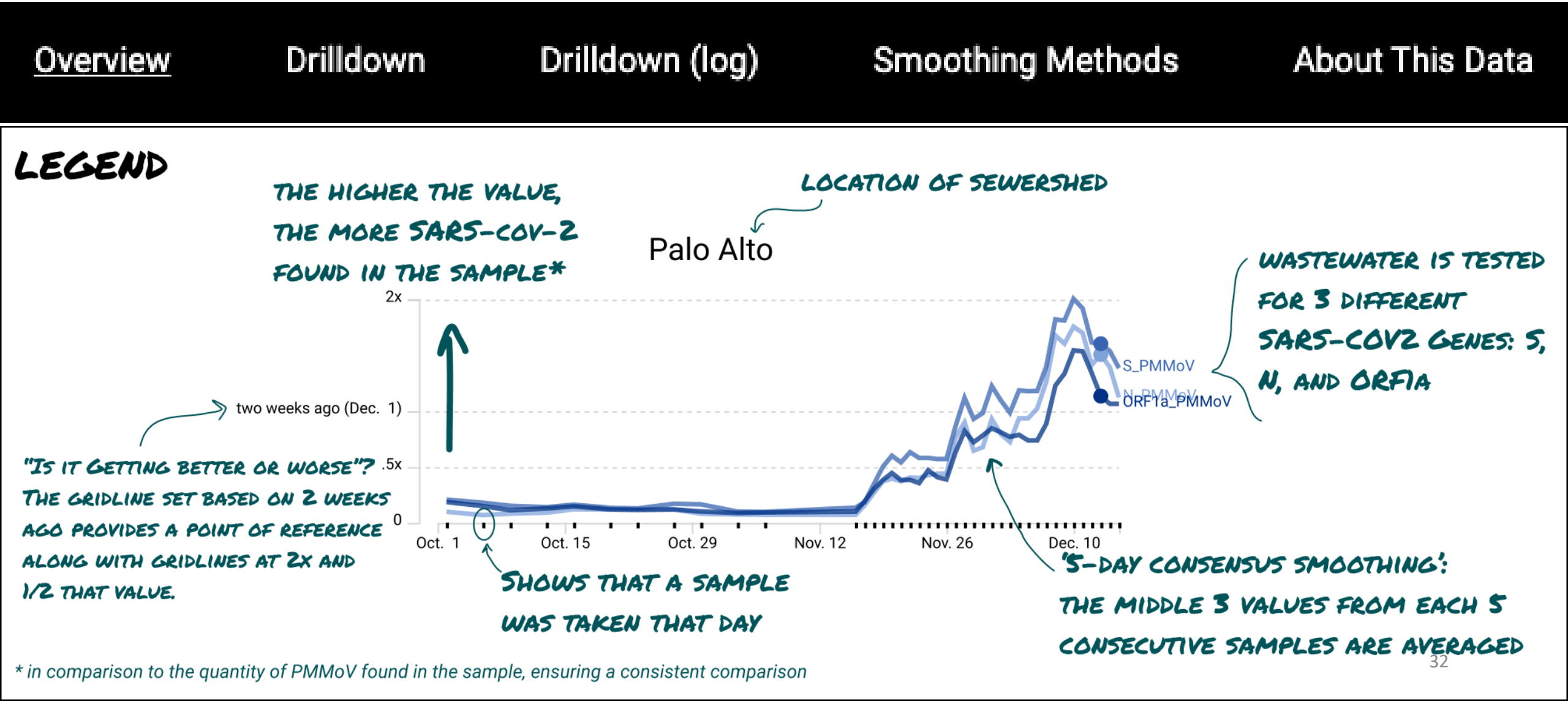


Real time monitoring Pilot

- 8 plants with daily samples
- Process solids with 10 replicates
- Measure:
  - SARS-CoV-2: N Gene, S Gene, ORF1a
  - Controls: BCoV, PMMoV
- Posted to dashboard in 24 hours
- Daily communication and weekly meeting with plants and health officers and CDC
- Partner with an industrial lab with robotics

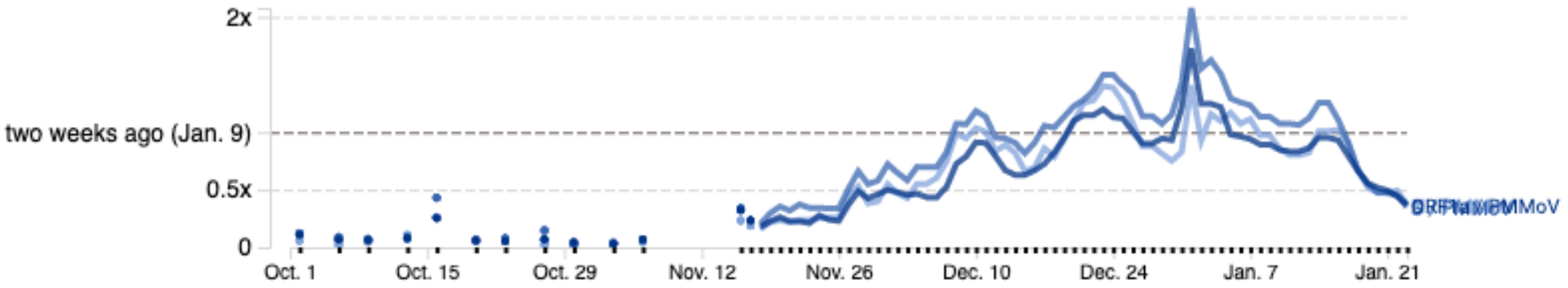
wbe.stanford.edu

Communicating with stakeholders via dashboard

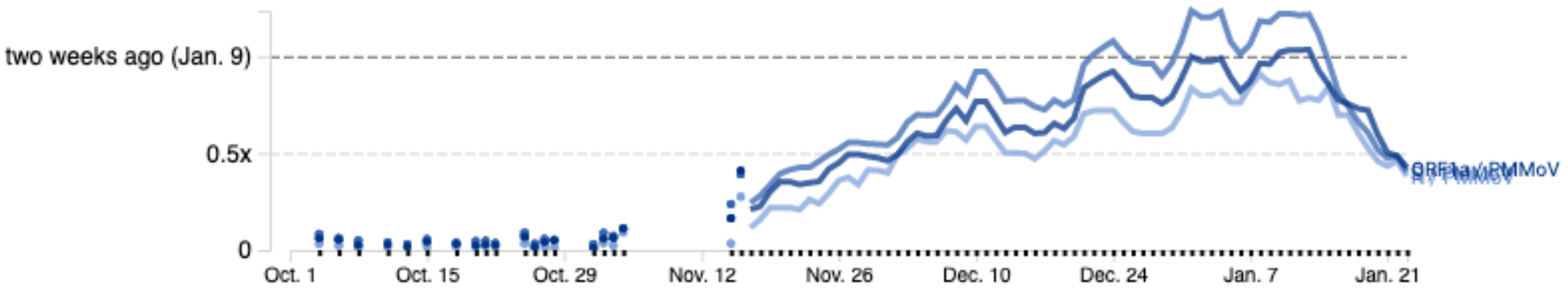


Overview | Drilldown | Drilldown (log) | Smoothing Methods | About This Data

Palo Alto

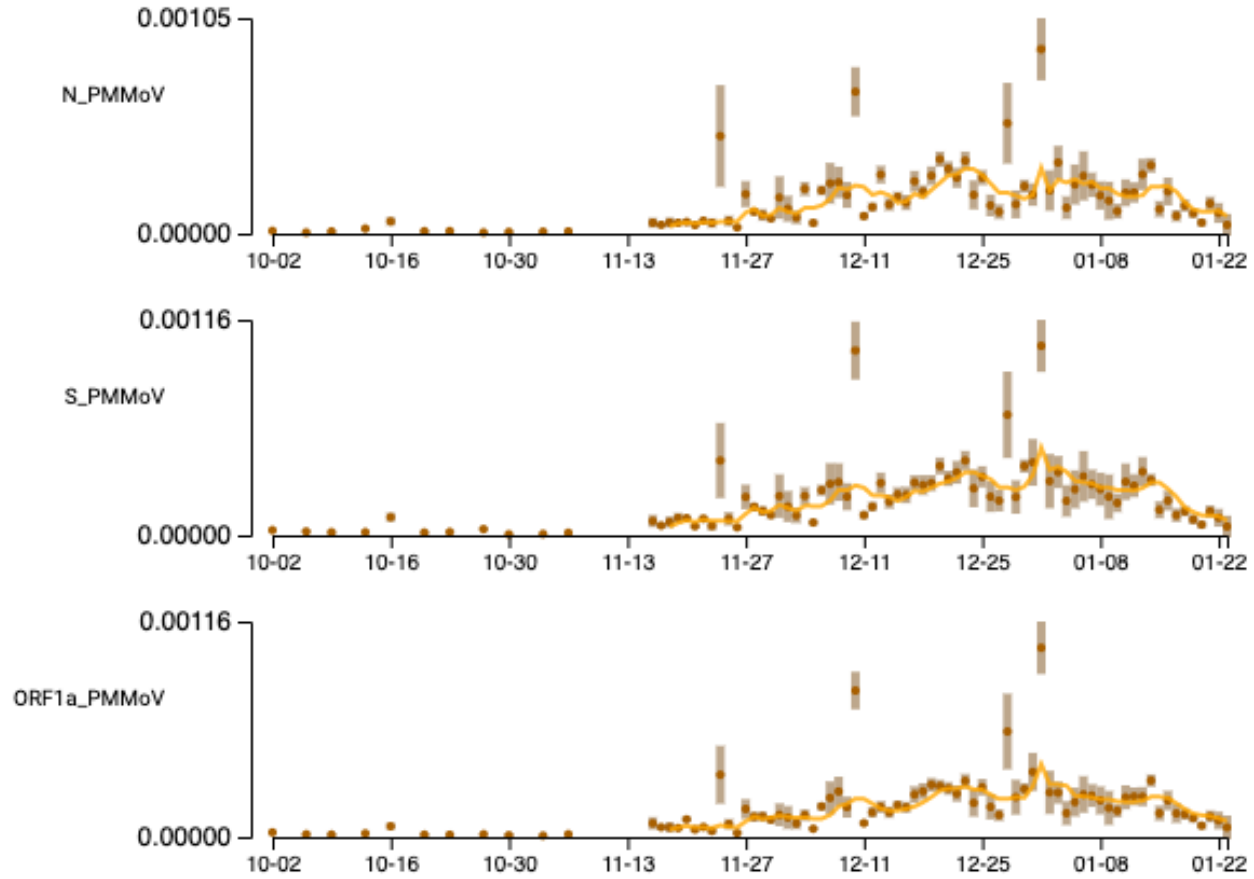


San Jose

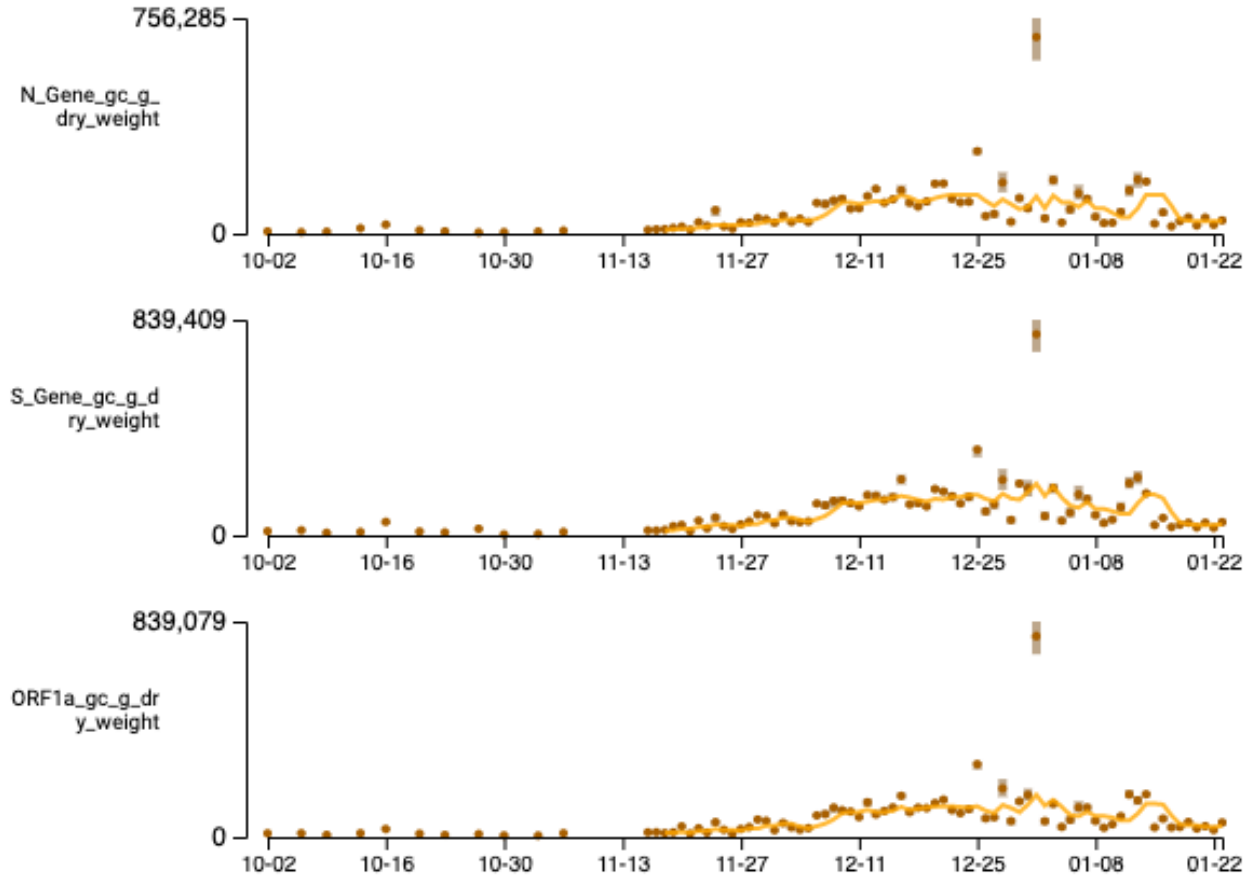


Palo Alto

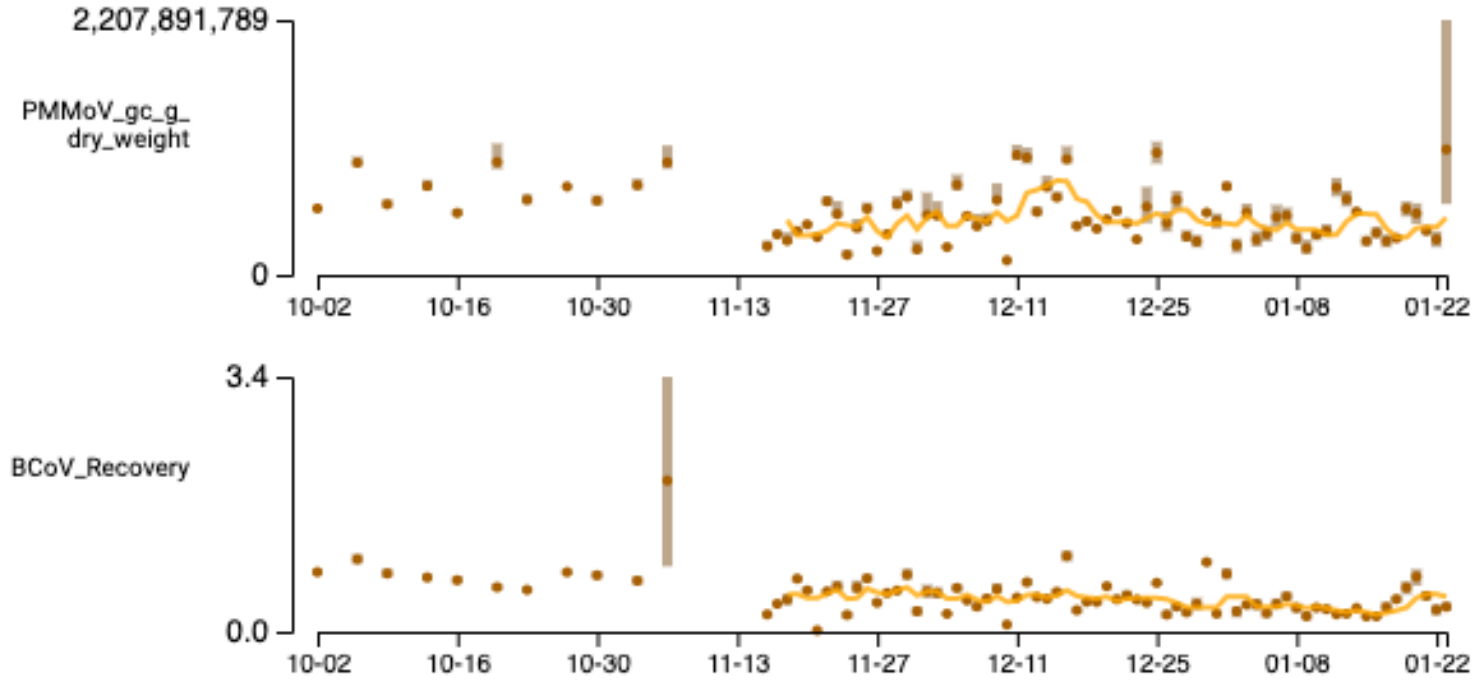
Normalized by PMMoV, Linear Scale



SARS-CoV-2 Genes Raw



Quality Control



Overview | Drilldown | Drilldown (log) | Smoothing Methods | About This Data

Palo Alto | San Jose | Sunnyvale | Gilroy | Davis | Oceanside | Silicon Valley | Sacramento

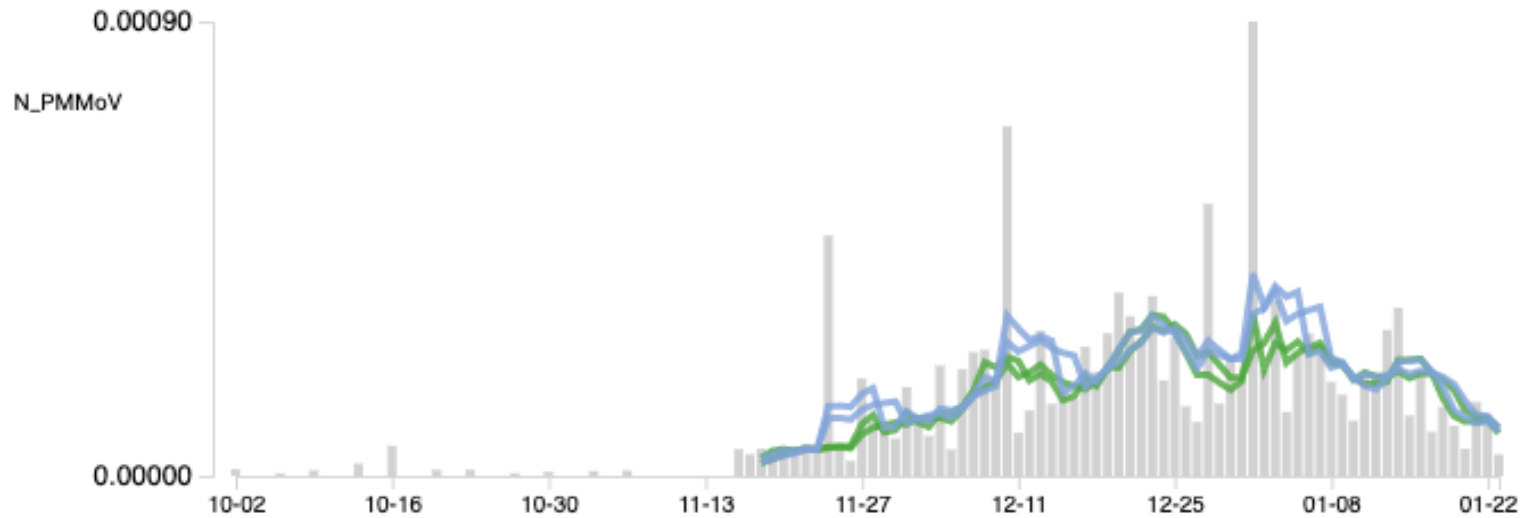
- [x] show consensus smoothing, 5 day window
- [x] show consensus smoothing, 7 day window
- [x] show moving average, 5 day window
- [x] show moving average, 7 day window

Palo Alto

On left, see the daily values as bar charts and the smoothed lines for four different methods.

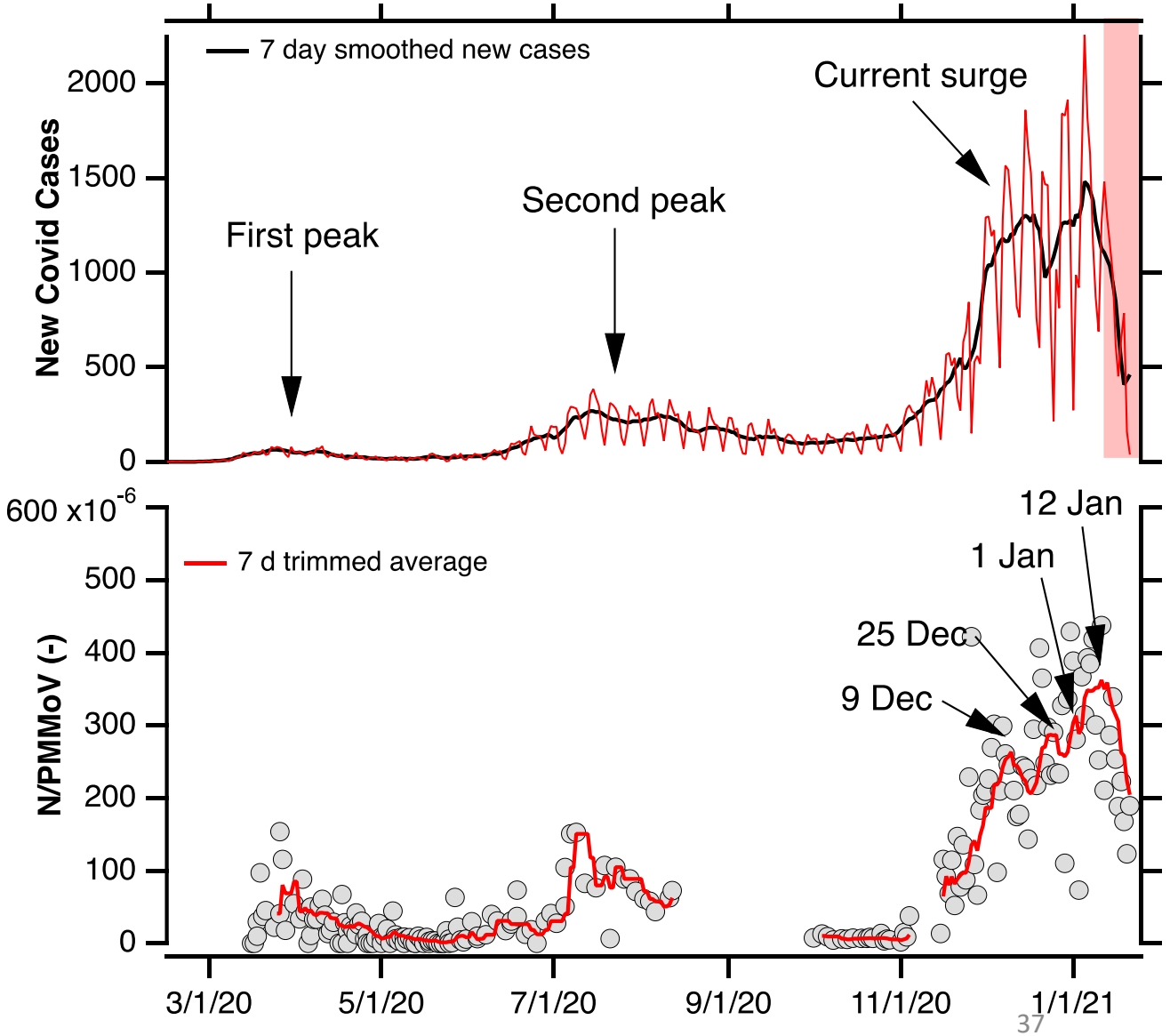
On right, see the same smoothed lines without the bar chart (and a different y-axis).

Normalized by PMMoV

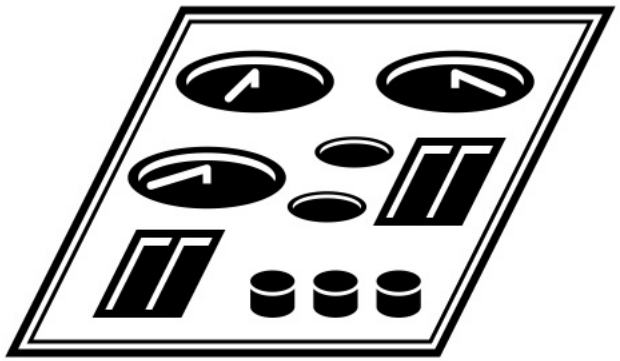


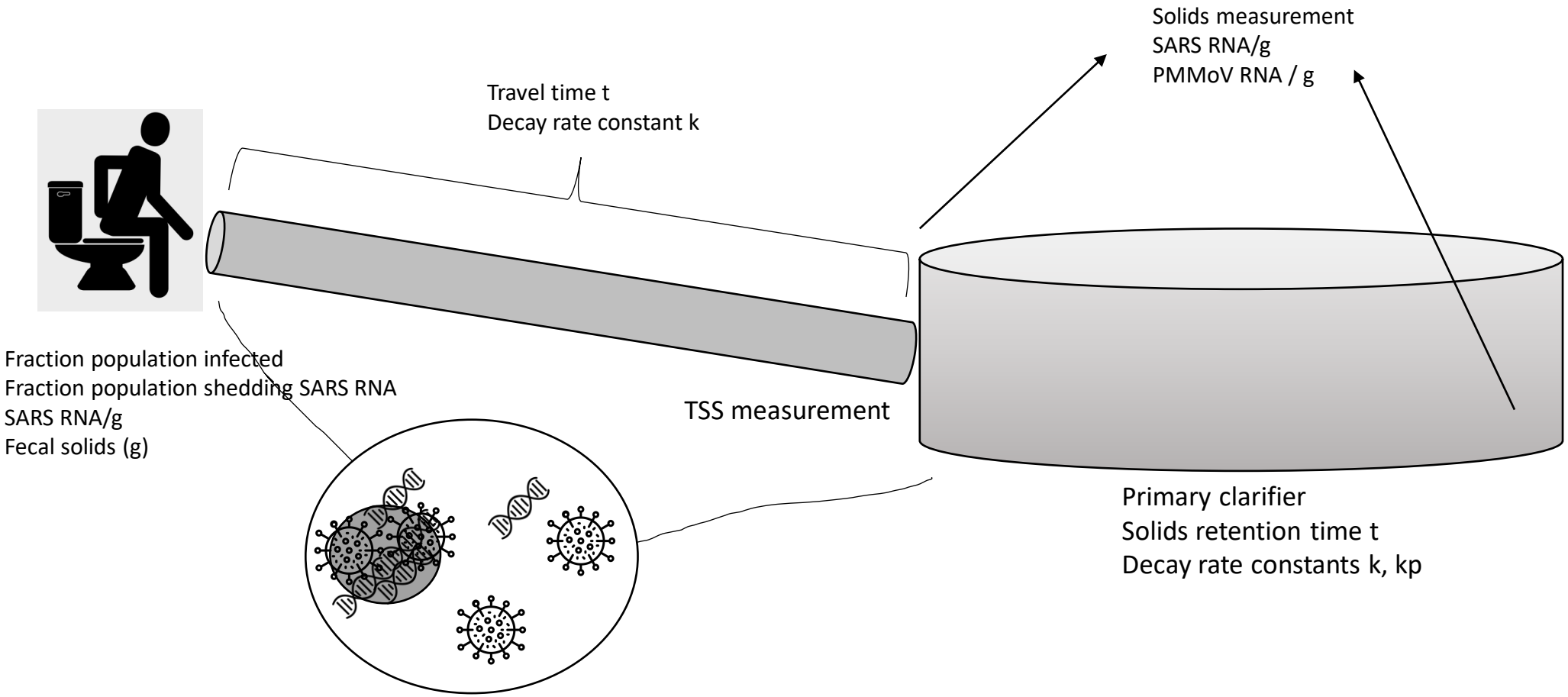
Challenges with using wastewater data

- Sources of variability are foreign to public health professionals
- How to best interpret and display data requires ongoing dialogue with stakeholders
- Case data for specific sewersheds is difficult to obtain
- Comparison of measurements across plants must be done thoughtfully (with the goal of comparing covid-19 case load)



5. Model to compare measurements across plants





Some assumptions

- Decay SARs and PMMoV RNA is minimal in the system
- A fraction of TSS is fecal solids
- Conceptually, SARS RNA is delivered to the system along with fecal solids
- Once in the waste stream, SARS RNA and SARS in solids and liquid reach equilibrium
    - Kd = Conc in solids/Conc in liquids [ml/g] = Cs/Cw = 1000 ml/g

$$\frac{C_s(1+K_dTSS)}{C_{PMMoV}(K_dTSS)} = \frac{C_{feces}}{C_{PMMoV\_feces}} A \frac{new\ cases}{P_{sewershed}}$$

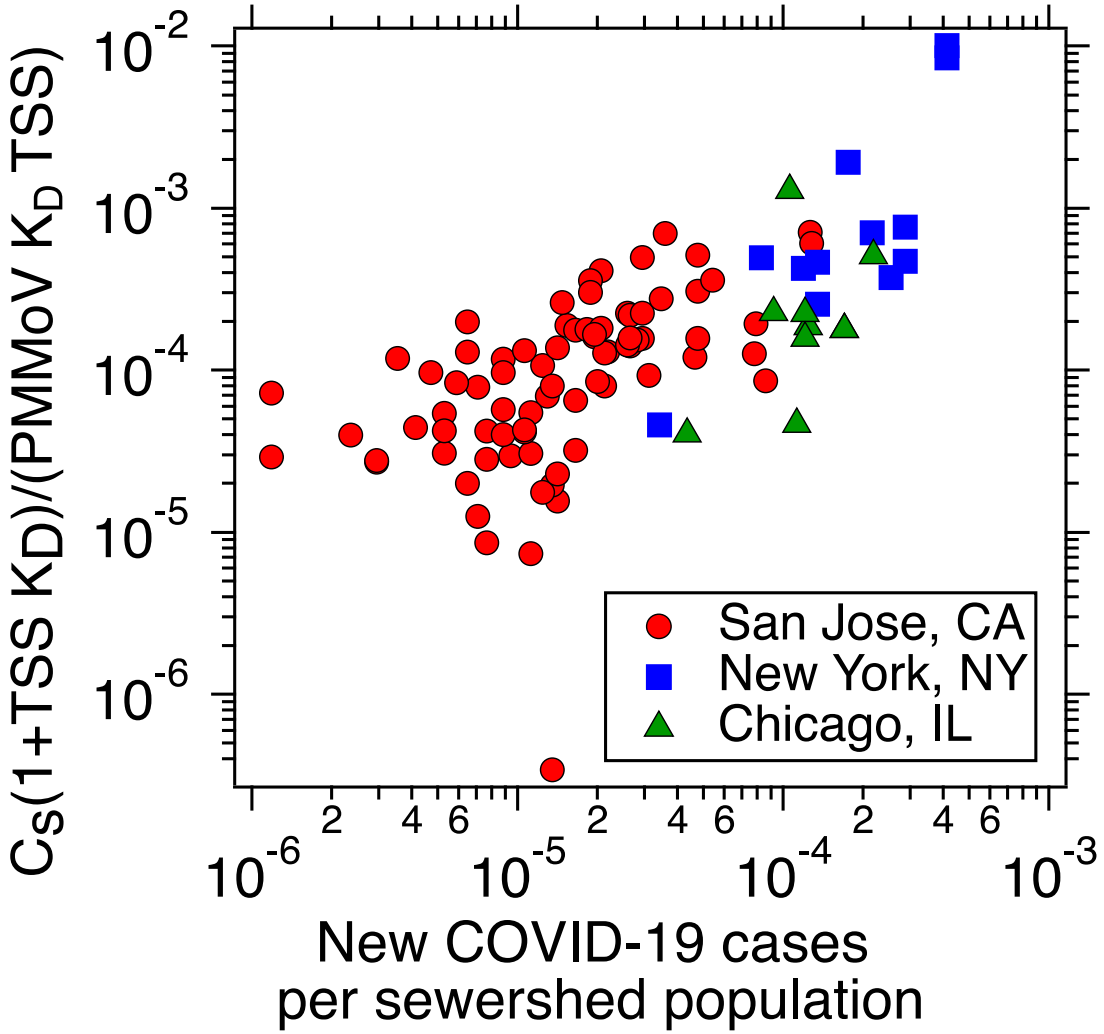
- wastewater specific measurements
- if TSS and Kd are the same among plants, then C_s/C_{PMMoV} can be directly compared between plants
- $KdTSS = 10^{-4}*TSS = 0.1 – 1.0$

Not expected to vary by sewershed

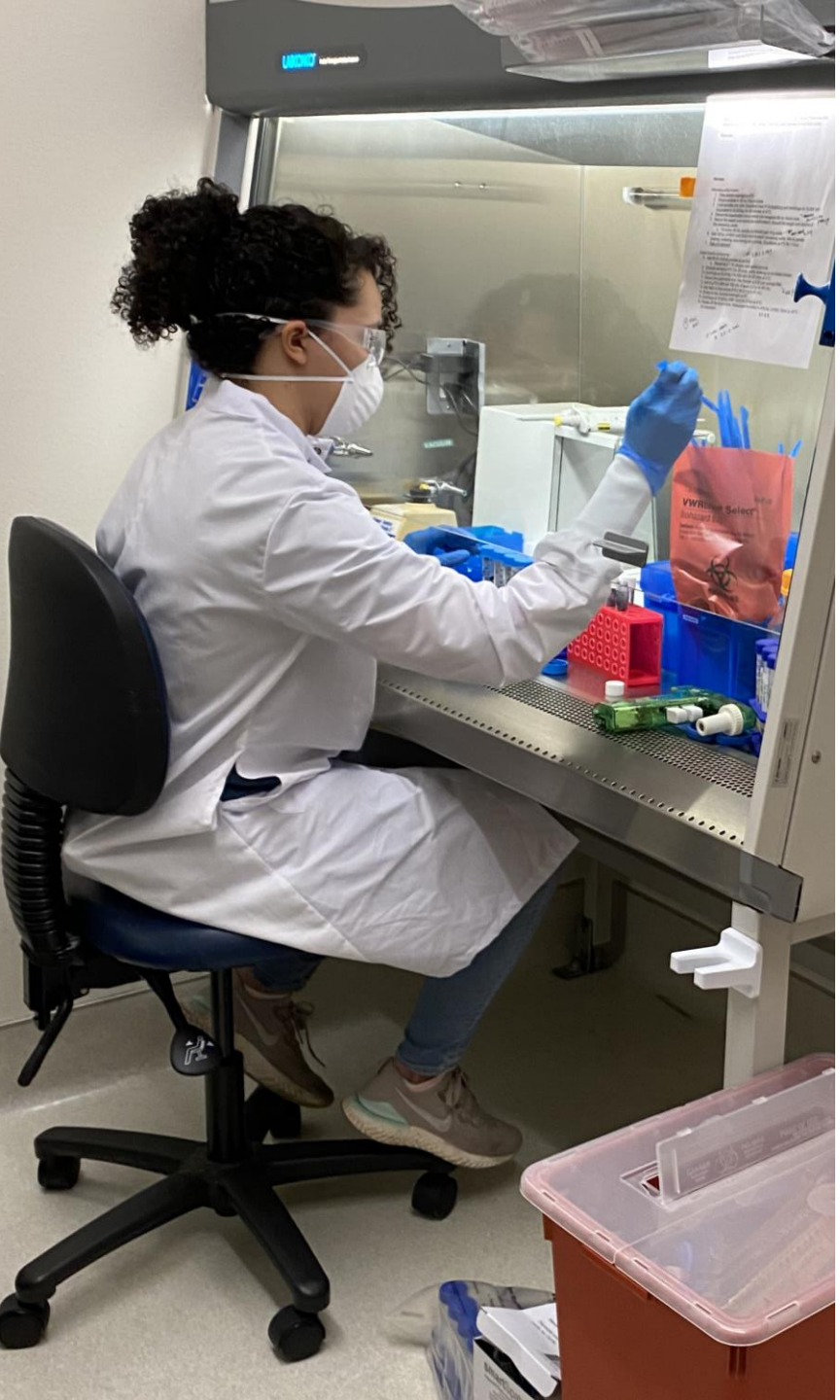
May vary by sewershed and time

Plotting LHS vs New cases/$P_{sewershed}$ should give a straight line with slope = $A*C_{feces}/C_{PMMoV\_feces}$

Application of model to 3 plants



- Suggestive that y-axis can be used to assess COVID-19 prevalence
- Further work is needed to determine if C_s/C_{PMMoV} would be sufficient for cross plant comparisons



Next Steps

- Refining data visualization to aid in interpretation
  - Very different sources of uncertainty and variability compare to clinical data
  - Add clinical data to dashboard
- Application of model to:
  - Allows for between-plant comparisons
  - Estimate of community transmission
- Expansion of testing
  - Potentially adding additional plants to real-time monitoring
  - Adding assays for new variants of concern
  - Sequencing SARS-CoV-2 in sewage
- Continued outreach and networking

Thank you!

- POTW staff including MWRD for sample collection and sharing
- Sam Dorevitch, Cook County Dept of Public Health, and Chicago Dept of Public Health for sewershed level case data in Stickney sewershed
- Linlin Li and Michael Balliet at Santa Clara County Public Health

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