



THE
Water
Research
FOUNDATION®



Global Update on Wastewater Surveillance for SARS-CoV-2

Christobel Ferguson, PhD



A close-up photograph of a blue microcentrifuge rack containing several clear plastic microcentrifuge tubes. Some tubes are open, showing a dark brown or black liquid inside. The rack is set against a white background.

Overview

Rapid Development & Adoption

Wide Variety of Approaches

Use Cases

Trends, Variants, and Early Warning

Lessons Learned

Global Update

Knowledge gaps

Next Steps

A close-up photograph of a blue microcentrifuge rack containing several clear plastic microcentrifuge tubes. Some tubes are open, showing a dark brown or black liquid inside. The rack is set against a white background.

Rapid Development & Adoption

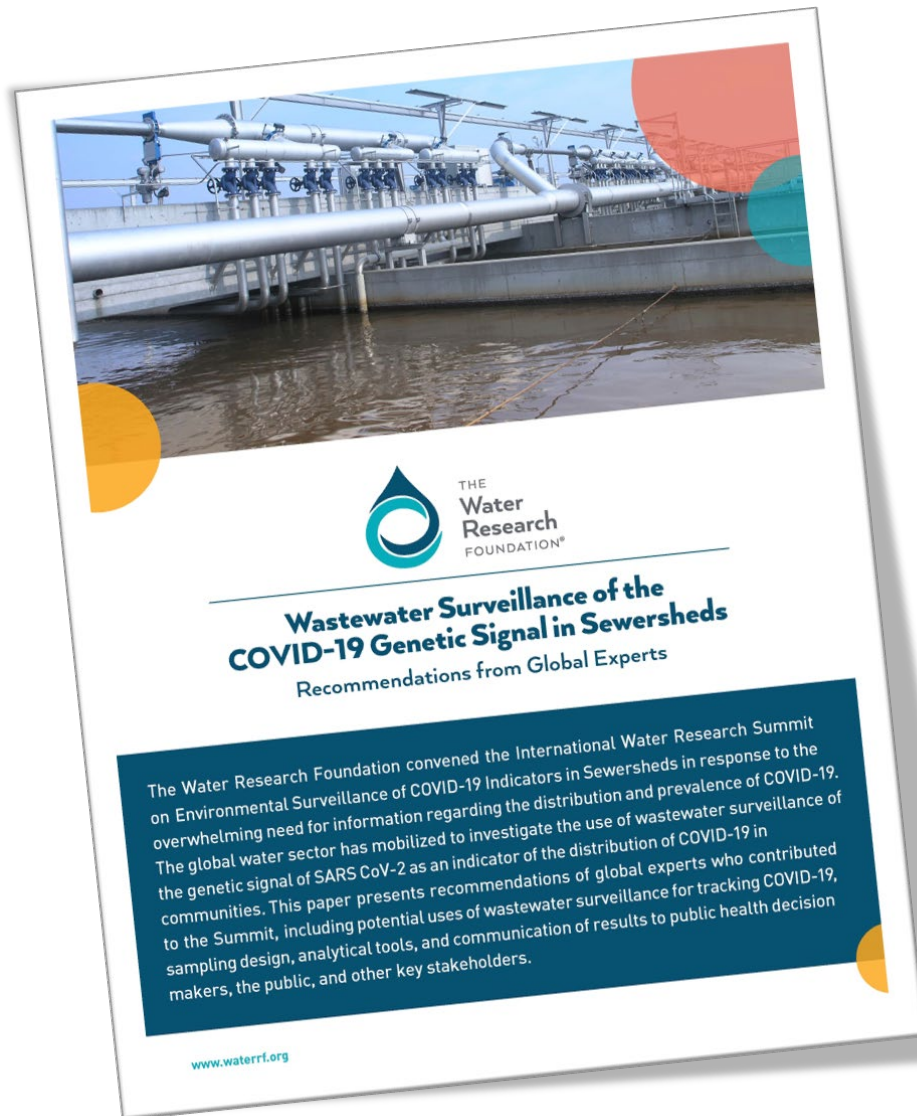
Wide Variety of Approaches

Methods to detect SARS-CoV-2 using wastewater surveillance.



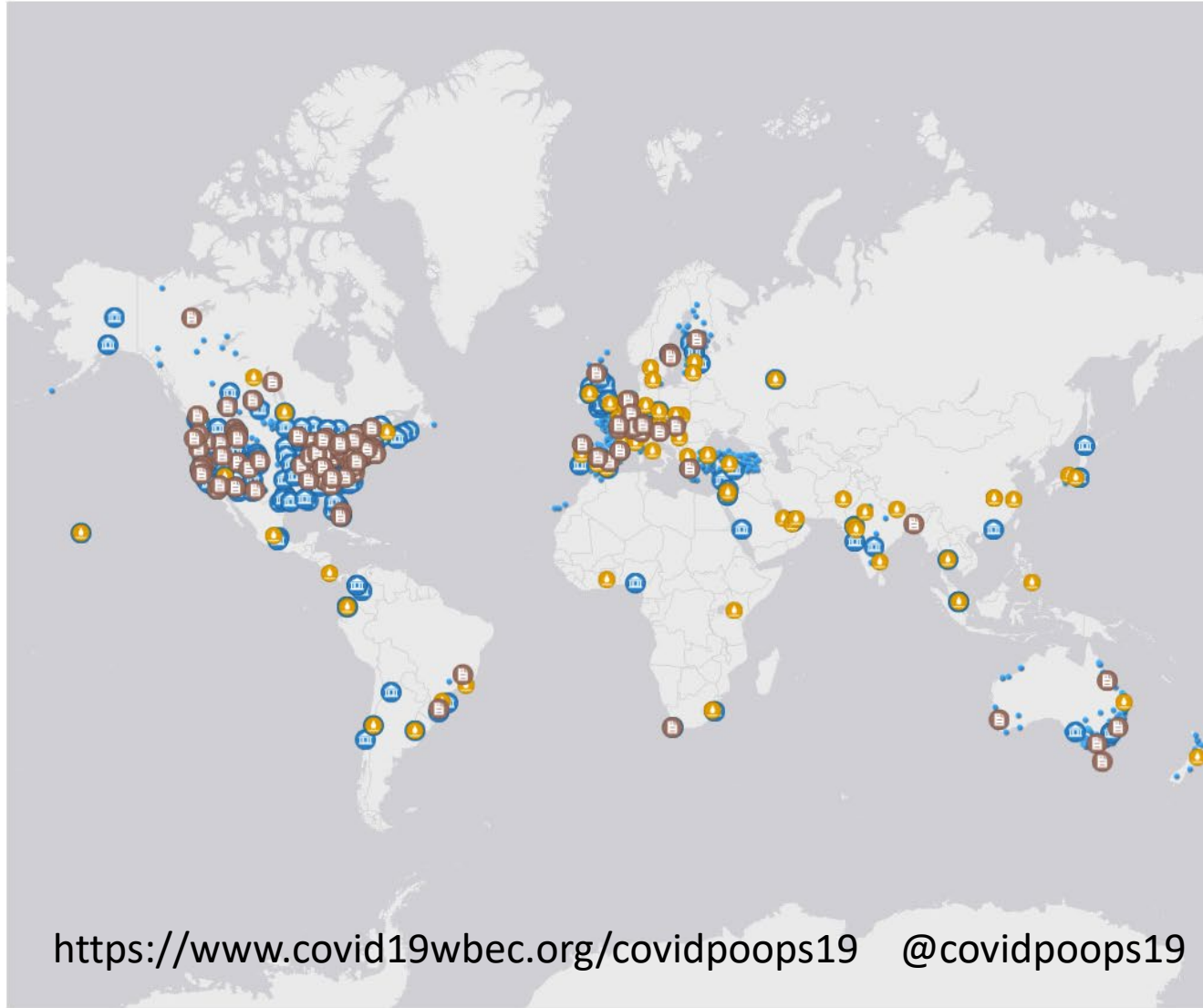
Rapid Water Sector Response & Collaboration

- Interlaboratory and Methods Assessment of the SARS-CoV-2 Genetic Signal in Wastewater ([#5089](#)) [DOI: 10.1039/d0ew00946f](https://doi.org/10.1039/d0ew00946f)
- Method SOPs available on the WRF website <https://www.waterrf.org/research/projects/interlaboratory-and-methods-assessment-sars-cov-2-genetic-signal-wastewater>
- Understanding the Factors that Affect the Detection and Variability of SARS-CoV-2 in Wastewater ([#5093](#)) – due August 2021
- DHS and CDC Project with AquaVitas
- NSF Research Coordination Network on SARS-CoV-2 wastewater surveillance
- CDC National Wastewater Surveillance System
- Water Research Australia ColoSSuS project
- US EPA Research with Cincinnati Ohio
- WRC (South Africa) Pilot Projects on method development
- Canadian Water Network Method evaluation
- EU Umbrella study under European Health Emergency Preparedness and Response Authority (HERA)



Implementation across the globe

COVIDPoops19 Summary of Global SARS-CoV-2 Wastewater Monitoring Efforts by UC Merced Researchers



Dashboards

 86

Last update: an hour ago

Universities

 263

Last update: an hour ago

Countries

 55

Last update: an hour ago

Sites

 2,287



Use Cases

Trends, Variants and Early Warning

Selecting the approach that meets the community need.



RT-qPCR analytical method Overview



French National Network
Obépine

	Concentration	Extraction	Genetic Target
Veolia Lazuka <i>et al.</i> (submitted)	Ultrafiltration	Silica spin column filter or Magnetic silica beads	N1 ¹ , N2 ¹ , E ²
KWR Medema et al. (2020)	Clarification using centrifugation + Ultrafiltration	Silica spin column	N1 ¹ , N2 ¹ , E ²
Eaux de Paris Wurtzer et al. (2020)	Ultracentrifugation	Silica spin column filter	N1 ¹ , N2 ¹ , E ² , RdRp (IP2 & IP4) ³
CNRS-LCPME Bertrand et al. (2020)	Ultrafiltration	Magnetic silica beads	
CNRS-LCPME Bertrand et al. (2020)	Desorption + Precipitation PEG	Magnetic silica beads	

¹ US CDC; ² [Corman et al. 2020](#); ³ Centre National de Référence des virus des infections Respiratoires, Institut Pasteur, Paris

- Viral recovery yield was 5.5 % +/- 0.5% using heat-inactivated SARS-CoV-2.
- N1 appeared to be the most sensitive biomarker.
- Protocol is operational, quick and easy to implement

Lazuka, Soyeux & Lacroix, Veolia, France, 2021

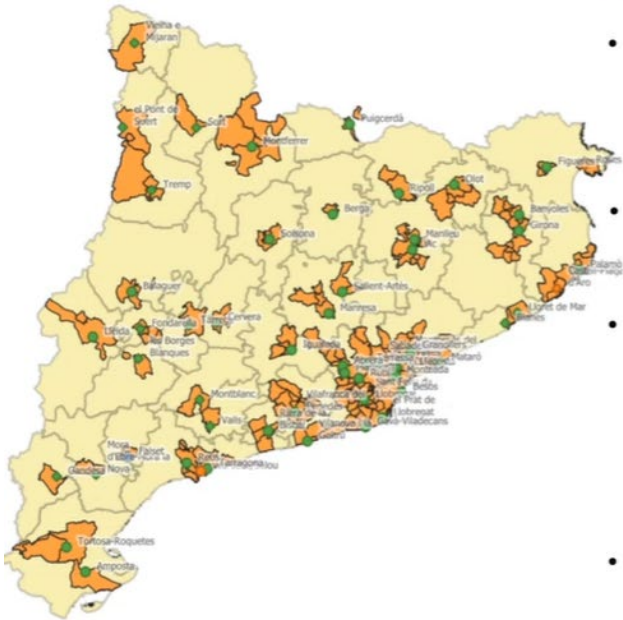
Trends

Activity



Sampling strategy, frequency and analysis

- A total of **56 WWTPs** selected based on two criteria:
 - 80% coverage of served population
 - Territorial evenness (41 out of 42 regions)
- **Sampling frequency** 36 WWTPs weekly & 18 WWTPs fortnightly
- **Sample collection and analysis**
 - Collecting flow-proportional, 24h-composite (every 20min) INLET samples
 - Shipment and distribution to the labs
 - Results within 48 hours
- **Quantification of three gene targets**
 - N1 (*N* gene)
 - N2 (*N* gene)
 - IP4 (*RdRp* gene)

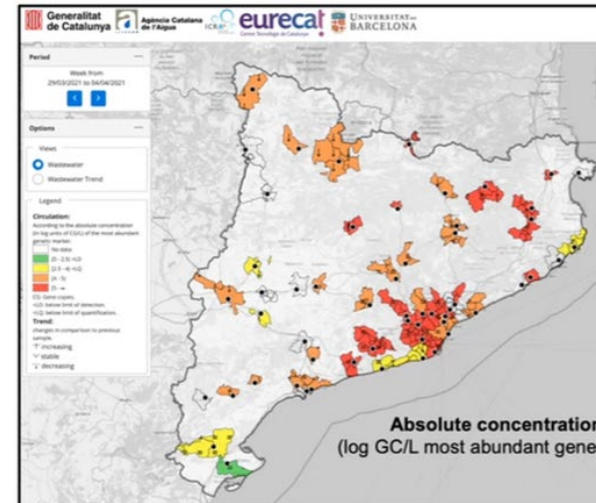


On-going since July 2020

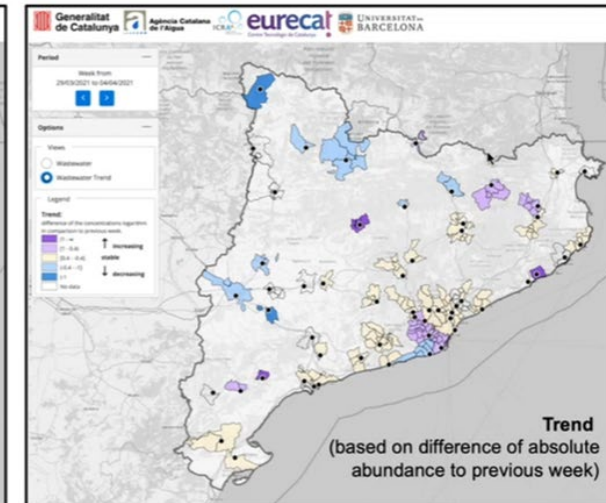
Borrego, Collado, Corominas, Guerrero & Pueyo
Catalan Surveillance Network, 2021

Visualization dashboard

<https://sarsaigua.icra.cat>



Absolute concentration
(log GC/L most abundant gene)



Trend
(based on difference of absolute abundance to previous week)





0:02:02



11:13



Science Brief for Phase 1: Proof of Concept

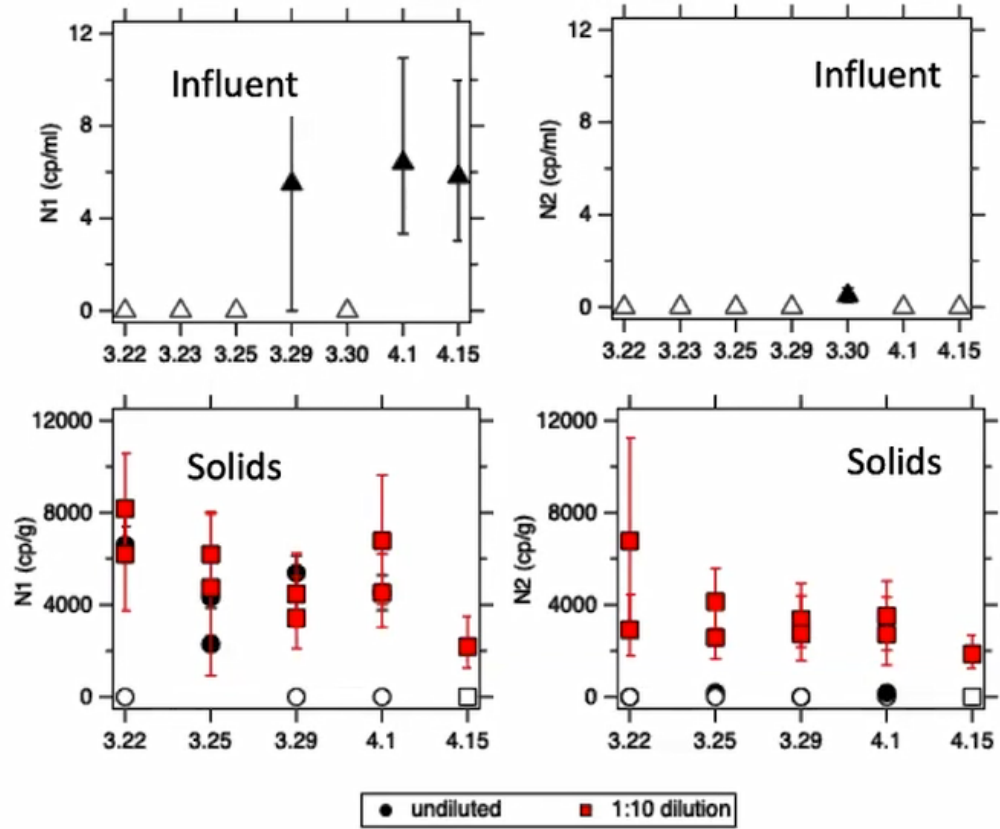
- Detected SARS-CoV2 RNA in 98% of wastewater samples from upstream and downstream WWTW of prisons, hospitals, industries and mines
- Positive gene amplification observed in environmental (NSS) samples – ie river water samples
- WBE proven to be a useful complementary surveillance tool for management of COVID-19
- Wastewater surveillance – cost effective, less invasive continuous screening approach
- Correlation between increase in viral load and increase in case numbers with time
- Method efficiencies – Skimmed milk flocculation and $Al(OH)_3$ adsorption-flocculation cost effective and faster than PEG/NaCl precipitation;
- Built a robust collaborative platform of scientists, laboratories and WSIs



No Notes.



We detected more positive signals by extracting directly from primary solids than with a PEG-based influent method.



- Limited variation among replicates
- Inhibition an issue for some samples

ENVIRONMENTAL
Science & Technology

These permissions are granted for the duration of the World Health Organization (WHO) declaration of COVID-19 as a global pandemic.

pubs.acs.org/est

SARS-CoV-2 RNA in Wastewater Settled Solids Is Associated with COVID-19 Cases in a Large Urban Sewershed

Katherine E. Graham, Stephanie K. Loeb, Marlene K. Wolfe, David Catoe, Nasa Sinnott-Armstrong, Sooyeol Kim, Kevan M. Yamahara, Lauren M. Sassoubre, Lorelay M. Mendoza Grijalva, Laura Roldan-Hernandez, Kathryn Langenfeld, Krista R. Wigginton,* and Alexandria B. Boehm*

Graham, et al. Environmental science & technology (2020) 55, 1, 488–498.

Krista Wigginton, University of Michigan, California, USA 2021





METHODS AND TARGETS

SAMPLE – PRIMARY INFLUENT, 24-HOUR FLOW
WEIGHTED COMPOSITE SAMPLE

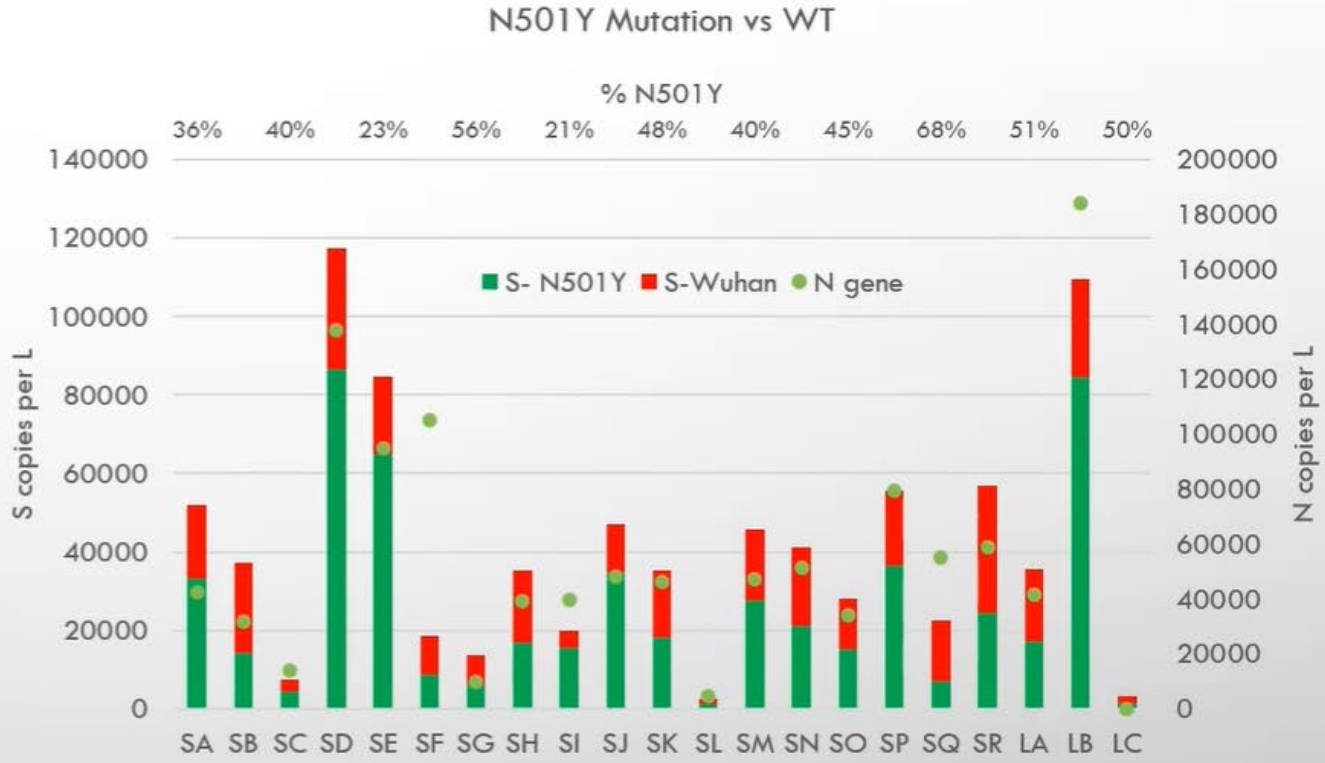
CONCENTRATION METHOD – INNOVAPREP
CONCENTRATING PIPETTE

RNA EXTRACTION – QIAAMP VIRAL RNA KIT

QUANTIFICATION – DDPCR

TARGETS

- SARS-COV-2 – N1
- RECOVERY – BCOV
- INDICATORS – PMMOV AND F⁺ BACTERIOPHAGE (GROUP II)
- VARIANT ASSAY – S GENE N501Y MUTATION AND WILD TYPE



Wastewater COVID-19 Tracking

Filter selection by city

Filter selection by utility

Boulder (Boulder_Gunbarrel) ✕

Filter selection by monitoring site

Finding site data

Select Parameter

SARS-2 Copies/L (uncorrected)

Select Health Statistic

New Cases (County)

Select Demographic

Race

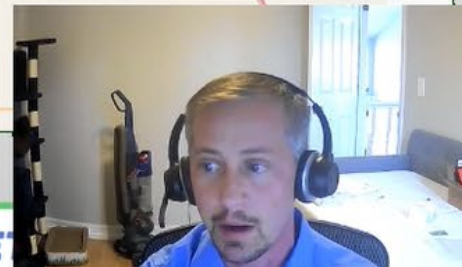
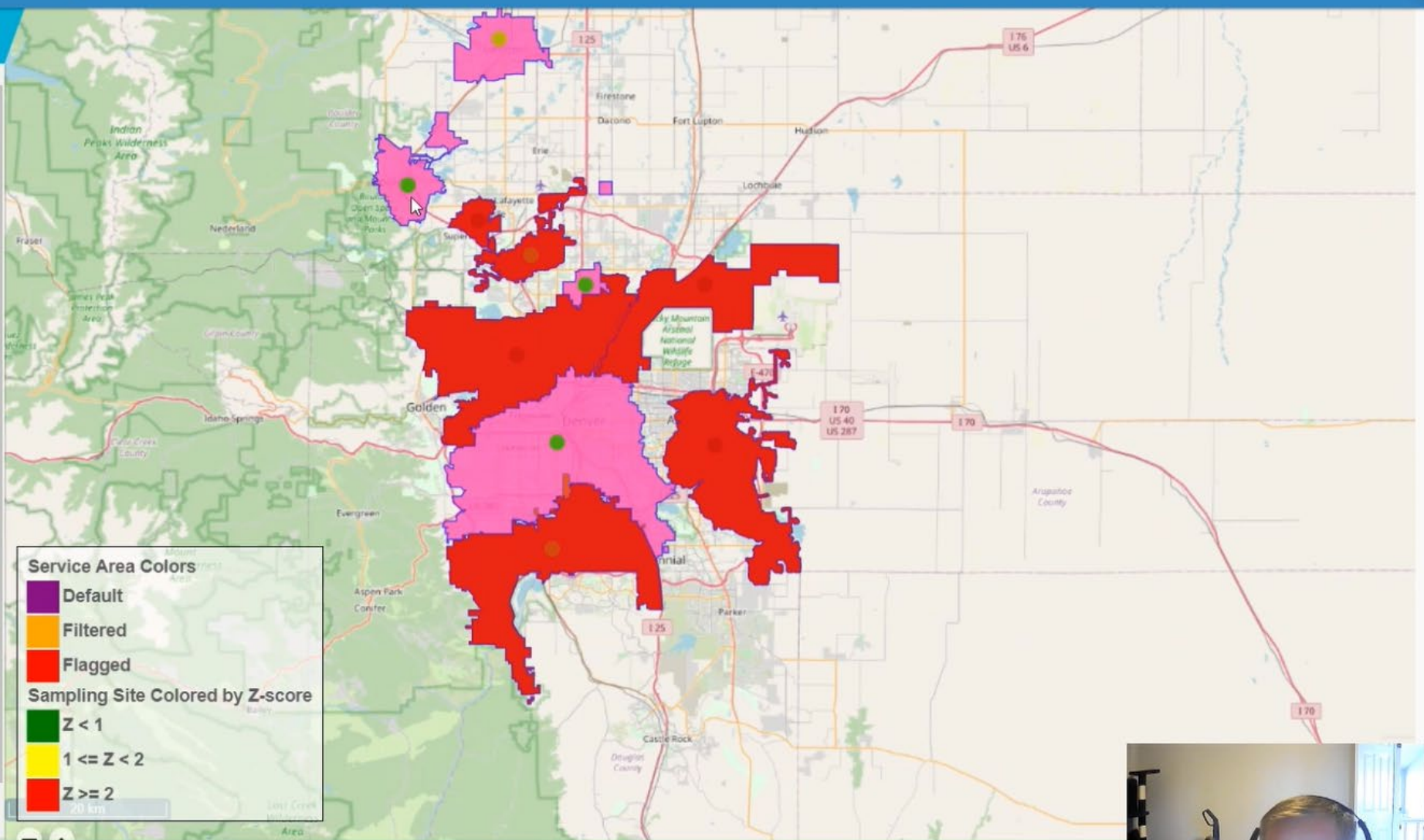
Include available microsewershed data

Make emergency facility level visible

Start date (YYYY/MM/DD) 2021/03/01

End date (YYYY/MM/DD) 2021/03/31

Download CDPHE Sample Report

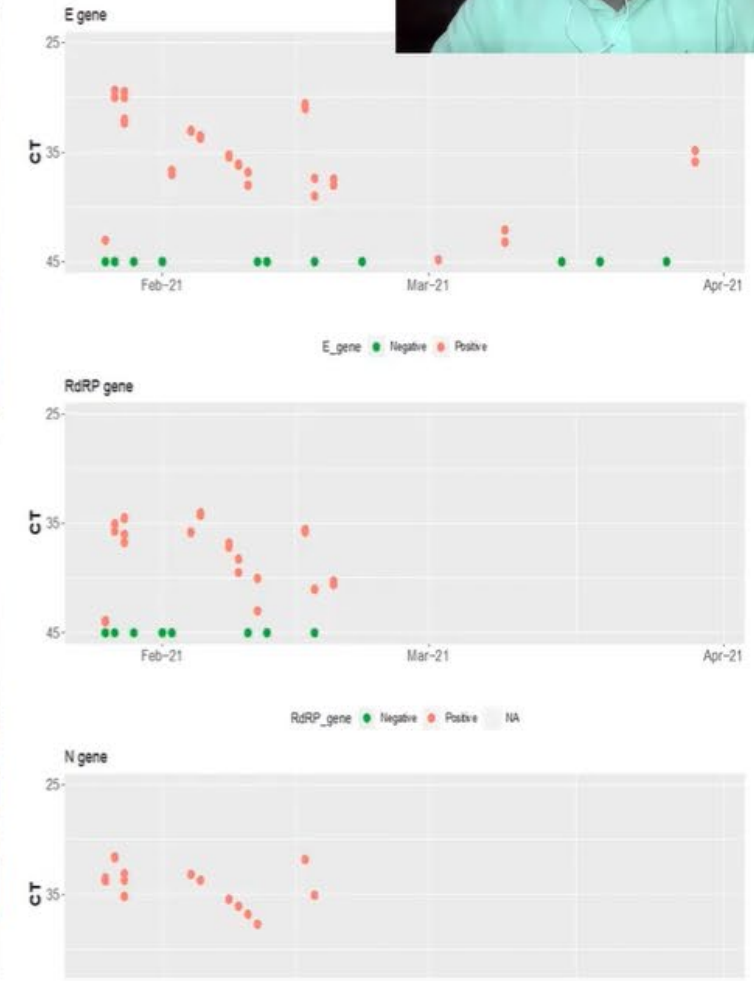


RESULTS

- SARS-CoV-2 RNA was detected from multiple onsite public toilet septic systems at Osu Alata within Korle Klottey
- SARS-CoV-2 RNA was detected in Community Two within the Tema sewer system



Tema Community 2

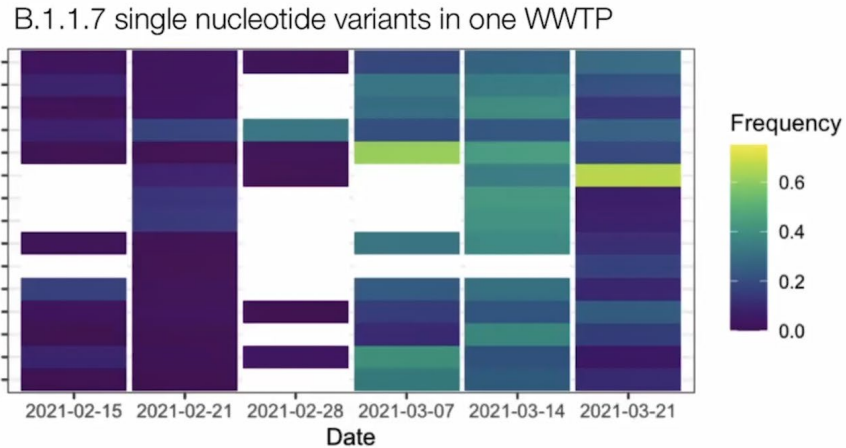
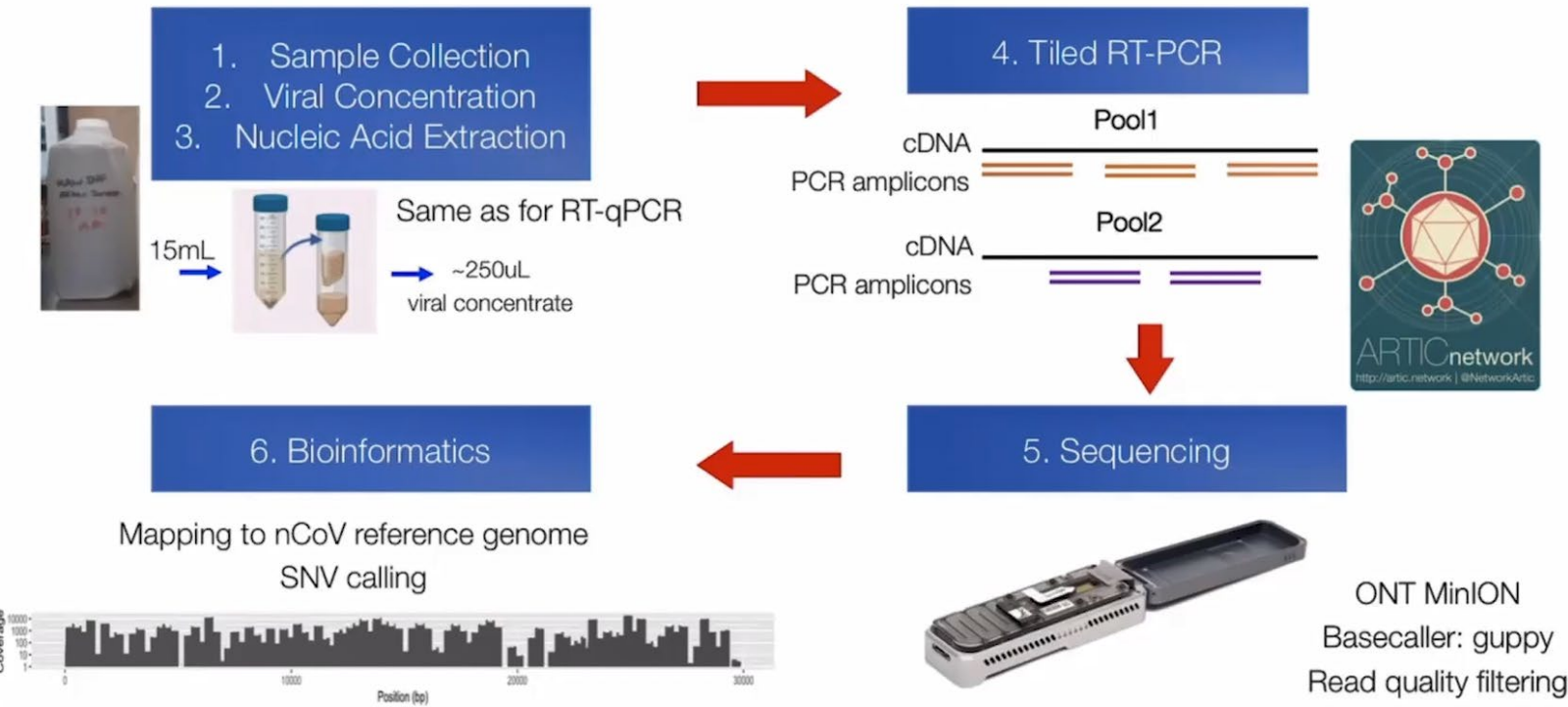


Habib Yakubu, Emory University, Ghana, 2021

Approach to tracking nCoV variants:



Variants



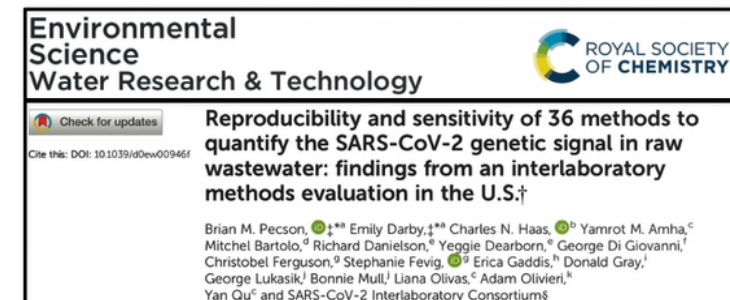
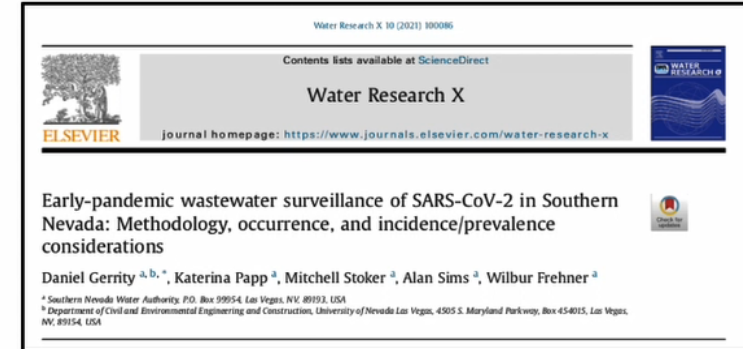
Ryan Ziels, University of British Columbia, British Columbia, Canada 2021





Southern Nevada Wastewater Surveillance

- **No hits in treated wastewater or local drinking water supply**
- **Wastewater influent samples collected weekly**
 - 1 site since March 2020 (100 mgd and 872k people)
 - 1 site since April 2020 (5 mgd and 86k people)
 - 4 sites since August 2020 (6-40 mgd and 115k-757k people)
 - 1 site since December 2020 (0.8 mgd and 16k people)
 - **UK variant of concern (B.1.1.7) detected in wastewater prior to clinical confirmation**
- **Short-term monitoring of a homeless shelter manhole**
 - 4 weekly samples from Late November – Early December (all positive)
 - Sampling coincidentally occurred during facility-wide outbreak
 - **California variant of concern (B.1.427/429) detected in final sample**
- **Participated in WRF round robin methods comparison**



Daniel Gerrity, Southern Nevada Water Authority, Nevada, USA 2021



COVID-19 Wastewater Surveillance Symposium
A Global Update



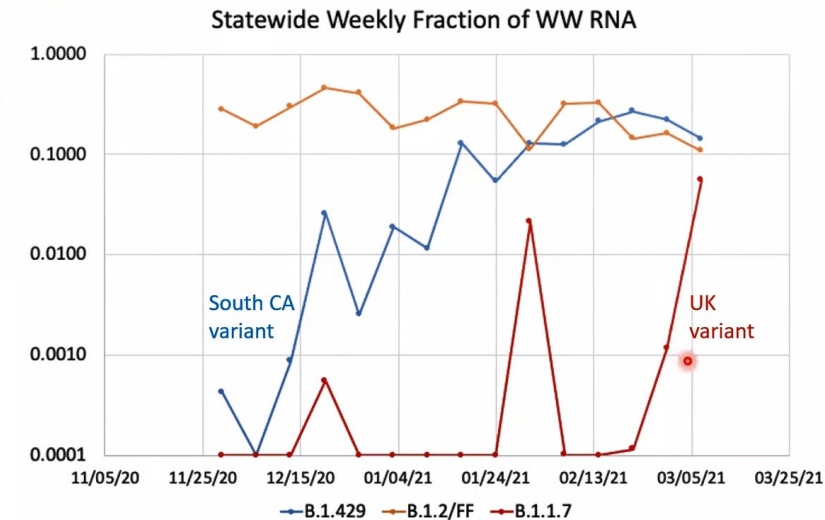
Covid-19 Wastewater Surveillance Efforts in Oregon

Devrim Kaya, Ph.D.

School of Chemical, Biological & Environmental Engineering



- 1) **NSF-RAPID:** Microsewershed surveillance in collaboration with Clean Water Services (CWS).
- 2) **TRACE-Oregon State University (OSU)** (Internally funded project): monitoring for SARS-CoV-2 at the building/dorm level & at the campus level for all 3 OSU campuses.
- 3) **TRACE-Community** (founded by David & Lucile Packard Foundation): combines WBE with random house-to-house nasal swab sampling to determine the "true" COVID-19 prevalence of a community
- 4) **OHA/U.S. CDC** : state-wide monitoring for SARS-CoV-2 and genotypes at 40+ WWTPs across the state of Oregon on a weekly basis.



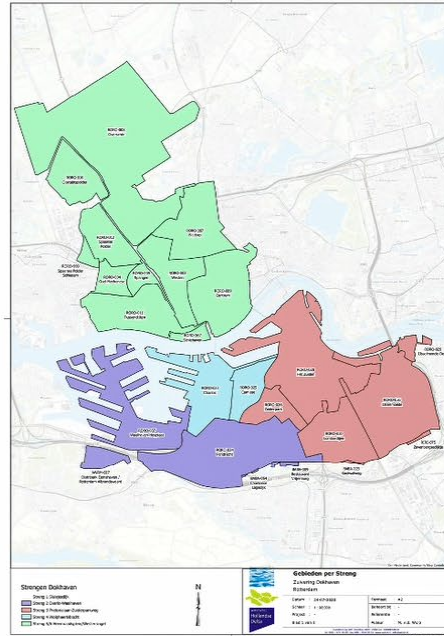
Devrim Kaya, Oregon State University, Oregon, USA 2021



City of Rotterdam

- 2 WWTP
 - Including 4 individual influents
- 4 pumping stations (neighbourhoods)
- Sampling since August 2020

Below ground



Above ground

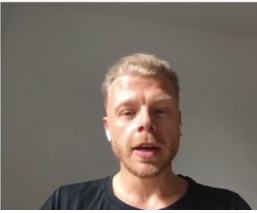
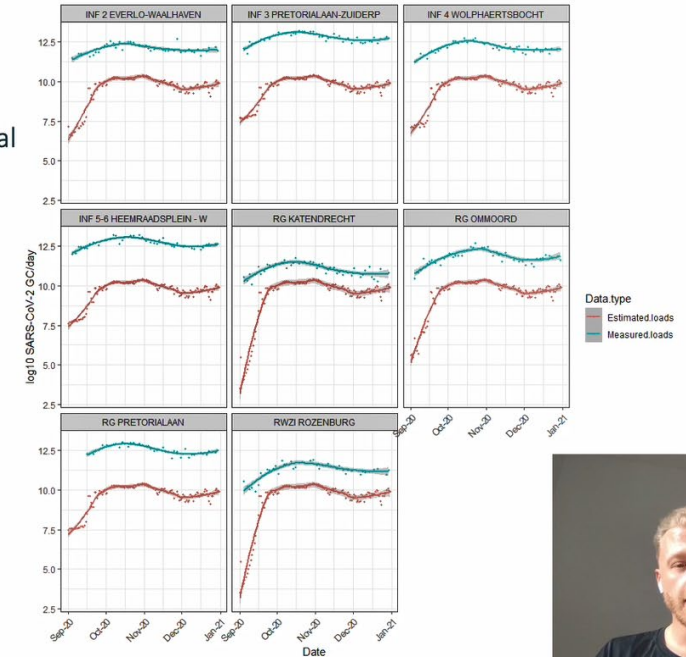
- Patient testing in same catchments
- Metadata collection
- Faecal samples for viral shedding and sequencing

Optimize shedding parameters to "fit" wastewater observations

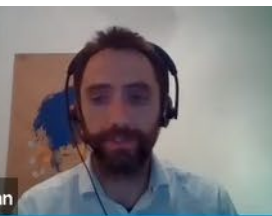
- Use Monte Carlo simulations and kinetic models to derive (estimates) of realistic viral shedding

Goals:

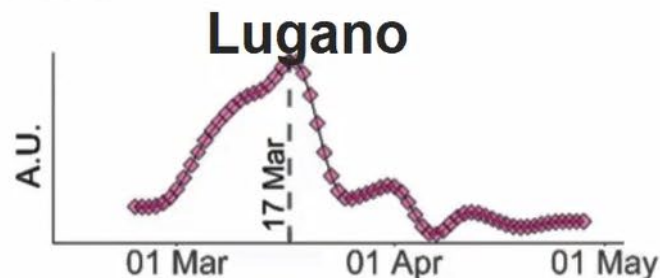
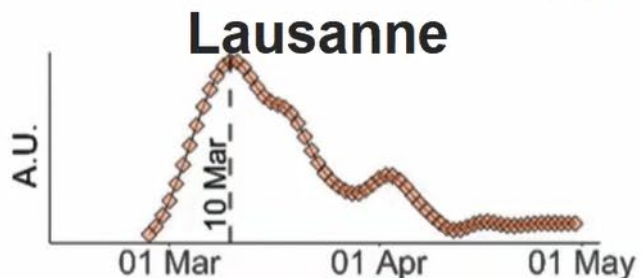
- Observed trends
- Early warning
- Link with what happens above ground
- Incidence estimation



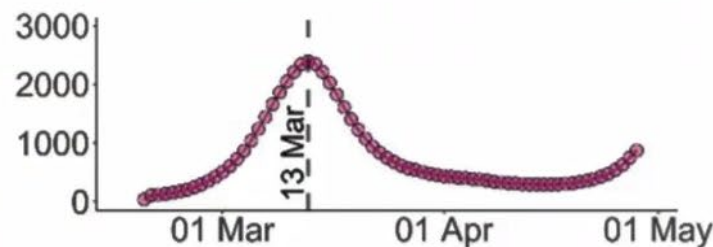
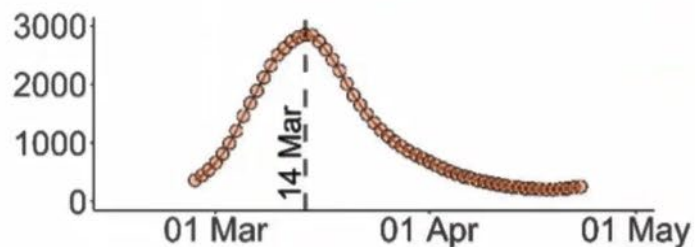
Show Trends (better than cases?)



First Wave

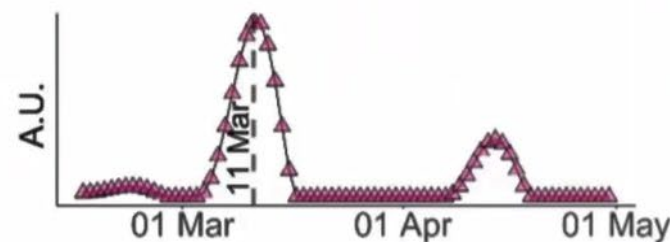
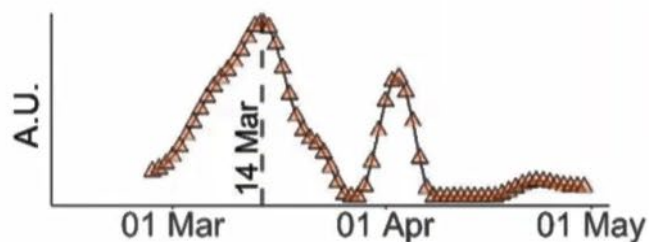


Case-based
incidence estimation



Reference
(SEIR model)

Seroprevalence



Wastewater-based
incidence estimation

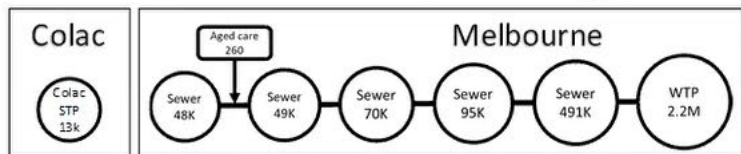
Fernandez-Cassi et al. (preprint)

Tim Julian, Eawag, Switzerland 2021

Early Warning



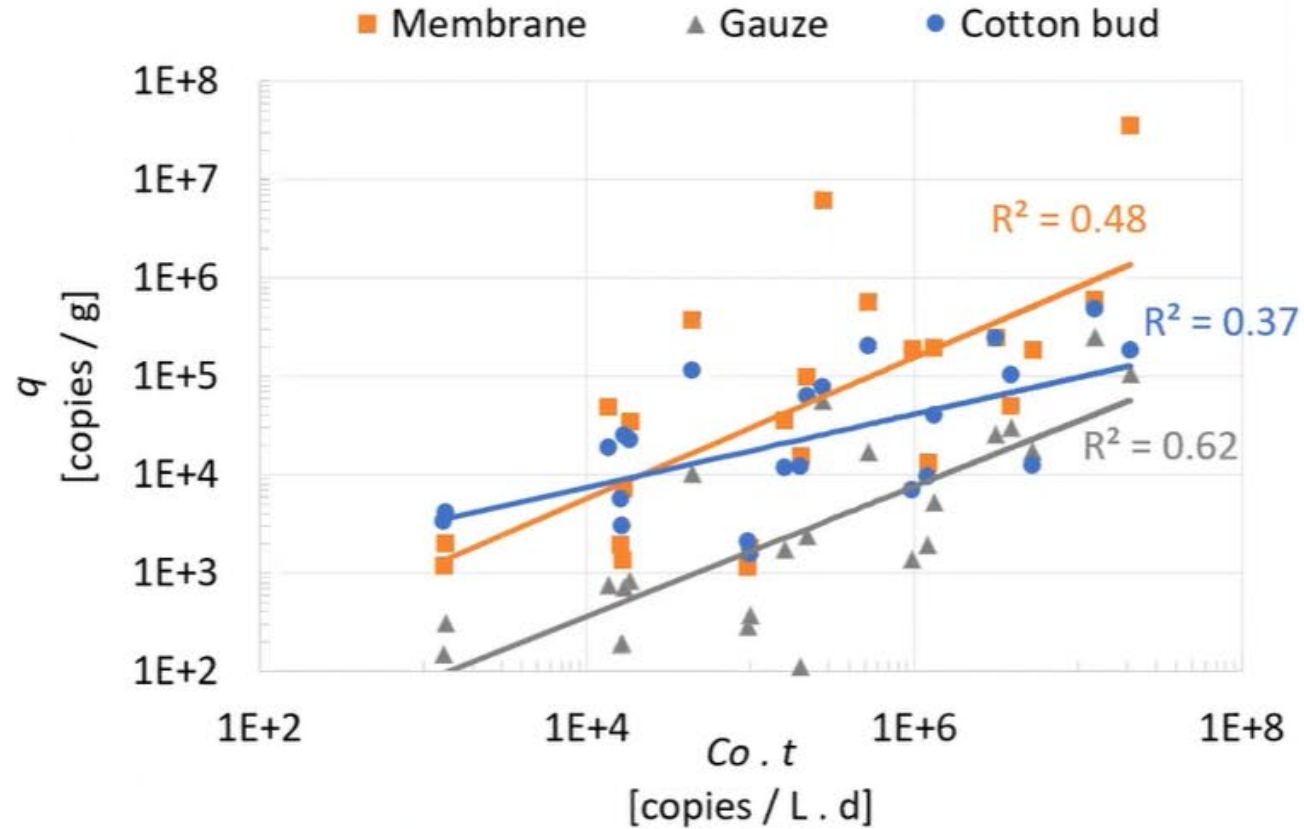
Passive sampling of SARS-CoV-2 in Wastewater: field



- Passive samplers had detection on 100% of days when wastewater concentrations were $> DL$
- Passive samplers detected SARS-CoV-2 on another 50% of occasions even though WW conc. $< DL$

McCarthy, Crosbie, Poon & Nolan, Monash Uni, Australia, 2021

Passive sampling of SARS-CoV-2 in Wastewater: lab

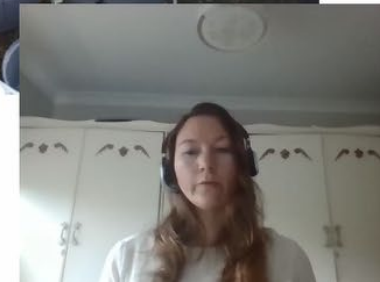


McCarthy, Crosbie, Poon & Nolan, Monash Uni, Australia, 2021



NSW program development

- February 2020 Sydney Water method development, collaboration with ColoSSoS partners
- March - July 2020 storing samples and analysis for method validation
- July 2020 started analysis of routine WWTP samples
- September 2020-February 2021 breaking down larger catchments in Sydney for routine network samples

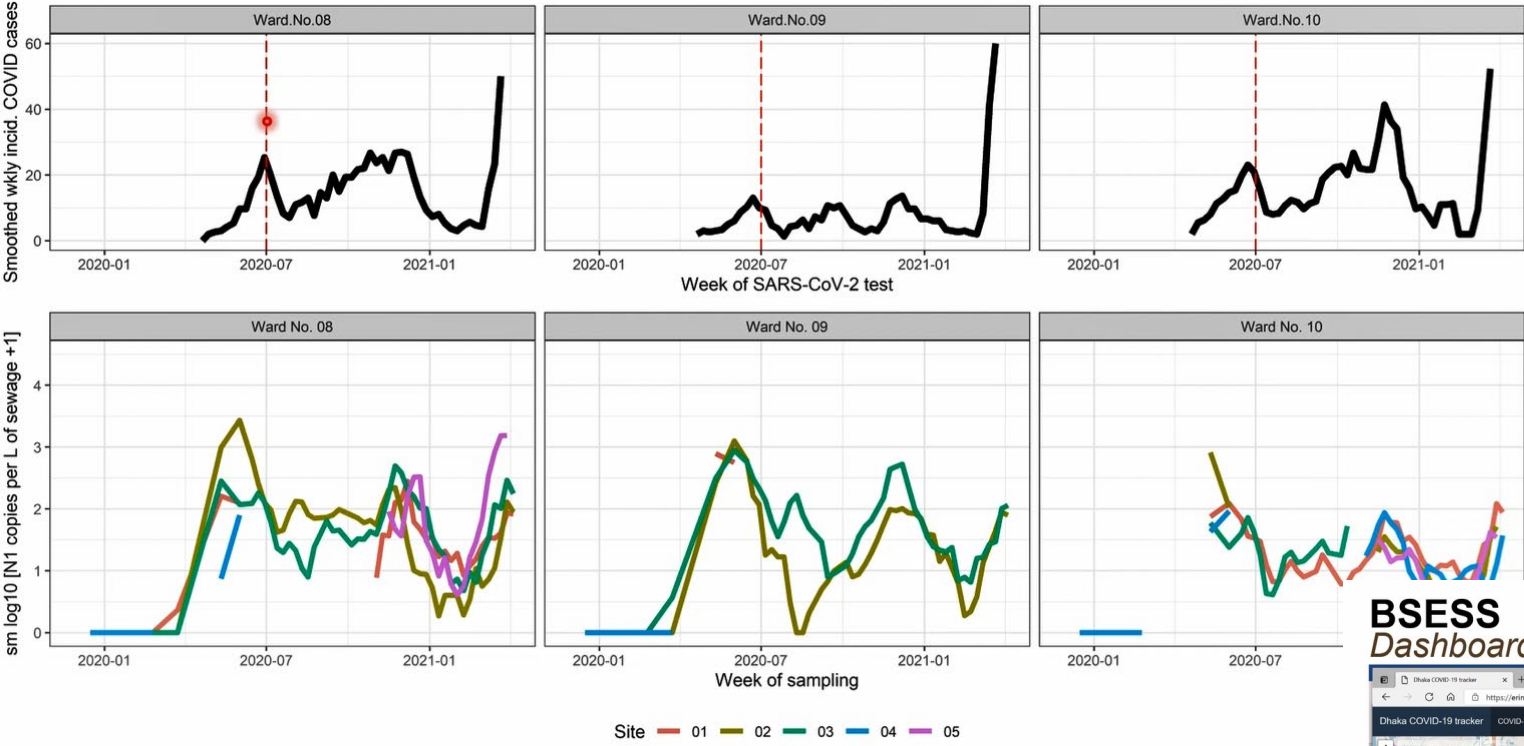


Get tested immediately if COVID-19 symptoms develop.
Find a testing clinic near you: nsw.gov.au/COVID-19

HAWKESBURY
SOUTH WESTERN SYDNEY

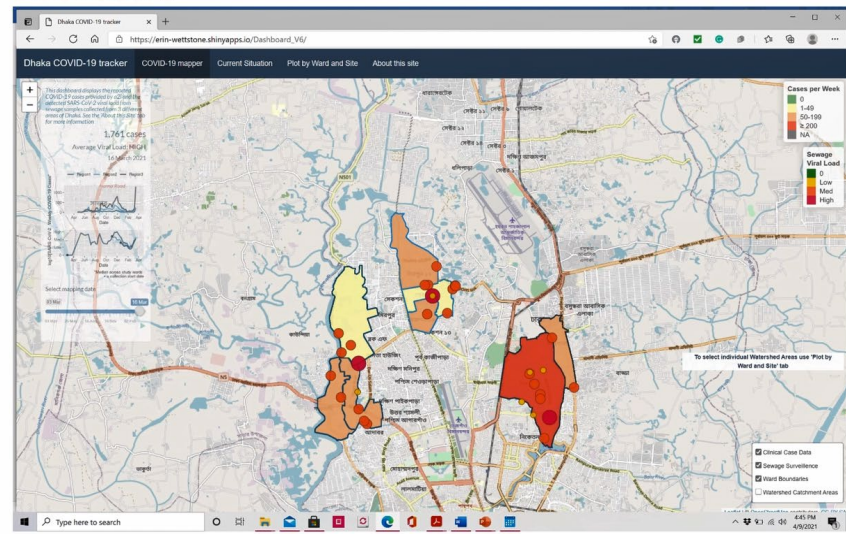
Zenah Bradford-Hartke, NSW Health, Australia 2021

SEWAGE AND COVID-19 CASE DATA



Started surveillance for SARS-CoV-2 in December 2019

BSESS Dashboard



Developed by 4th year biomedical engineering undergrads at UVA

33 sites over 3 study area

Dashboard displays weekly sewage and case data to aid Bangladesh public health stakeholders on mitigation efforts

Current situation shows a marked increase in the amount of SARS-CoV-2 in sewage markets 3rd wa



Mami Taniuchi, University of Virginia, Bangladesh 2021

https://erin-wettstone.shinyapps.io/Dashboard_V6/

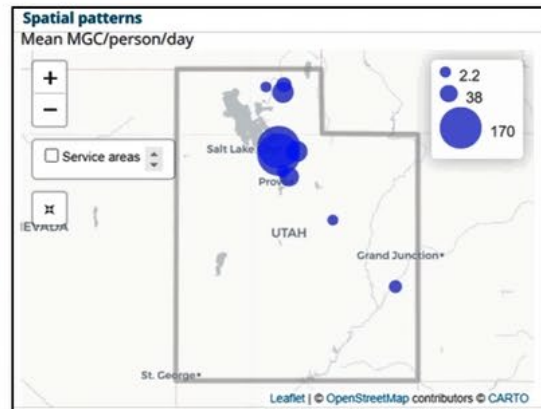
Utah Surveillance Program (wastewatervirus.utah.edu)

Phase I: Proof of concept
March 23 to April 6, 2020



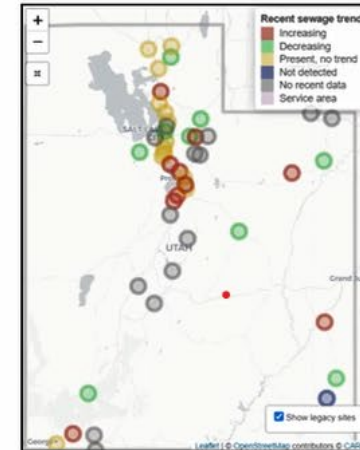
- **2 facilities** sampled daily for two weeks (**17% population**)
- U of U
- Urban center & ski location
- U of U seed funding

Phase II: Pilot
April 13 to May 24, 2020



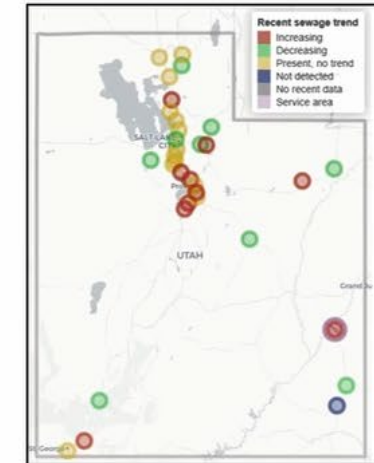
- **10 facilities** sampled weekly (**39% population**)
- U of U, BYU, USU
- Urban centers, tourist locations and rural areas
- Weidhaas, et al. 2021, Sci Total Env, 775: 145790
- DEQ seed funding

Phase III: Monitoring
May 24 to Dec 31, 2020

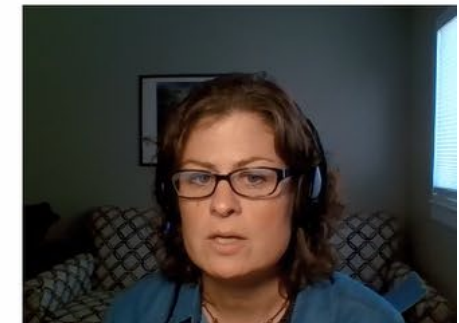


- **43 facilities** sampled weekly (**87% population**)
- U of U, BYU, USU, Soft Cell Bio
- CARES act funding

Phase IV: Optimization
Jan 1, 2021 to current



- **32 facilities** sampled bi-weekly (**86% population**)
- UDOH/CDC funding



~2500 samples processed for Utah environmental surveillance to date

Roper and Weidhaas, Utah State University and University of Utah, Utah, USA 2021

Results of Utah State University (USU) monitoring wastewater on campus



“Our actions have ‘protected our community from wider spread infections’.”

Utah State Univ. President Noelle Cockett quoting Bear River Health Director Lloyd Berentzen



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Informed USU – BRHD interventions

- Quarantine + required testing (4 dorms)
- E-mail advisories (specific housing areas)
- Targeted directives to test (hot spots)
- Deployment of mobile testing unit

Validated actions

- Reduced clinical cases in monitored dorms
- Identified clusters (specific housing areas)
- Monitored isolated cases & quarantines
- Feedback & guidance to contact tracing

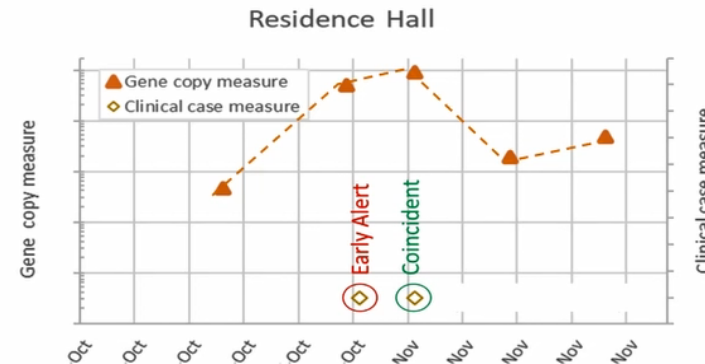


Improved Fall 2020 to Spring 2021



	Fall 2020	Spring 2021
	<i>Oct 21-Nov 20</i>	<i>Jan 14-Feb 12</i>

Living areas	11	11
Samples/wk	18	30
Early Alert (%)	44	78
Coincident (%)	5	5



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<https://www.usu.edu/today/storv/usu-biological-engineers-monitor-coronavirus-in-sewage>
~2500 samples processed for Utah environmental surveillance to date

Roper and Weidhaas, Utah State University and University of Utah, Utah, USA 2021



Lessons Learned

Global Update

Innovation through Collaboration



Lessons Learned

- Early warning (KWR & CDC ~ 6 days, WRC ~ 2 weeks)
- Objective population surveillance, independent of human test behavior
- Feasible for emergence of variants (signature mutations of)
- Fast (ddPCR within days, compared to 3-4 weeks for clinical surveillance)
- Efficient: on population sample, allowing high resolution surveillance
- Population size affects sewer signal dynamics
- Decentralized wastewater systems will not be captured
 - ~25% of US residences are not connected to sewer
 - Onsite treatment increasingly common at correctional facilities, universities
- Negative results do not indicate absence of cases
- Low incidence may be below the limit of detection
- Cannot be used to “clear” a community or facility
- May be impacted by pre-treatment of sewage for odor or worker safety

Medema, KWR,
Netherlands, 2021

Amy Kirby, CDC, 2021



Method Changes over time

- By April 2021 approx 50% of survey respondents were using WBE with recovery controls to track trends and ~60% of those were using Bovine CoV, with human OC43 next most common
- Most results are not adjusted for recovery efficacy
- Most groups use at least one of the CDC N gene primers
- Groups needing high throughput and short TAT are using ddPCR
- Many WBE programs are using commercially available kits
- Passive samplers are being used to detect low levels and/or to capture infrequent events to “monitor” for hotspots

Knowledge gaps

Next steps

Priorities for further Research



Research Needs

- Define ways to account for factors that impact interpretation at different scales and across different methodologies
- Improve TAT for real-time management of early warning use case
- Increased sensitivity to detect decreases in levels as case loads decline and to pick up hot spots for early warning
- Standardization of internal controls and reporting – should results be adjusted for matrix recovery and/or fecal strength – or not?
- Development of standard reporting – Effective Reproductive number (R_e) value?



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Thank You

