SUMMARY REPORT

Pilot Program for Onsite Testing of SARS-CoV-2 in Correctional Facility Wastewater

Prepared by: Water Environment Federation

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FINAL

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Abbreviations

ADF	average daily flow
CA	California
CDC	U.S. Centers for Disease Control and Prevention
CDCR	California Department of Corrections and Rehabilitation
CF	correctional facility
COVID-19	coronavirus disease of 2019
Ct	cycle threshold
DCLS	Virginia Division of Consolidated Laboratory Services
DEQ	Department of Environmental Quality
DNA	deoxyribonucleic acid
DOC	Department of Corrections
GLP	good laboratory practice
gpd	gallons per day
gc	gene copies
GU	gene units
L	liter
LOD	limit of detection
LOQ	limit of quantification
mL	milliliters
MOU	memorandum of understanding
no.	number
OK	Oklahoma
PCR	polymerase chain reaction
POC	point of care
PPE	personal protective equipment
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
RT-qPCR	real-time quantitative polymerase chain reaction
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
uL (or µL)	microliters
VA	Virginia
WA	Washington
WBS	Wastewater-based surveillance
WEF	Water Environment Federation
WRRF	water resource recovery facility

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Acknowledgments

This pilot program was a collaborative effort made possible only by participation of numerous people across multiple agencies. The Water Environment Federation (WEF) is deeply grateful to the U.S. Centers for Disease Control and Prevention (CDC) for their funding of, and collaboration during, the pilot. WEF is also profoundly appreciative for the hard work many people from the following organizations committed to this pilot:

- California Department of Corrections and Rehabilitation
- Oklahoma Department of Corrections
- Oklahoma Department of Environmental Quality
- Virginia Department of Corrections
- Washington Department of Corrections
- Washington Department of Health
- Hach Company
- LuminUltra Technologies Ltd.

In particular, the teams of testing technicians at each facility deserve special thanks for taking on new responsibilities related to this pilot despite their already busy day jobs.

EXECUTIVE SUMMARY

The coronavirus disease of 2019 (COVID-19) pandemic highlighted the value of wastewater-based surveillance (WBS) for complementing clinical testing data and providing a more complete picture of COVID-19 infection levels and trends in a population. Most WBS participants send samples to offsite laboratories for analysis. For remote communities and institutions—such as correctional facilities (CFs)—sample shipment can pose a logistical challenge and prevent rapid turnaround of test results. Onsite methods for wastewater testing have the potential to provide more timely data to clinical and public health professionals.

In this pilot, the Water Environment Federation (WEF) collaborated with the U.S. Centers for Disease Control and Prevention (CDC) to evaluate the use of onsite wastewater testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as a means for supplementing case surveillance data and possibly providing an early warning for COVID-19 outbreaks in CFs. The pilot effort was supported by funding from the CDC to WEF under Cooperative Agreement CK20-2003 (Improving Clinical and Public Health Outcomes through National Partnerships to Prevent and Control Emerging and Re-Emerging Infectious Disease Threats).

A total of 18 CFs across four states participated in the pilot, with an average participation duration of 25 weeks (range: 16 to 32) and an average of 44 tests performed per site (range: 14 to 126). Sites used a magnetic bead-based method to concentrate wastewater samples and extract ribonucleic acid (RNA) before reverse transcription quantitative polymerase chain reaction (RT-qPCR) for detection of the N2 gene target in the SARS-CoV-2 RNA. Out of the 795 samples analyzed across all sites during the pilot, 427 (54%) were produced during RT-qPCR analytical runs for which all quality control metrics were passed. Data quality generally improved over the course of pilot participation.

Based on participant observations during startup and implementation of the pilot, the following eight **barriers** were identified related **to onsite wastewater testing technology use**:

1. Lack of dedicated staff time
2. Testing method complexity
3. Challenges associated with COVID-19
4. Supply issues
5. Test performance
6. Training deficiencies
7. Challenges related specifically to CFs
8. Lack of communication of value

Overcoming these barriers during the pilot was achieved with a combination of leadership commitment, additional training, weekly meetings, and responses to questions outside of trainings and meetings.

The following four **barriers** were identified **to the acceptance and use of wastewater data**:

1. Loss of actionable window
2. Insufficient data density
3. Lack of plans for data use
4. Skepticism about whether the test was giving reliable results

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These data acceptance barriers were overcome by comparing wastewater data to COVID-19 case counts during check-in calls and holding refresher trainings to improve data quality.

Correlations between daily SARS-CoV-2 RNA concentrations (N2 gene copies/L) and cumulative weekly COVID-19 cases reported for incarcerated residents of CFs (total cases reported in the 7 days after the wastewater sample was collected) were significant (at $\alpha = 0.01$) at three CFs (out of three) in California and one CF (out of five) in Washington. (Correlation calculations were not performed for wastewater and case data from Oklahoma and Virginia.) Overall, the pilot was considered a success because:

- There were instances of the wastewater data being used to complement other COVID-19 surveillance, such as the wastewater being the first indication of an outbreak at one California CF and prompting the shift to point-of-care rapid antigen testing when the clinical staff realized the PCR testing of residents resulted in a significant lag relative to wastewater;
- All participants indicated they would participate in the pilot again, if given the chance, due to the opportunity to develop new collaborations, gain experience, learn what did and did not work, and generate data that was of sufficient quality and seemed to reflect the COVID-19 status of their facility; and
- Three out of four states plan to continue with their own wastewater surveillance programs for the foreseeable future.

The pilot demonstrated that test kits for quantification of SARS-CoV-2 in wastewater could be used by individuals without prior public health laboratory experience, provided sufficient training and troubleshooting support is provided. Further, the pilot indicated that wastewater testing can assist with early identification of COVID-19, provided that high-quality, timely wastewater data are generated. The following are recommended to maximize the success of future onsite testing programs in CFs:

- Roles, responsibilities, plans, protocols, and success metrics should be clearly defined at the outset of the program;
- Data should be timely, high quality, and understandable;
- The burden on corrections staff should be minimized;
- Training should be multimodal, repeated, and responsive;
- The CF's sewer system should be understood; and
- Challenges and delays should be expected.

This report summarizes the pilot scope, outcomes, and lessons learned so that the barriers to establishment of onsite testing programs can be understood. Understanding these barriers, and how best to overcome those barriers, is critical for expanding WBS to remote locations.

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1. INTRODUCTION: Why was the pilot implemented?

1.1 Background

The COVID-19 pandemic highlighted the value of WBS for complementing clinical testing data and providing a more complete picture of COVID-19 infection levels and trends in a population. Wastewater samples can be collected and tested for the presence and quantity of SARS-CoV-2 genetic material using PCR laboratory methods, which amplify specific sequences of the SARS-CoV-2 RNA. The relative concentrations of SARS-CoV-2 gene copies found in a wastewater source, when compared over time, can offer a timely and cost-effective indication of when community transmission is increasing, plateauing, or decreasing.

Most communities and institutions participating in wastewater surveillance activities send samples to state, commercial, or university laboratories for analysis. For remote communities and institutions, sample shipment can pose a logistical challenge and prevent rapid turnaround of test results. Onsite methods for wastewater testing have the potential to provide data to clinical and public health professionals more quickly than if samples were sent off site for analysis. Correctional facilities (CFs) have faced an additional set of challenges related to the COVID-19 pandemic. Outbreaks in CFs, including prisons, jails, and detention centers, have high attack rates and are challenging to control. Security related to collecting and shipping samples places an added burden on these facilities.

In this pilot, WEF collaborated with the CDC to evaluate the use of onsite wastewater testing for SARS-CoV-2 as a means for supplementing case surveillance data and possibly providing an early warning for COVID-19 outbreaks in CFs. The pilot effort was supported by funding from the CDC to WEF under Cooperative Agreement CK20-2003 (Improving Clinical and Public Health Outcomes through National Partnerships to Prevent and Control Emerging and Re-Emerging Infectious Disease Threats). This report provides a summary of the pilot program and offers guidance for correctional facilities and other congregate settings seeking to incorporate WBS to their COVID-19 response plan.

1.2 Pilot Objectives

The objectives of the onsite wastewater testing pilot were to (1) assess how field test kits can be used by individuals not working in public health labs and (2) learn how effectively onsite wastewater testing can assist with the early identification of COVID-19. To that end, we aim to answer the following four questions in this pilot summary report:

1. What is the quality of the data produced by the rapid testing platform?
2. What barriers exist to using on-site wastewater testing technology in correctional facilities?
3. What barriers exist to acceptance and use of wastewater testing data in CFs?
4. How well did the wastewater SARS-CoV-2 concentrations correlate with COVID-19 incidence?

A protocol for wastewater surveillance at the facility level to be shared with facilities, correctional or otherwise, with populations living in congregate settings is also provided.

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2. SCOPE: How was the pilot set up?

2.1 Pilot Sites

In coordination with CDC, WEF considered the following criteria when selecting the states for inclusion in the pilot:

1. Geographic location: states representing different regions of the country, and CFs from urban and rural settings.
2. Wastewater surveillance experience: states with varied levels of wastewater surveillance experience.
3. Willingness to participate: states demonstrating interest and support from the leadership of their department of corrections.
4. Ability to participate: states for which the participating agency would be able to receive shipment of supplies and manage distribution of supplies to the testing sites.

Based on the selection criteria, California (CA), Oklahoma (OK), Virginia (VA), and Washington (WA) were selected to participate in the onsite wastewater testing pilot. The Water Environment Federation signed separate Memoranda of Understanding (MOUs) with the California Department of Corrections and Rehabilitation (CDCR), Oklahoma Department of Environmental Quality (OK DEQ), Virginia Department of Corrections (VA DOC), and Washington State Department of Corrections (WA DOC). Based on the MOU, WEF agreed to provide the supplies—which included training from the supplier—required for initiation of onsite wastewater testing in up to five CFs, while the partner agency agreed to identify sites for inclusion in the testing program, initiate and sustain testing, and share data files with WEF. The Water Environment Federation left it to the discretion of these partner agencies whether they wanted to coordinate with additional state agencies relevant to wastewater surveillance for the pilot project. **Table 1** shows the state agencies that were directly involved in the pilot—either as a partner with CDC and WEF (through execution of an MOU) or through participation in ongoing pilot project meetings at the invitation of the partner agency—and the organization role of the pilot project lead(s) for each state.

Within each state, it was the responsibility of the partner agency to select the specific CFs at which to perform the testing pilot, and a total of 18 CFs were selected for participation in the pilot. The characteristics of the CFs identified by each partner agency are summarized in **Table 2**. Table 2 also contains some information on the sample collection location, which is discussed further in the next section.

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Table 1. State Agencies and Points of Contact Participating in Pilot

Agencies in bold are the partner agencies that signed an MOU with WEF; see text for details.

State	Participating agency(ies)	Role of pilot project lead(s) at partner agency(ies)
California	• **California Department of Corrections and Rehabilitation**	(1) Research epidemiologist in the Public Health Branch of the Medical Services Division (CDCR)
Oklahoma	• Oklahoma Department of Corrections • **Oklahoma Department of Environmental Quality**	(1) Director of Water Quality Division (OK DEQ) (2) Municipal wastewater enforcement specialist in the Water Quality Division (OK DEQ)
Virginia	• **Virginia Department of Corrections**	(1) Utilities Plant Administrator (VA DOC) (2) Environmental and Energy Administrator (VA DOC)
Washington	• **Washington Department of Corrections** • Washington Department of Health	(1) Infectious disease physician (WA DOC) (2) Environmental manager (WA DOC)

2.2 Testing Approach

Each partner agency was provided with a complete set of supplies needed for testing wastewater for SARS-CoV-2 for at least 4 months, from sample collection to concentration to analysis (see supply list in **Appendix A**), at up to five sites. Samples were collected with autosamplers programmed to collect composite samples (either 4-hour or 24-hour; see Table 2) of untreated wastewater from a manhole or from the headworks of the CFs' water resource recovery facility (WRRF).

The pilot project leads at each partner agency identified agency staff to perform the testing. These testing technicians used the GeneCount SARS-CoV-2 Wastewater RT-qPCR Kit (Hach Company, Colorado, USA and LuminUltra Technologies Ltd., New Brunswick, Canada) to concentrate wastewater with the magnetic bead method, extract the RNA, and quantify the SARS-CoV-2 RNA using reverse-transcription quantitative PCR (RT-qPCR) with the GeneCount Q16 device (LuminUltra Technologies Ltd., New Brunswick, Canada). This testing approach was selected because it was the only commercially available rapid testing option suitable for SARS-CoV-2 RNA quantification in wastewater at the time the pilot was initiated (August 2021). The workflow for the full method (from concentration to RNA extraction to RT-qPCR) is detailed in **Appendix B**.

The GeneCount Q16 has 16 wells and can therefore run approximately 12 samples at a time, after accounting for the necessary process controls. The N2 PCR assay was used, which targets the N2 region of the SARS-CoV-2 nucleocapsid gene, and the overall method concentration factor, limit of quantification (LOQ), and limit of detection (LOD) were 5, 100 000 gene copies (gc)/L, and 50 000 gc/L, respectively.

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Table 2: Pilot Site Characteristics

Site	Institution type	Approximate number of residents during pilot	Facility wastewater fate	Approximate wastewater average daily flow (gpd)*	Wastewater sample collection point	Wastewater sample type
California						
CA-1	Mixed custody	2,960	Onsite WRRF	500,000	Inlet headworks, outer perimeter of institution	24-hr composite
CA-2	Maximum security	1,850	Onsite WRRF	500,000	Influent channel at WRRF headworks	24-hr composite
CA-3	Minimum to medium security	1,970	Onsite WRRF	400,000	WRRF headworks	24-hr composite
Oklahoma						
OK-1	Minimum to medium security	1,250	Onsite WRRF	300,000	WRRF headworks	4-hr composite (6-10 am)
OK-2	Minimum security	825	Onsite WRRF	100,000	Influent flow channel	4-hr composite (6-10 am)
OK-3	Minimum security	775	Onsite WRRF	80,000	Manhole upstream of WRRF	4-hr composite (6-10 am)
OK-4	Minimum security	920	Onsite WRRF	60,000	Manhole upstream of WRRF	4-hr composite (6-10 am)
OK-5	Medium security	950	Onsite WRRF	120,000	Manhole upstream of WRRF	4-hr composite (6-10 am)
Virginia						
VA-1	Level 1 and level 2	1,485	Onsite WRRF	110,000	Influent headworks before grinder	Composite
VA-2	Minimum security	600	Onsite WRRF	50,000	Influent headworks before grinder	Composite
VA-3	Level 3	1,200	Onsite WRRF	70,000	Influent headworks before grinder	Composite
VA-4	Level 2/medium security	1,190	Onsite WRRF	70,000	Influent headworks before grinder	Composite

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Site	Institution type	Approximate number of residents during pilot	Facility wastewater fate	Approximate wastewater average daily flow (gpd)*	Wastewater sample collection point	Wastewater sample type
VA-5	Maximum security	1,250	Onsite WRRF	140,000	Influent headworks before grinder	Composite
Washington						
WA-1	Minimum security	480	Onsite WRRF	26,000	Headworks to WRRF	Mix of grab and composite
WA-2	Mixed custody	1,820	Local municipal WRRF	210,000	Manhole on facility grounds	24-hr composite
WA-3	Mixed custody	1,270	Local municipal WRRF	Unknown	Outside secure perimeter at a manhole	3-hr composite (6-9am)
WA-4	Mixed custody	740	Local municipal WRRF	Unknown	Outside secure perimeter at headworks of decommissioned onsite WRRF	3-hr composite (6-9am)
WA-5	Mixed custody	1,930	Local municipal WRRF	290,000	Outside secure perimeter at Parshall flume vault at decommissioned onsite WRRF	24-hr composite

Abbreviations: gpd = gallons per day; WRRF = water resource recovery facility

*Multiply gpd by 0.0038 to convert to m^3/day

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2.3 Training Program

Testing technicians and other pilot participants received initial in-person or virtual training from experienced trainers at Hach Company, the technology supplier for the GeneCount Q16 PCR device, as described in **Table 3**. These initial trainings focused on good laboratory practices; how to set up, program, and use the autosamplers; how to concentrate the wastewater samples with the magnetic bead technique; how to run the GeneCount Q16 PCR device; and what process controls to use. The complete training syllabus is shown in **Appendix C.** In-person training attendees were given as much "hands-on" time as possible to practice the challenging steps in the testing process, such as pipetting small volumes. The actual hands-on time, however, depended on the number of participants in each training session, with more participants translating into less hands-on time per person.

The extent of previous laboratory experience held by the testing technicians trained on the onsite method varied across sites (see Table 3). Although many of the testing technicians had previous wastewater laboratory experience, none had experience working in public health laboratories.

As pilot testing got underway in each state, additional training and support was provided as needed:

- For California, Hach provided additional virtual training via videos showing specific steps of the testing process and ran two virtual meetings to review process control procedures and answer any questions from the testing technicians
- In Oklahoma, a representative from the partner agency worked one-on-one with the CF testing teams to refresh the material covered in the initial trainings and help troubleshoot any challenging steps. Hach responded to specific follow-up questions via telephone calls when needed.
- In Virginia, Hach conducted a question-and-answer follow-up session a few weeks after the original training.
- In Washington, Hach was available for questions as needed following the initial training.

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Table 3: Schedule, Format, and Participants for Initial Training Sessions

State	Date	Training location, format, and number of participants*	Number of technicians trained by pilot site	Previous lab experience of site technicians
CA	November 2021	• CA-3, in person, 4 • CDCR HQ, in person and virtual, 35	• CA-1: 2 • CA-2: 3 • CA-2: 1	• CA-1: daily controls process testing • CA-2: some laboratory experience, not certified • CA-3: none
OK	August 2021	• OK-4, in person, 10 • OK-5, in person, 30	• OK-1: 1 • OK-2: 2 • OK-3: 1 • OK-4: 1 + 1 resident • OK-5: 3	• OK-1: A laboratory license • OK-2: C laboratory license, basic process control testing • OK-3: C laboratory license, basic process control testing • OK-4: C laboratory license, basic process control testing • OK-5: C laboratory license, basic process control testing
VA	October 2021	• VA-2, in person, 16 • VA-3, in person, 4 • VA-5, in person, 4	• VA-1: 2 • VA-2: 2 • VA-3: 2 • VA-4: 2 • VA-5: 1	• VA-1: basic water/wastewater laboratory testing • VA-2: basic water/wastewater laboratory testing • VA-3: one had basic water/wastewater laboratory testing, one had more experience and a degree in chemistry • VA-4: basic water/wastewater laboratory testing • VA-5: basic water/wastewater laboratory testing
WA	October 2021	• WA-1, in person, 6 • WA-2, in person, 1	• WA-1: 4 • WA-2: 0 (one operator was trained by individuals incarcerated in facility WA-1) • WA-3: 0 • WA-4: 0 • WA-5: 0	• WA-1: basic water/wastewater controls process testing • WA-2: none • WA-3: N/A • WA-4: N/A • WA-5: N/A

Abbreviations: HQ = headquarters; N/A = not applicable

*Includes people other than the technicians who performed the testing

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2.4 Quality Assurance and Quality Control

Quality assurance for SARS-CoV-2 analysis with the GeneCount Q16 involved meeting a set of quality control metrics, as shown in **Table 4** and described in more detail in **Appendix D.** In addition, duplicate samples were recommended. These could either be field duplicates (two samples collected at the same time and taken through all RNA concentration, RNA extraction, and PCR steps in parallel) or RNA extraction duplicates (two aliquots of eluted RNA run through PCR in parallel). The Water Environment Federation supported monitoring of the overall quality of the pilot data in collaboration with CDC, as described in **Section 1.6**.

Table 4: GeneCount Q16 SARS-CoV-2 Quality Control Metrics

*See **Appendix D** for additional details.*

Quality control metric	Purpose	Matrix	Recommended frequency
Negative control	Verify no contamination is present and prevent reporting of false positives	Nuclease-free water	One per PCR run (one per 16 wells)
Positive control	Confirm that reverse transcription and/or PCR reactions are proceeding normally and prevent reporting of false negatives	Positive control DNA	One per PCR run (one per 16 wells)
Matrix spike	Confirm that there is no interference from the wastewater matrix with reverse transcription and/or PCR reactions	Positive control DNA spiked into unconcentrated wastewater	One per PCR run (one per 16 wells)

Abbreviations: DNA = deoxyribonucleic acid; PCR = polymerase chain reaction

2.5 Data Analysis and Sharing

After completion of the PCR runs, the GeneCount output files were either uploaded directly to a secure cloud-based platform (by California, Virginia, and Washington testing teams) or mailed in prepaid bubble mailers to WEF headquarters (by the Oklahoma testing team). Once received, WEF transferred the data from the GeneCount output files to a facility-specific data summary file, plotted the measured SARS-CoV-2 concentrations (N2 gene copies per liter), and recorded whether the quality control metrics had been passed for each PCR run. The Water Environment Federation shared each state's set of facility summary files with the state teams, also via the cloud. An example of a SARS-CoV-2 concentration plot and the associated raw data log are shown in **Figure 1** and **Table 5**, respectively.

Two state teams (California and Washington) opted for weekly virtual check-in meetings with WEF and CDC, facility clinical staff, and facility pilot program leads. Discussion items during these weekly meetings included quality control issues, feedback from testing technicians, and a comparison of wastewater data with reported COVID-19 incidence. These meetings allowed the state teams to receive and discuss timely data and provided an opportunity for WBS knowledge sharing.

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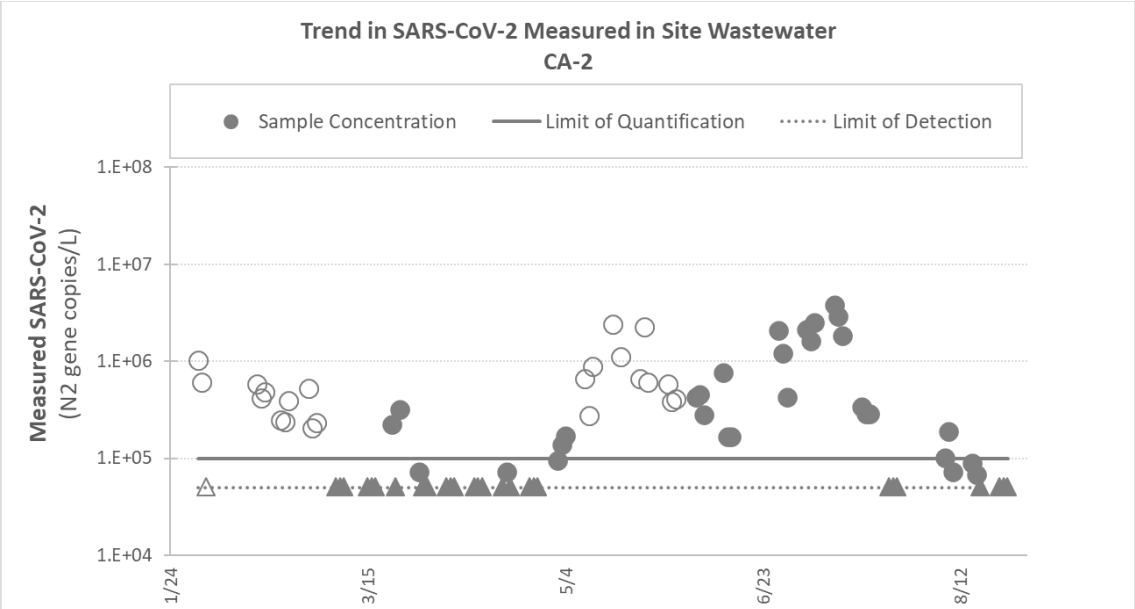
Figure 1: Example of Time Series Plot of Wastewater SARS-CoV-2 RNA Concentrations

*Time series plots for wastewater concentrations at all participating facilities are provided in **Appendix F.***



Notes:
(1) Open symbols correspond to samples for which not all quality control metrics were passed during analysis.
(2) Triangles correspond to sampling events for which both duplicate results were reported by the GeneCount as non-detects.

Table 5: Example of Raw Data Log With Quality Control Information

*See **Section 2.4** and **Appendix D** for definitions and more information.*

Date	Ct (Replicate 1)	Ct (Replicate 2)	Ct (unitless)	Concentration (Replicate 1) (GU/mL)	Concentration (Replicate 2) (GU/mL)	Concentration (GU/mL)	Concentration (gene copies/L)	Positive Control	Negative Control	Internal Sample Control	Spike (gene copies/mL)	Internal Spike Control
1/31/2022	34.36	34.33	34.35	1.02E+03	1.03E+03	1025.00	1.03E+06	Pass	Pass	Pass	No spike	N/A
2/1/2022	34.33	35.02	34.68	6.71E+02	5.48E+02	609.50	6.10E+05	Pass	Pass	Pass	No spike	N/A
2/2/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	No spike	N/A
2/7/2022	20.03	18.43	19.23	7.57E+06	2.04E+07	1.40E+07	1.40E+10	Fail	Fail	Fail	No spike	N/A
2/8/2022	18.2	18.02	18.11	2.36E+07	2.63E+07	2.50E+07	2.50E+10	Fail	Fail	Fail	No spike	N/A
2/9/2022	18.02	18.74	18.38	2.64E+07	1.69E+07	2.17E+07	2.17E+10	Fail	Fail	Fail	No spike	N/A
2/15/2022	34.9	35.78	35.34	7.23E+02	4.24E+02	573.50	5.74E+05	Pass	Pass	Pass	No spike	N/A
2/16/2022	35.78	0	17.89	4.19E+02	Below LoD	419.00	4.19E+05	Pass	Pass	Pass	No spike	N/A
2/17/2022	35.6	35.53	35.57	4.67E+02	4.89E+02	478.00	4.78E+05	Pass	Pass	Pass	No spike	N/A
2/21/2022	36.67	36.6	36.64	2.40E+02	2.51E+02	245.50	2.46E+05	Pass	Pass	Pass	No spike	N/A
2/22/2022	36.25	37.32	36.79	3.11E+02	1.60E+02	235.50	2.36E+05	Pass	Pass	Pass	No spike	N/A
2/23/2022	36.81	35.33	36.07	2.21E+02	5.52E+02	386.50	3.87E+05	Pass	Pass	Pass	No spike	N/A
2/28/2022	34.98	36.03	35.51	6.90E+02	3.59E+02	524.50	5.25E+05	Pass	Pass	Pass	No spike	N/A
3/1/2022	36.69	37.23	36.96	2.38E+02	1.70E+02	204.00	2.04E+05	Pass	Pass	Pass	No spike	N/A
3/2/2022	36.75	0.00	18.38	2.29E+02	Below LoD	229.00	2.29E+05	Pass	Pass	Pass	No spike	N/A
3/7/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	9.02E+02	Pass
3/8/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	9.02E+02	Pass
3/9/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	9.02E+02	Pass
3/15/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	1.98E+03	Pass
3/16/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	1.98E+03	Pass
3/17/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	1.98E+03	Pass
3/21/2022	37.34	36.34	36.84	1.58E+02	2.95E+02	226.50	2.27E+05	Pass	Pass	Pass	6.31E+02	Pass
3/22/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	6.32E+02	Pass
3/23/2022	36.63	35.86	36.25	2.46E+02	3.98E+02	322.00	3.22E+05	Pass	Pass	Pass	6.33E+02	Pass
3/28/2022	38.15	41.08	39.62	9.55E+01	5.00E+01	72.75	7.28E+04	Pass	Pass	Pass	5.22E+02	Pass
3/29/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	5.22E+02	Pass
3/30/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	5.22E+02	Pass
4/4/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	2.91E+02	Pass
4/5/2022	0.00	0.00	0.00	Below LoD	Below LoD		5.00E+04	Pass	Pass	Pass	2.91E+02	Pass

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To enable interpretation of the wastewater data by each facility, a spreadsheet file was provided for each site that required manual input of the latest wastewater results, but automatically trended and summarized all the data for that facility following data entry. These files were included on the desktop of the laptops sent to each state. A document that could be printed out and filled in by hand to evaluate the data trends was also provided. A screenshot of the spreadsheet and a copy of the manual data logging sheet are shown in **Appendix E**.

2.6 Feedback

The Water Environment Federation received feedback from pilot participants related to technology and data use barriers via

- Conversations during pilot startup and ongoing pilot meetings
- Discussions after project completion with
    1. Pilot project lead and epidemiologist (California)
    2. Facility clinical staff using wastewater data (California)
    3. Facility clinical staff using wastewater data (California)
    4. Pilot project champion and water quality division director at state environmental agency (Oklahoma)
    5. Pilot project lead and engineer (Oklahoma)
    6. Pilot project lead and utilities plant manager (Virginia)
    7. Environmental services manager performing testing (Virginia)
    8. Wastewater operator performing testing (Virginia)
    9. Wastewater superintendent performing testing (Virginia)
    10. Pilot project lead and infectious disease physician interpreting wastewater data (Washington)
    11. Pilot project coordinator and environmental manager (Washington)
    12. Epidemiologist at state health department (Washington)
    13. Wastewater coordinator at state health department (Washington)
    14. Concentration method co-developer and technology trainer (Hach)
    15. Concentration method co-developer and technology trainer (Hach)

3. OUTCOMES: What data were produced and what barriers were identified?

This section contains a presentation of both the qualitative pilot outcomes (to answer questions 2 and 3 from **Section 1.2**) and quantitative pilot outcomes (to answer questions 1 and 4 from **Section 1.2**). We first present a summary of the data generated in the pilot to provide the context for the qualitative discussion on barriers to technology use and data acceptance, and then conclude with a summary of the correlation between the wastewater data and COVID-19 case data.

3.1 Data Quantity and Quality

A total of 795 wastewater samples were collected and analyzed across 18 facilities during the pilot. Of these, 427 (54%) were reported from PCR runs for which all quality control metrics were passed and 230 (29%) contained quantifiable levels of SARS-CoV-2. The maximum RNA concentration measured was 2.5

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x 10^{10} gc/L (see **Tables 6** and **7**). The average pilot participation duration across all sites was 25 weeks (range: 16 to 32) and an average of 44 tests were performed per site (range: 14 to 126).

3.1.1 Data Quantity

Figure 2 shows the months in which testing was performed at each of the 18 facilities participating in the pilot, while **Table 6** provides details on the number of weeks of testing at each site, the total number of samples collected and analyzed during the pilot, the sample density (average number of samples collected and analyzed per week), whether the analysis was performed on-site at the facility (or off-site at another facility), the number of clinical reporting events (defined as the number of times the results were discussed with clinical and/or public health staff), and the average clinical reporting density (number of times the results were discussed with clinical staff and/or public health per week). Time series plots of measured SARS-CoV-2 RNA concentrations for all 18 facilities are included in **Appendix F**.

Figure 2. Testing Duration at 18 Facilities During Pilot

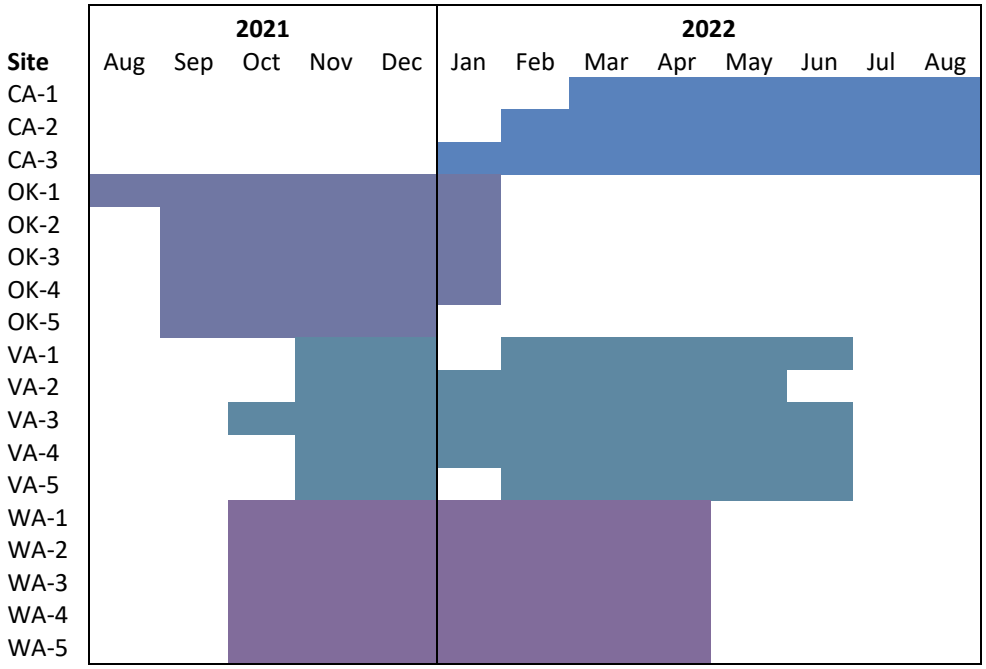

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Table 6. Summary of Testing Duration and Sample and Reporting Densities at 18 Facilities During Pilot Project

*See **Section 3.1.1** for definitions of reporting events and reporting density.*

Site	Testing start date	Testing stop date	Total testing duration (weeks)	Total samples collected & analyzed (#)	Average sample density (#/week)	Where analysis was performed	Total clinical reporting events (#)	Average clinical reporting density (#/week)
CA-1	3/1/2022	8/17/2022	24.1	72	3.0	Onsite	24	1.0
CA-2	1/31/2022	8/17/2022	28.3	81	2.9	Onsite	28	1.0
CA-3	1/3/2022	8/17/2022	32.3	67	2.1	Onsite	32	1.0
OK-1	8/17/2021	1/20/2022	22.3	38	1.7	Onsite	0	0
OK-2	9/22/2021	1/13/2022	16.1	35	2.2	Onsite	0	0
OK-3	9/1/2021	1/20/2022	20.1	67	3.3	Onsite	0	0
OK-4	9/13/2021	1/13/2022	17.4	36	2.1	Onsite	0	0
OK-5	9/6/2021	12/27/2021	16.0	25	1.6	Onsite	0	0
VA-1	11/11/2021	6/24/2022	32.1	22	0.68	Onsite	0	0
VA-2	11/1/2021	5/3/2022	26.1	38	1.5	Onsite	0	0
VA-3	10/26/2021	6/2/2022	31.3	24	0.77	Onsite	0	0
VA-4	11/8/2021	6/6/2022	30.0	29	1.0	Onsite	0	0
VA-5	11/19/2021	6/21/2022	30.6	21	0.68	Onsite	0	0
WA-1	10/12/2021	4/7/2022	25.3	126	5.0	Onsite	25	1.0
WA-2	10/28/2021	4/29/2022	26.1	17	0.65	Onsite	26	1.0
WA-3	10/19/2021	4/5/2022	24.0	43	1.8	Off-site (at WA-1)	24	1.0
WA-4	10/19/2021	4/6/2022	24.1	40	1.7	Off-site (at WA-1)	24	1.0
WA-5	10/28/2021	4/26/2022	25.7	14	0.54	Off-site (at WA-2)	26	1.0
Total				***795***				

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3.1.2 Data Quality

As shown in **Table 7**, pilot data quality (expressed as percent of samples passing all quality control metrics) varied from state to state (range: 22% for Virginia to 81% for Washington) and from site to site (range: 0% for sites Virginia-1, Virginia-3, and Virginia-5 to 93% for California-1), with an overall pilot average of 54%. It is notable that the two states (California [65%] and Washington [81%]) with weekly check-in meetings during the pilot had higher data quality than the two states (Oklahoma [30%] and Virginia [22%]) for which no such regular check-in meetings occurred. Details on the breakdown of quality control failures are provided in **Figure 3**. The most challenging quality control metric to meet was adequate detection of the positive control deoxyribonucleic acid (DNA) in unconcentrated wastewater (the "matrix spike"): 63% of all samples met this quality control metric. In comparison, 84% and 77% of samples, respectively, achieved the negative control and positive control quality control metrics. There does not seem to be an association between the degree of prior laboratory experience and the proportion of site's samples meeting all three quality control metrics. For example, data from site Washington-2 (no prior laboratory experience) were generally of higher quality than the data from site Oklahoma-2 (prior laboratory experience).

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Table 7: Summary of Pilot Data Quality, Samples With Detectable SARS-CoV-2 RNA, and Maximum Observed RNA Concentrations by State and Site

State	Site	Total samples (#)	Samples passing all quality control metrics (# [% of total])	Samples with detectable SARS-CoV-2 RNA (#[% of total])	Maximum concentration measured (N2 gc/L)
California	CA-1	72	67 [93]	42 [58]	3.7×10^{7}
	CA-2	81	45 [56]	50 [62]	2.5×10^{10}
	CA-3	67	30 [45]	22 [33]	8.5×10^{5}
	Total	220	142 [65]	114 [52]	2.5×10^{10}
Oklahoma	OK-1	38	24 [63]	12 [32]	3.1×10^{6}
	OK-2	35	12 [34]	3 [8.6]	1.3×10^{10}
	OK-3	67	17 [25]	22 [33]	8.4×10^{7}
	OK-4	36	1 [2.8]	3 [8.3]	2.4×10^{5}
	OK-5	25	7 [28]	8 [32]	1.0×10^{8}
	Total	201	61 [30]	48 [24]	1.3×10^{10}
Virginia	VA-1	22	0 [0]	0 [0]	--
	VA-2	38	10 [26]	4 [11]	9.8×10^{4}
	VA-3	24	0 [0]	0 [0]	--
	VA-4	29	19 [66]	0 [0]	--
	VA-5	21	0 [0]	0 [0]	--
	Total	134	29 [22]	4 [3]	9.8×10^{4}
Washington	WA-1	126	107 [85]	32 [25]	1.4×10^{6}
	WA-2	17	12 [71]	8 [47]	5.2×10^{5}
	WA-3	43	37 [86]	16 [37]	5.7×10^{5}
	WA-4	40	33 [83]	4 [10]	9.8×10^{4}
	WA-5	14	6 [43]	4 [29]	2.9×10^{5}
	Total	240	195 [81]	64 [27]	1.4×10^{6}
Overall Total		**795**	**427 [54]**	**230 [29]**	**2.5×10^{10}**

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Figure 3. Compliance With Quality Control Metrics by Site and Sample

Samples are shown in the order in which they were analyzed at each site. A **purple** bar indicates the negative control was run and did not have any detectable SARS-CoV-2 in it. An **orange** bar indicates the positive control was run and enough of the DNA control was detected. A **blue** bar indicates a matrix spike was run and enough of the DNA control was detected. For a given sample to "pass all quality control metrics", all three bars need to be present. In addition, samples needed to pass an internal process control, which they typically did; those data are not shown here. See Section 2.4 and Appendix D for more details. A solid border around a graph indicates the technicians running the samples at that site **did have** prior laboratory experience, whereas a dashed line indicates the laboratory technicians **did not have** prior laboratory experience.

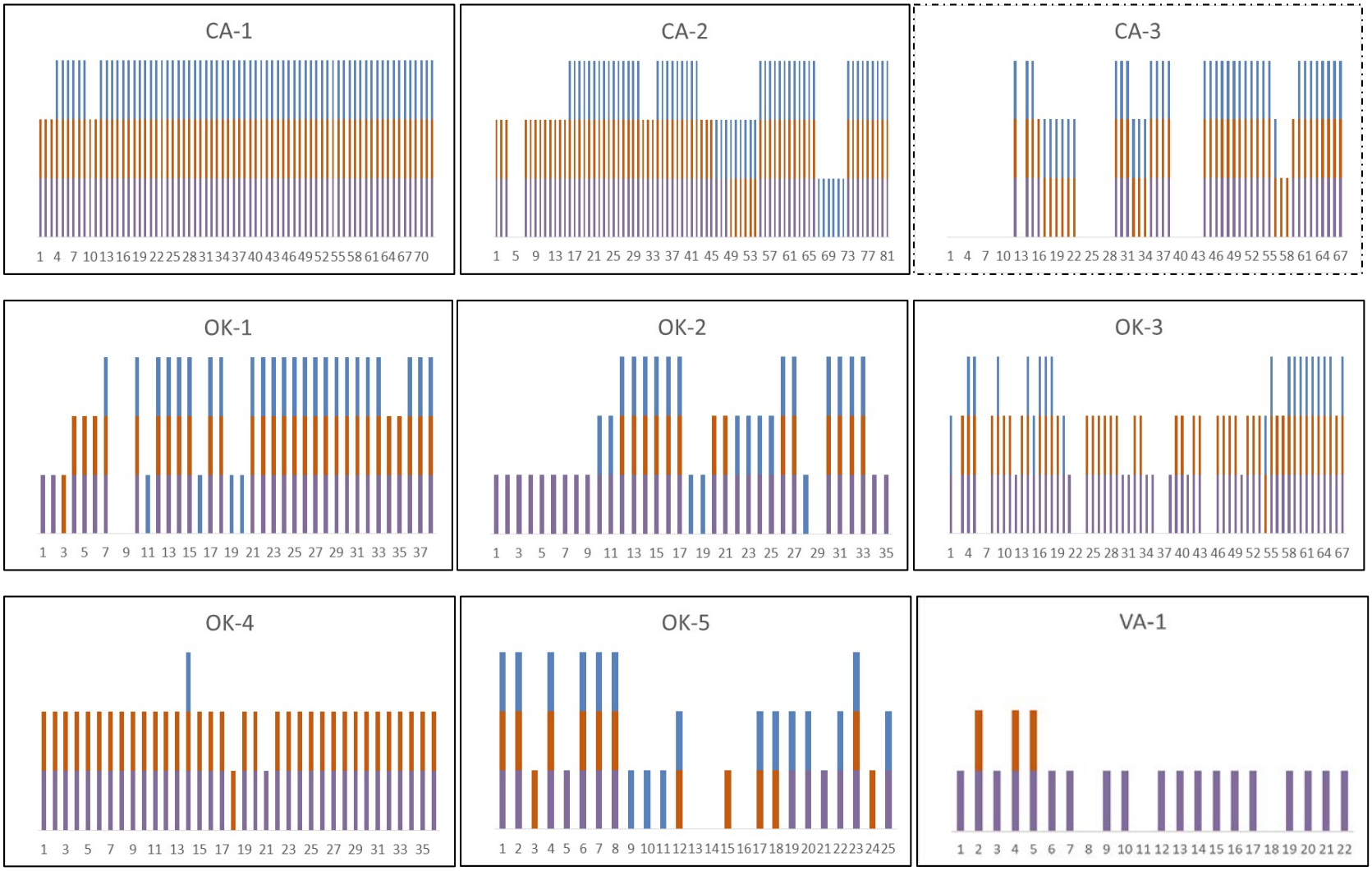

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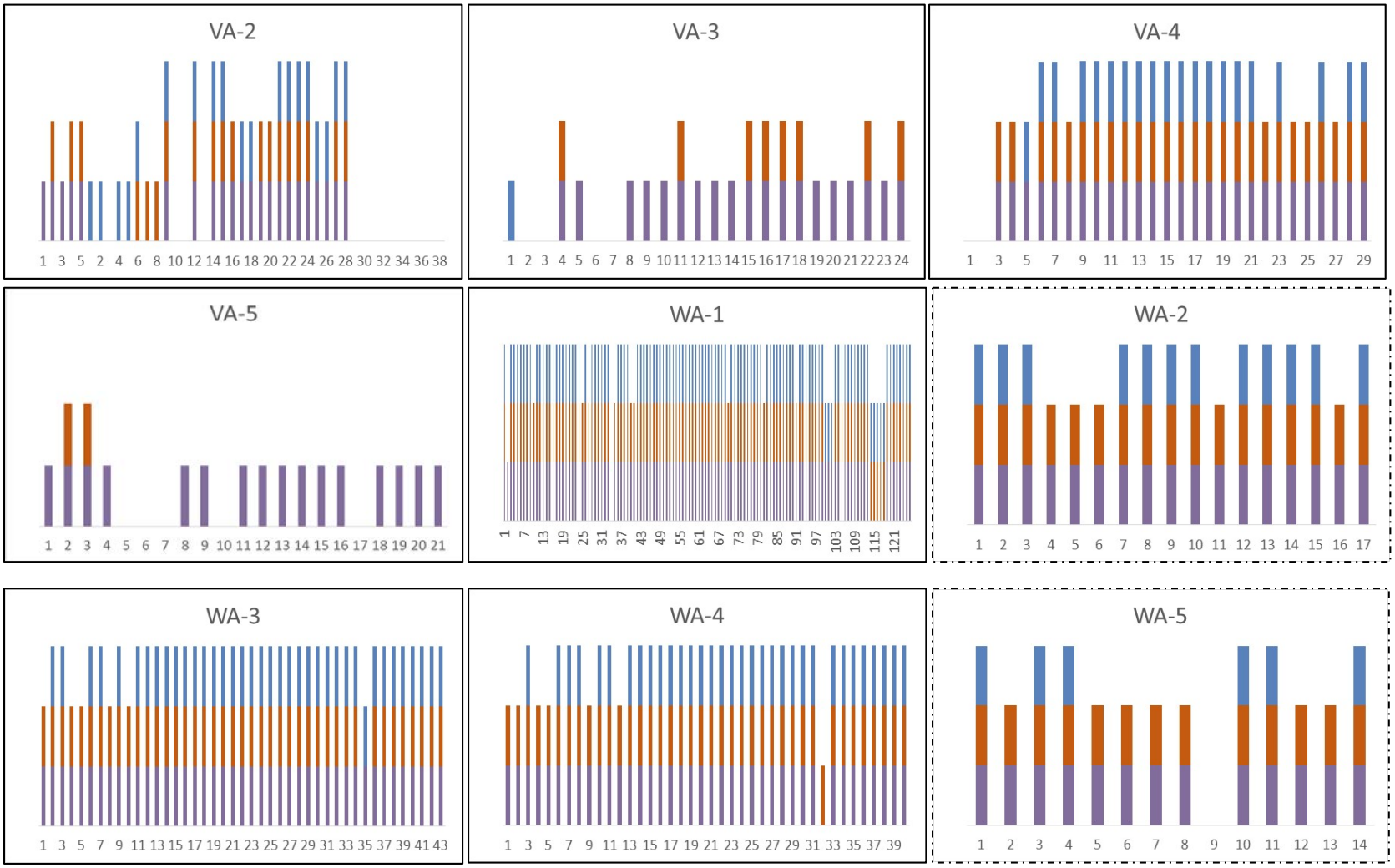
Figure 3 (continued). Compliance With Quality Control Metrics by Site and Sample

Samples are shown in the order in which they were analyzed at each site. A **purple** bar indicates the negative control was run and didn't have any detectable SARS-CoV-2 in it. An **orange** bar indicates the positive control was run and enough of the DNA control was detected. A **blue** bar indicates a matrix spike was run and enough of the DNA control was detected. For a given sample to "pass all quality control metrics", all three bars need to be present. In addition, samples needed to pass an internal process control, which they typically did; those data are not shown here. See Section 2.4 and Appendix D for more details. A solid border around a graph indicates the technicians running the samples at that site **did have** prior laboratory experience, whereas a dashed line indicates the laboratory technicians **did not have** prior laboratory experience.



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3.2 Onsite Testing Technology Use

Based on discussions with pilot participants and observations during ongoing pilot meetings and activities, eight major barriers to technology use were identified. These are presented below in order from most to least commonly mentioned or observed. The discussion of barriers is followed by a discussion of how they were overcome during the pilot.

3.2.1 Barriers to Technology Use

Lack of dedicated staff time

The most cited barrier was the lack of staff time dedicated to the testing. Whether the testing was performed by wastewater operators, environmental laboratory staff, or maintenance staff, it was "[e]xtra work that" had to be done "on top of already busy schedules" by individuals who were "already overburdened with what they need to do". It was challenging to dedicate time to testing "given all other operational duties" and, in California specifically, was "an even bigger ask of the facilities" than other projects previously undertaken. One trainer was told by an institution staff member: "We're not doing this test. We're too busy". Challenges related to identifying suitable testing staff at the outset of the pilot appeared to delay initiation of the pilot program, particularly in California.

The lack of previous laboratory experience was identified as another potential barrier. However, it was noted that "even those without previous laboratory experience could get the method with enough practice" and, conversely, even those with "some laboratory experience" found the steps to be "onerous". Therefore, the lack of previous laboratory experience is not considered a direct barrier. The barrier is, instead, the lack of dedicated staff time needed to practice the method due to its complexity.

Testing method complexity

The lack of dedicated staff time was especially an issue because the test took "so long" and "was extremely complicated". Method complexity was mentioned as a barrier by everyone directly involved in testing. Specific challenges included accurately pipetting small volumes—not to mention understanding the different pipette tip sizes—and completely removing the ethanol during RNA concentration and extraction (see **Appendix B**). Testing staff reported that the RNA concentration and extraction and qPCR preparation steps required from about 2 to 4 hours total (not including time to run the GeneCount PCR), and that these steps did not necessarily get "quicker with more experience" because moving too fast could cause "missteps". Moreover, it was possible to be pulled away from the test to "take care of something in the plant" as part of regular duties and then have to start over entirely because the timing of a specific step was missed. Even with laboratory experience, the steps were found to be "onerous", and it was "probably overreaching for the average person to come in and start this procedure". Because the testing process was so involved, in most cases it "took too much time to do it as frequently as … [was] needed to make it clinically useful".

Challenges related to COVID-19

Staffing challenges were exacerbated by COVID-19. Testing started a month later than expected at one facility and was temporarily suspended at three other facilities in the middle of the pilot, because the testing staff were all out with COVID-19. In locations where the testing was being performed by incarcerated residents (*e.g.*, Washington), testing would be halted during an outbreak because the

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residents were not able to get to the laboratory, even though they had not tested positive for COVID-19. And even if the testing technicians were available to perform the wastewater tests, COVID-19 could prevent the sample from being collected. At one facility, the individual with the key to the sampler was out with COVID-19 for 14 days, "which meant no sample for 14 days". In general, COVID-19 caused work forces to be "decimated" and distracted by "competing priorities".

Supply issues

The variety and volume of supplies required to start testing were substantial, requiring abundant storage space and time to unpack and re-kit everything for each facility. The reagents and supplies were not packed in a way that mapped them to different steps of the process (*e.g.*, color coding or numbering reagents and supplies to correspond with different steps). And not all reagents required for the process were included in the GeneCount SARS-CoV-2 Wastewater RT-qPCR Kit (such as some of the pipettes, the isopropyl alcohol, and the ethanol), resulting in shipments from multiple manufacturers.

Six specific issues related to supplies were noted:

- When supplies shipped from LuminUltra to CDCR were unpacked for training, they were missing the binding beads—a critical reagent for the testing process.
- The GeneCount Q-16 taken out of the box for one training in Oklahoma did not work, requiring that someone drive an hour each way to the next closest facility to retrieve that facility's GeneCount Q-16 device to use for the training.
- The GeneCount software stopped working at multiple facilities, requiring downloading and reinstalling the software from the Internet. That was a particular issue at an Oklahoma facility that did not have Internet access.
- Some Washington facilities had issues programming the autosamplers and using them to achieve reliable sample collection.
- Although not a supply deficiency per se, the single magnetic rack provided in the GeneCount SARS-CoV-2 Wastewater RT-qPCR Kit made it impossible to process an entire batch of samples in parallel.
- As the California pilot extended into the summer of 2022, the expiration dates of the some of the reagents passed, and it was unclear which past-due reagents would affect test performance.

Test performance

In addition to frustration over the method complexity, some participants expressed concern about the test performance. In one Washington facility, wastewater testing consistently showed results below the limit of detection, even during a period with a known COVID-19 outbreak in the facility. It was believed the method was "hypersensitive" to chemicals "used to treat water and wastewater", potentially resulting in non-valid results and false negatives and requiring repeated testing for samples with "non-conclusive results". And, even when all internal controls were passed, "there wasn't 100% confidence that the test was reliable based on case counts". A summary of the test performance is provided in **Section 3.1.2**. The relationship between the wastewater SARS-CoV-2 RNA concentrations and COVID-19 incidence is explored further in **Section 3.4**.

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Training deficiencies

Training issues were mentioned by four participants. As described in **Section 2.3**, testing staff initially received mostly in-person trainings, but also some virtual training. All follow-up trainings were done virtually. Virtual trainings, in combination with videos provided by the trainer, were found to be helpful, but it was more difficult to learn the method online. Specifically, it was challenging to provide technical assistance over the telephone because it was necessary to "be there in person to troubleshoot". Some of the in-person trainings had too many attendees. Even 16 attendees (at Virginia-2) were thought to be too many because it limited the amount of hands-on time each trainee could have. The training setup in general did not give the "at bats" and repetition needed to solidify an understanding of the testing method at the outset of the pilot. For some facilities, there was a lag of 4 to 6 months between training and startup of the testing—further eroding the training efficiency.

It should be noted that the success of the initial training did not appear to wholly dictate the success of the testing (as expressed by the testing density and the fraction of test results passing quality control over the duration of the program). It was thought that "everyone can learn [the method] through repetition". California-1 and California-2, for example, ultimately produced data that passed all quality controls (**Figure 3**), even though technicians in both facilities were trained virtually. Therefore, training deficiencies should be considered a barrier to initial technology use only, and one that does not necessarily affect technology use in the long term. Training deficiencies can, however, delay the start of the program that may reduce motivation and commitment and result in inefficient use of testing program funds.

Challenges related to correctional facilities

The CF setting presented unique challenges for the pilot testing program. Accepting shipments of supplies, such as ethanol, was challenging at some CFs. In some cases, the lack of Internet access made it impossible to share the PCR output in a timely manner or update GeneCount software as needed. In facilities that relied on incarcerated residents to perform the testing, the testing program needed to be suspended during an outbreak when the facility quarantine prevented the residents from accessing the testing lab. In general, the CF challenges, especially those related to implementing training sessions, were more noticeable in maximum security facilities relative to minimum security prisons. At minimum security prisons, the training could be performed "where the testing was going to take place", which meant the trainers could assist with getting the whole "system up and running".

Lack of communication of value

The challenges associated with finding adequate dedicated staff to perform the testing were exacerbated by the fact that not all facilities were aware of the potential value in the wastewater testing approach. The communication of the pilot plan and engagement of the local facility staff was done differently across the states and facilities as dictated by the local corrections organization structure. In some cases, the communication did not reflect a motivation to do the testing "from the top down". Moreover, staff with knowledge of the system were not always consulted when selecting the facilities to participate in the pilot and it would have been helpful to have consistent protocols around "roles and responsibilities and communications".

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3.2.2 Overcoming Technology Use Barriers

The numerous barriers to use of the testing technology appeared to have been overcome in the eight facilities that were able to produce high-quality results (> 50% of all samples passing all quality control; see **Section 3.1.2**) at a sufficient testing density (> 1 sample/week; see **Section 3.3.1**). Overcoming these barriers was achieved with a combination of the following:

- Leadership commitment: Having one or more people willing to serve as the pilot program lead and liaison between the different groups involved to make sure all questions were answered, and all issues were addressed in a timely manner (done in all states).
- Additional training: Following up the initial in-person or virtual trainings with virtual refresher trainings and sharing of testing method demonstration videos (done in California, Virginia).
- Weekly meetings: Discussing testing results with clinical, environmental, and, ideally, testing staff to review any quality control issues or inconsistencies with clinical testing data and provide immediate feedback on test performance (done in California and Washington).
- Responses to questions outside of the trainings and meetings: Making expertise available for rapid responses to any questions from testing staff (done in California, Virginia, and Washington).

3.3 Acceptance and Use of Onsite Wastewater Testing Data

Based on discussions with pilot participants and observations during ongoing pilot meetings and activities, four major barriers to acceptance and use of the wastewater data were identified. These are presented below in order from most to least commonly mentioned or observed. The discussion of data use barriers is followed by a discussion of how they were overcome during the pilot.

3.3.1 Barriers to Data Acceptance and Use

Loss of actionable window

For wastewater surveillance data to be useful for CFs, healthcare and public health staff needed to receive the results as soon after sampling as possible. In Oklahoma, results were never shared with health staff, while in California and Washington, a week could elapse between sample collection and data sharing with health staff. Although clinical staff in Virginia CFs used wastewater data to make decisions about testing of individuals who are incarcerated, it was unclear whether the data used were from this pilot or from a parallel wastewater surveillance program (for which samples were being sent to an outside laboratory) already underway.

Several factors contributed to the long duration between sample collection and data sharing—or the lack of data sharing altogether:

- In California, three samples were collected and analyzed each week at each of the three participating facilities. In all cases, all three weekly samples were batch processed through the RNA concentration and extraction, qPCR preparation, and PCR analysis steps on a single day. Once those results were uploaded to the secure cloud-based data sharing site, WEF plotted and summarized the data and shared the results summary with the clinical staff at each facility within a few hours. However, samples collected on a Monday were not analyzed until Thursday or Friday, which meant that results were usually not available until Friday or Monday—a one-week delay from sample collection to reporting.

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- The lack of WiFi access in Oklahoma facilities necessitated mailing the GeneCount output files to WEF headquarters. Upon receipt of the mailed files, WEF staff would transfer the files to the cloud-based data sharing site. Even if the mailing process worked smoothly, this meant that results were available no sooner than a week after the testing was performed. In many cases, the mailing did not go smoothly. Although a data action template was provided to Oklahoma testing staff (see **Appendix E**) this template was never used.
- In Washington, samples from five facilities were processed at two laboratories—one on the west side of the state and one on the east side of the state. Samples from the other three facilities where laboratory testing was not taking place had to be transported to the facilities with the testing labs. There were often delays in sample transport, which had to be done by car. Further, the GeneCount files could not be uploaded to the secure cloud-based sharing site by the CF residents doing the testing. Instead, the GeneCount files had to be copied from the laptop at the CF by DOC staff and driven offsite to upload to the cloud. Therefore, there were also often delays between when the testing was complete and when the GeneCount files were uploaded to the cloud so that WEF could share the data summary with the clinical staff.

Insufficient data density

The original recommendation for the pilot sites was to collect samples twice per week and perform the analysis twice per week—as soon after sample collection as possible. Actual average sample collection density varied across the sites from 0.37 to 5.0 samples per week, while sample reporting density (that is, the interval at which results were reported to clinical staff—which was a function of how frequently the PCR analysis was run and how frequently data were uploaded to the cloud-based data sharing site) was 0 times per week in Oklahoma and Virginia and 1 time per week in California and Washington. It was thought that sampling and testing once per week was not sufficient, because it is not possible to "catch everything" with an interval of 7 days between sample collection. Collecting samples three times per week (as was done in California) was "great", but it would be preferable to have performed the analysis three times a week as well to have more "real-time" results. One participant reported that, ideally, each facility would sample daily, and results would be reported on the same day as sample collection. Data density is discussed further in **Section 3.1.1**.

No plan for data use

Given the novelty of rapid onsite wastewater testing for SARS-CoV-2 in a facility setting, there were no standard practices for evaluating trends in wastewater data at the start of the pilot, nor were there published clinical or public health guidelines for use of wastewater data to guide decision making. To enable interpretation of the wastewater data by each facility, WEF collaborated with CDC to provide a spreadsheet (see **Appendix E**) that required manual input of the latest wastewater results, but automatically trended and summarized all the data for that facility following data entry. The Water Environment Federation also provided a pdf document (also provided in **Appendix E**) that could be printed out and filled in by hand to evaluate the data trends. None of the facilities used these files independently. And two of the four states chose to have weekly meetings, where their processed data from the previous week was presented back to them for discussion.

Nonetheless, "coordinated" and "consistent" communication of results was mentioned as a challenge by two participants. And two different participants mentioned that they would have been interested in

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receiving “more clinical guidance” and “hand holding” from the CDC or WEF on how to interpret the wastewater data.

Skepticism about whether test was giving reliable results

As discussed further in **Section 3.1.2**, 54% of all sample runs during the pilot passed all analytical quality control metrics. However, the fraction of quality control-passing samples varied over time at each site, as well as from site to site (range across sites: 0% to 93%). Further, there was also a sense that “even if you ran the test well, it may have missed some cases” and that PCR inhibition could have played a role in the consistent non-detects at Washington-4.

3.3.2 Overcoming Data Use Barriers

It was challenging to address barriers related to producing results within the actionable window and increasing data density, because both were dictated by how the testing program was set up at each site. However, steps were taken to understand how to use the data for action and improve trust in the results.

To better understand how to use the data for action, the weekly California and Washington check-in calls included a comparison of wastewater results to the COVID-19 status in each facility. The COVID-19 status was either described qualitatively by clinical staff or quantitatively by presentation of COVID-19 incidence data (new daily positive cases). Although no correlation or regression calculations were performed for these meetings, this comparison framed discussions around whether the wastewater and COVID-19 case data were generally consistent with each other. In some cases (such as in California-3 in April 2022), there was “no indication that anything was going up apart from the wastewater”. And, once testing of residents became optional and there was little incentive for an individual to take a test, the wastewater helped fill in clinical testing gaps. One participant noted that the wastewater helped the clinical staff “make good decisions”.

Specific actions taken in response to the wastewater data during the pilot included:

- Shifting from PCR to point-of-care rapid tests for clinical testing once wastewater highlighted how delayed the PCR clinical testing signal was (California-2).
- Diverting resources from daily clinic operations to perform individual testing once the wastewater increased above the LOQ (California-2).
- Confirming, when used in combination with clinical testing results, that it was suitable to declare the outbreak over and end facility quarantine (Washington-1).

To improve the reliability of the results required improving compliance with the quality control metrics. This was achieved by holding virtual refresher trainings to clarify the purpose and mechanics of running the negative control, positive control, and matrix spike. These additional trainings appeared to pay off, especially in California-3, where the data quality improved starting with the 44th sample (see **Figure 3**).

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3.4 Comparison Between Wastewater and COVID-19 Case Data

In addition to understanding the feasibility of using onsite testing in non-public health labs, the other objective of the pilot testing was to evaluate how onsite wastewater testing can assist with the early identification of COVID-19. To that end, we compared the COVID-19 case data with the wastewater concentration data.

3.4.1 Data Comparison Approach

Numerous approaches exist for correlating COVID-19 case data with wastewater RNA concentrations. Different types of case data can be used in correlations, such as daily incidence, the rolling average of daily incidence over a 7-day period (or another interval), or cumulative incidence. Similarly, different types of wastewater data can be used, such as daily concentrations, the rolling average of daily concentrations over a 7-day period (or another interval), daily loads (*e.g.*, flow-normalized concentrations), or the rolling average of daily loads. The correlation itself can involve calculating a Pearson (R) or Spearman (rho) correlation coefficient. The latter is typically more suitable given the skewed distributions common to case and wastewater data. **Table 8** shows scatterplots for different correlation approaches for one pilot facility (California-1), and the corresponding correlation coefficients.

To evaluate the relationship between COVID-19 case and wastewater data for the pilot facilities, we compared the rank of 7-day prospective cumulative resident case numbers with the rank of daily wastewater data using Spearman's rho. The 7-day prospective cumulative case number on any given day was defined as the total number of positive cases reported in the subsequent week. This cumulative case number was used because it typically had a strong correlation with the wastewater data, and it is a meaningful number from a clinical and public health perspective in that it is useful to know how many cases are expected in the facility in the coming week. The Spearman's rho was used because the datasets are skewed due to the presence of many days with no cases and non-detectable wastewater RNA concentrations. For days with non-detectable RNA concentrations, the wastewater value used in the correlation calculation was one half the analytical method LOD (or 25 000 gc/L). Only wastewater data that passed all quality control metrics were used in the calculations, and only California and Washington facilities were included in the analysis because there were not enough daily wastewater data points from Oklahoma and Virginia for a meaningful correlation analysis. **Figure 4** displays different types of case data (daily, rolling 7-day average, and 7-day prospective cumulative cases) and wastewater data (daily, rolling 7-day average) for the California and Washington facilities. The results of the correlation analysis for cumulative cases versus daily wastewater data are provided in the next section.

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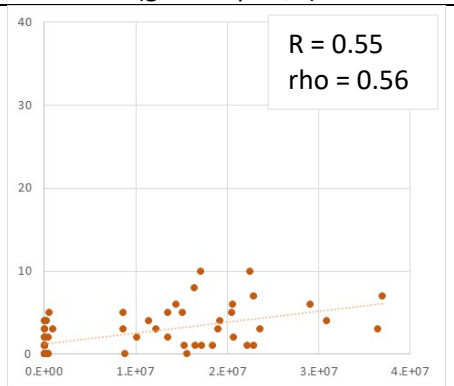
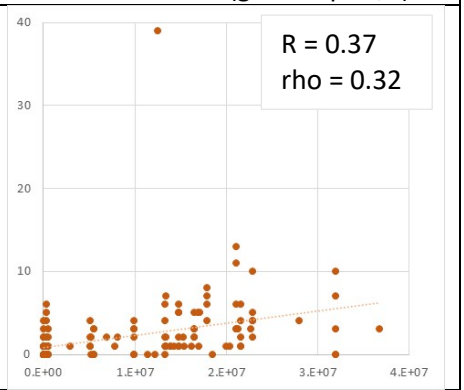
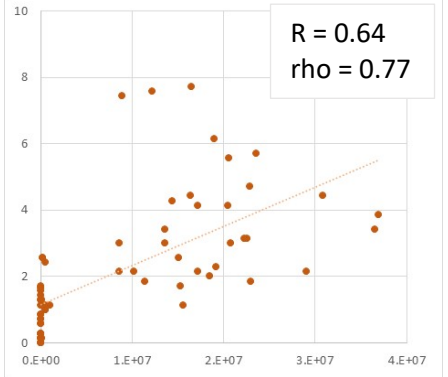
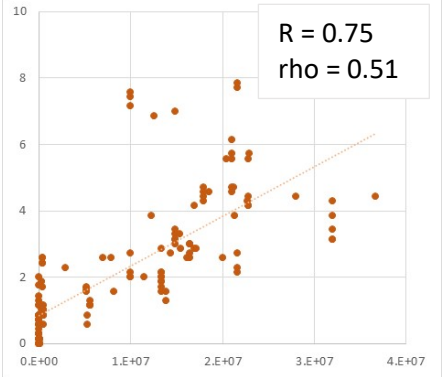
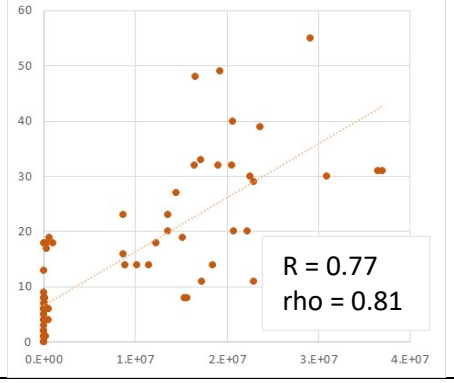
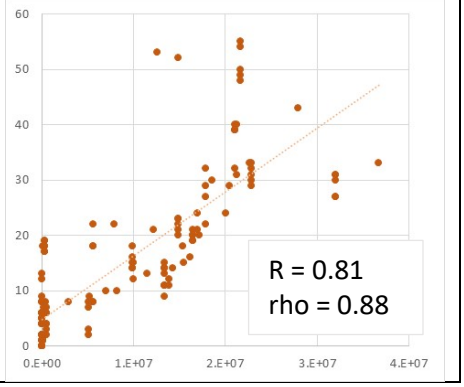
Table 8. Six Approaches to Correlating COVID-19 Case and Wastewater Data for One Pilot Facility (CA-1)

Case data are shown on the y-axis and wastewater data are shown on the x-axis of each scatterplot.

Case Data (y-axis)	Wastewater Data (x-axis)	
	Daily N2 Concentrations (gene copies/L)	Rolling 7-Day Average of Daily N2 Concentrations (gene copies/L)
Daily reported COVID-19 cases (no.)		
Rolling 7-day average of reported COVID-19 cases (no.)		
Prospective 7-day cumulative COVID-19 cases (or the sum of all cases reported in subsequent week) (no.)		

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3.4.2 Summary of Correlations

Table 9 shows the correlation coefficients for the California and Washington facilities. The correlation was significant (at α = 0.01) for all three California facilities and for one Washington facility (Washington-1) facility.

Table 9. Spearman's Rho Coefficients for Correlations Between 7-Day Cumulative COVID-19 Cases and Daily Wastewater N2 Concentrations at California and Washington Facilities

7-day cumulative COVID-19 cases are calculated as the sum of the total cases reported in the 7 days following the date of wastewater sampling; only wastewater data passing all quality control metrics included, and non-detect wastewater samples are set equal to one half the method limit of detection.

Facility	Rho	Count	Significant at α = 0.01?
CA-1	0.81	67	Yes
CA-2	0.72	45	Yes
CA-3	0.63	30	Yes
WA-1	0.62	86	Yes
WA-2	0.35	12	No
WA-3	0.38	37	No
WA-4	0.30	33	No
WA-5	-0.46	6	No

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Figure 4. Time Series Plots of COVID-19 Case and Wastewater Data at California and Washington Facilities

COVID-19 case data are shown in **blue** (bars are daily case counts; blue solid line is 7-day rolling average; blue dashed line is cumulative cases reported in the subsequent 7 days) and wastewater data are shown in **orange** (lighter orange line with circle symbols is daily wastewater concentrations; darker orange line is 7-day rolling average). *Please note the x- and y-axis scales vary from one plot to another.*

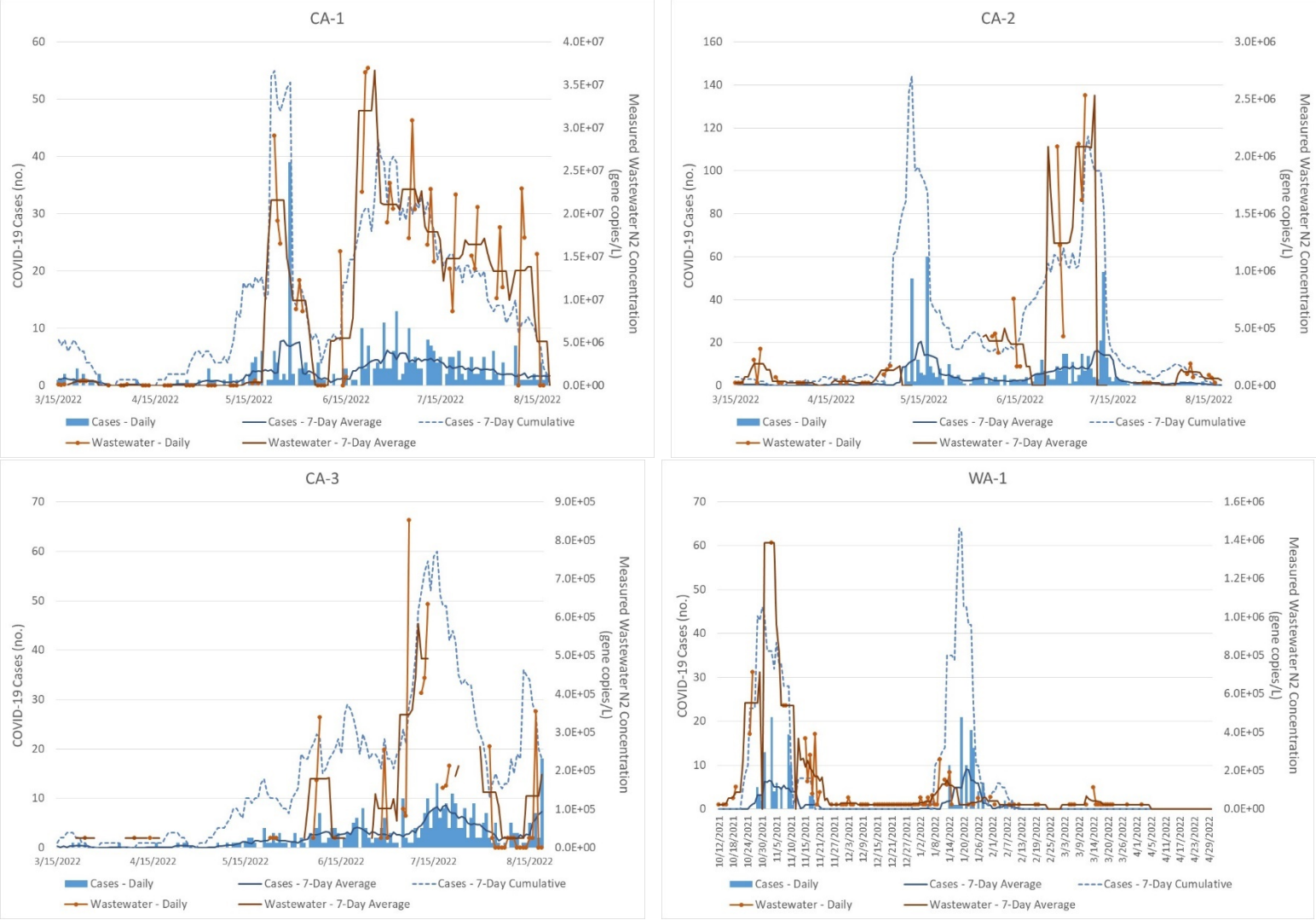

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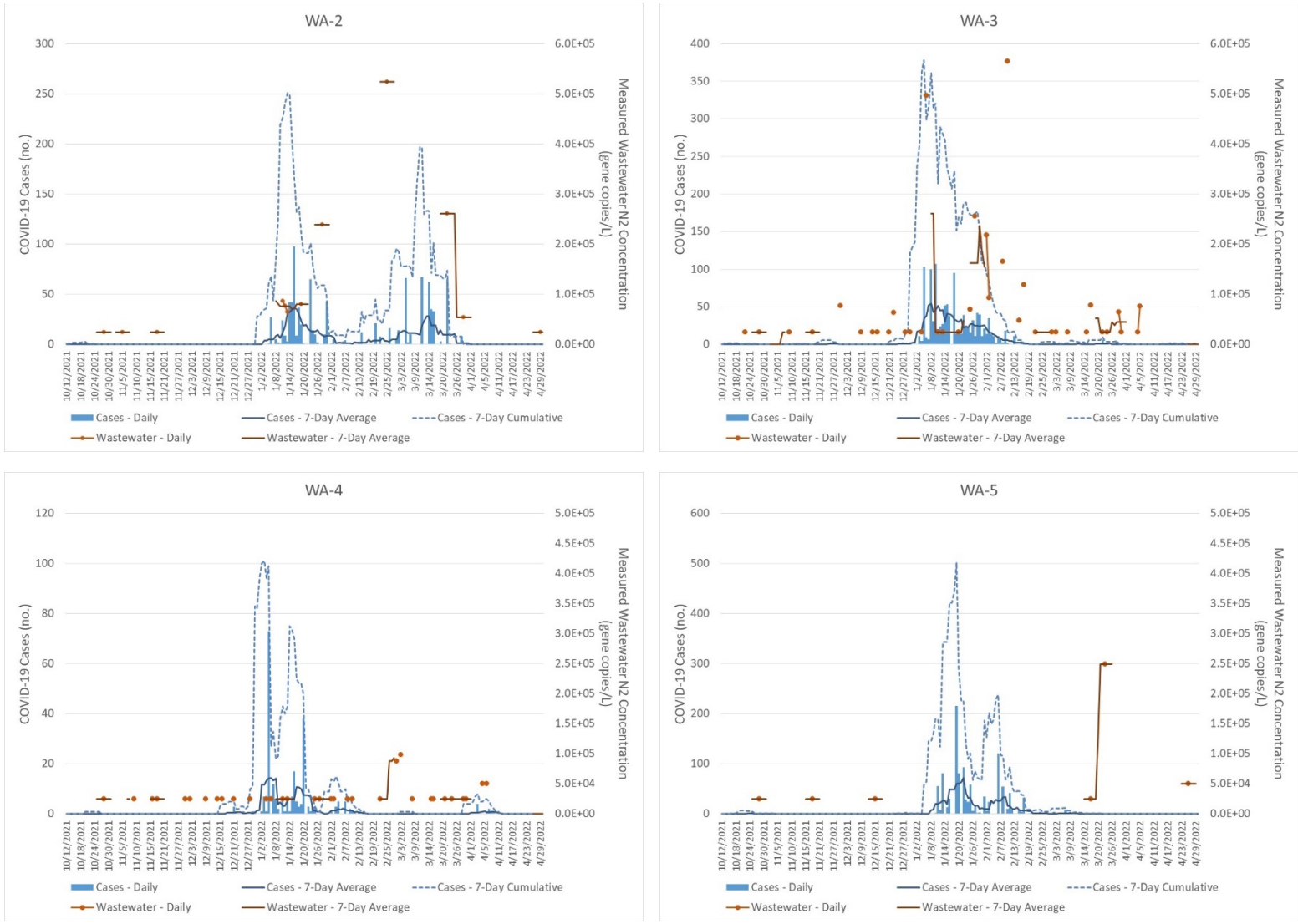
Figure 4 (continued). Time Series Plots of COVID-19 Case and Wastewater Data at California and Washington Facilities

COVID-19 case data are shown in **blue** (bars are daily case counts; blue solid line is 7-day rolling average; blue dashed line is cumulative cases reported in the subsequent 7 days) and wastewater data are shown in **orange** (lighter orange line with circle symbols is daily wastewater concentrations; darker orange line is 7-day rolling average). *Please note the x- and y-axis scales vary from one plot to another.*



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4. DISCUSSION: Was the pilot successful?

Despite the challenges associated with pilot implementation, and the issues with wastewater data quantity and quality, there were many successful components of the effort. These include

1. The wastewater data being used to guide specific clinical and public health decisions,
2. The pilot process being viewed as valuable by participants, and
3. Most states opting to continue wastewater surveillance beyond the pilot.

Each of these successes are described in more detail below.

4.1 Specific Actions Guided by Wastewater Data

Although neither WEF nor CDC provided detailed guidance on how to translate the wastewater data into clinical or public health action, the state testing teams discovered ways to fold the wastewater data into their COVID-19 surveillance efforts as follows:

- At California-2, the wastewater data were the first indication of an outbreak in April—before any positives had shown up in clinical testing. During the outbreak, the clinical staff realized that using PCR for individual testing resulted in a significant lag relative to the wastewater. As a result, they switched to using point-of-care rapid antigen testing to provide more real-time information.
- At Virginia facilities, wastewater data were used to prompt an increase in individual testing—although this may have been driven largely by the data generated from the parallel wastewater testing effort that involved sending samples to an outside laboratory for analysis.
- Similarly, at Washington-1, the wastewater data was a leading indicator for the start of an outbreak and helped support decisions to increase individual testing and segregate residents who tested positive from residents who tested negative.
- Also at Washington-1, a series of non-detect wastewater data was used—in concert with an absence of positive individual tests—to confirm the end of an outbreak.

4.2 Value of Pilot Experience

All participants indicated they would participate in the pilot again, even knowing what they did at the end of the process. The pilot value came from

- Fostering “a collaboration [between the corrections and health departments] that had not previously existed”;
- Being at the forefront and participating in a “new technology” rather than going “boldly where everyone has been”;
- Getting the facilities “started” in an area where they “would not have had capacity”;
- Having a chance to “learn a new technology”;
- Offering a “great collaborative experience” and a chance to “start the ball rolling … regardless of where we end up”;
- Institutions being able to “have experience with wastewater surveillance for 6 months”;
- Being “a step on the path towards access to this kind of tool for prisons”;
- Providing “pockets of good performance”;

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- Ultimately generating data that “passed quality metrics” and therefore was of use to “the chief medical executives” because it was of “sufficient quality”;
- Generating data that “really does seem to reflect what’s happening on the ground”;
- Learning “what didn't work”, namely that the “GeneCount is not feasible”, that “transport of [samples] between facilities [is not] feasible”, and therefore, “processing the data onsite is important”;
- Confirming that wastewater surveillance “is a useful tool”, even if it was not possible to “collect data on a regular enough basis”;
- Being “the best thing I did in the last year and a half” and “one of the most exciting things I've done in my career”; and
- Showing that “wastewater testing is promising”.

In short, “frustrating and negative pilots are just as important as ‘successful’ ones” and “it wasn't all success, but we did experience success”.

4.3 Willingness to Continue Wastewater Surveillance

Three states are continuing with wastewater surveillance.

- In California, the onsite testing approach will be replaced with contract laboratory testing for all CFs. Relying on an outside laboratory is expected to be more sustainable and enable more widespread participation in CDCR’s wastewater surveillance program. Clinical staff intend to use the wastewater data to guide resource allocation and targeted testing.
- In Virginia, the DOC’s Health Services Unit continues to be committed to sending wastewater samples to the Virginia Division of Consolidated Laboratory Services (DCLS) for testing. Currently, Virginia DOC delivers samples to DCLS on Tuesday and receives results back on Friday evening or Monday. While onsite wastewater testing could theoretically generate more actionable data, the plan is to continue with DCLS for now.
- In Washington, the DOC is purchasing 13 GeneXpert Rapid PCR devices so they can perform wastewater testing onsite in all Washington prisons. In addition, they have hired a dedicated staff member to run the program.

The exception is Oklahoma, for which there are no plans to continue wastewater surveillance at this time.

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5. LESSONS LEARNED: What is recommended for other onsite wastewater testing programs?

Along with the participants in the pilot program and CDC, WEF learned many valuable lessons that would apply to other onsite wastewater testing programs in CFs or other institutional settings.

Lesson 1: Roles, responsibilities, plans, protocols, and success metrics should be clearly defined at the outset of the program

Many stakeholders are relevant to any CF wastewater surveillance program, including environmental and utility staff, healthcare providers and public health staff, corrections officers, and incarcerated residents. There are also stakeholders outside the CF that may be involved, similar to how the CDC and WEF were involved in this pilot. And there are a wide variety of tasks that need to be performed during program implementation. Therefore, it is critical to spell out the roles and responsibilities—which representative from which stakeholder group will be performing which task—at the start of the program. The individual(s) responsible for completing the following tasks should be identified:

- Developing the sampling plan (where samples are taken, what type of samples are collected, how often they are collected, what metadata are needed);
- Developing the laboratory analysis protocol (what are the maximum hold times for samples before concentration, what concentration method will be used, what nucleic acid extraction method will be used, will extracts be analyzed with PCR immediately, if not immediately, how frequently will PCR be run, what is the plan for quality assurance, what is the plan for managing PCR inhibition if expected);
- Procuring any supplies and equipment needed for sample collection and/or analysis;
- Developing the data processing and sharing protocol (where will wastewater sample metadata be stored, how will wastewater concentration data be analyzed and trended, where will wastewater concentration data be stored and who will have access to it, who will collect the clinical testing data, how frequently will wastewater and clinical data be shared and with whom, should any data sharing agreements need to be in place, and should there be regular calls to discuss the data and, if so, with whom);
- Establishing the public health and clinical action protocols, or the process by which these protocols will be established (*e.g.*, how much wastewater data will need to be collected before action thresholds are developed, if any);
- Providing overall management of the wastewater surveillance program to monitor conformance with plans and protocols, address any issues as they arise, and foster communication among the different stakeholder groups;
- Collecting wastewater samples on an ongoing basis, including a contingency plan if the primary sample collector is unavailable;
- Documenting sample metadata (collection time, wastewater flow if possible) on an ongoing basis;
- Analyzing wastewater samples, including a contingency plan if the primary analyzer is unavailable;
- Evaluating and sharing the data in accordance with program protocols on an ongoing basis;
- Establishing the frequency of general check-in calls; and
- Making clinical/public health decisions based on the wastewater data, if any.

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When developing these plans and protocols, it is important to keep in mind that it is critical to have consistency in terms of who is sampling, who is doing the testing, and how the data are analyzed and interpreted. An example of an overall program protocol is provided in **Appendix G**.

As part of the development of plans and protocols, it is also helpful to identify specific success metrics at the outset of the program. The following metrics were identified during this pilot, but can be adapted for a site's specific needs:

- Samples are collected and tested regularly (2 to 3 times per week and even more frequently during an outbreak),
- Results are robust (≥ 90% pass all quality control metrics),
- Results are reported in a timely manner (within 24 to 48 hours of sample retrieval) to department of corrections and department of health partners,
- There is a protocol in place for the laboratory to alert department of corrections health partners when SARS-CoV-2 is detected after a period of non-detects or when a particularly high result is obtained, and
- Results are consistent with clinical surveillance data.

Lesson 2: Data should be timely, high-quality, and understandable

Healthcare and public health staff need to receive processed wastewater data within a few days of sample collection to be able to act on the wastewater signal. Moreover, these data need to be high quality and reliable, that is, produced in accordance with a robust quality assurance plan and meeting pre-defined quality control metrics. The likelihood of producing high-quality data can be increased by

- Having a clearly defined sample collection plan and laboratory analysis protocol,
- Providing multimodal and repeated training sessions for testing staff and a mechanism for testing staff to get a rapid response to any questions that arise during program implementation (see Lesson 5 for more information), and
- Holding regular check-in/feedback sessions with the CF "team" to discuss data, quality control, and challenges—ideally with clinical and public health staff, the wastewater surveillance program manager and the staff directly involved in testing.

In addition to minimizing the turnaround time between sample collection and data reporting, samples need to be collected and analyzed at an adequate frequency to be useful. Testing once per week is not sufficient for action. Rather, a minimum of three times per week is recommended, with testing results reported within 24 hours of sample collection. Ideally, wastewater testing would be performed five to seven days per week, with results available the same day as sample collection.

Finally, when evaluating and sharing data, care must be taken to present the data meaningfully. Simple time series plots of either flow-normalized loads or unnormalized concentrations are sufficient, although the plots should include clear notations on the magnitude of the wastewater viral signal relative to the method LOD and LOQ and which analytical runs (if any) had quality control issues. Including error bars for each data point (standard deviations or standard errors of replicate analyses, for example) is also helpful. Presenting the quintile for the latest result or the general trend (increasing, decreasing, stable)

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can also assist with putting the data into context. This may be unnecessary, though, especially if weekly check-in calls are held to discuss the data trends.

Note that it should be assumed that the value of the data produced from a wastewater surveillance program will be higher when the clinical testing coverage is lower.

Lesson 3: The burden on corrections staff should be minimized

The environmental or utility staff likely to be tasked with wastewater sample collection and analysis are most probably already busy meeting the demands of their existing jobs. Further, the public health and clinical staff needed to translate the wastewater data into action can similarly be overwhelmed, especially if in the middle of an outbreak response. It can be challenging to incorporate any time-consuming tasks related to wastewater surveillance into the workload of corrections employees.

The following steps can be taken to minimize the burden on staff from a wastewater surveillance program:

- Perform wastewater surveillance at CFs that discharge wastewater to their own treatment system to increase the likelihood that sample collection can be performed efficiently;
- Optimize sample collection by collecting samples for wastewater surveillance from the same locations already being used for wastewater sample collection for other purposes (process control, compliance;)
- Separate the responsibilities of wastewater sample collection from wastewater analysis from data processing, so that no one person or team is responsible for all three; and
- Select an analytical method that is less time consuming and complex than the method used in this pilot because the GeneCount method was found to be too onerous for nearly all testing teams who participated.

It may be preferable to centralize the analysis in one or two laboratories to minimize the number of staff dedicated to laboratory work. This would involve having multiple facilities collect their own samples, but then transporting those samples to one or two CFs serving as the dedicated wastewater surveillance laboratory(s). However, this analysis centralization should only be done if there are pre-existing transportation routes (such as shuttle buses that already transport compost or other environmental streams) that can be leveraged for sample transport. Relying on commercial shipping services (FedEx, UPS) or the use of personal cars for sample transport is not recommended.

Lesson 4: Training should be multimodal, repeated, and responsive

A comprehensive training program that covers best practices related to sample collection and analysis is critical to the success of an onsite wastewater testing program for disease surveillance. Such a training program would have multiple components to support the testing team during the program duration.

Recommendations related to training include the following:

- The initial training should be given by the technology provider in person in the location where the analysis is going to be performed. Virtual events are not recommended for the initial training but may be appropriate for follow-up training. Attendance at each in-person training event should be capped at 10 to give attendees adequate opportunity to practice any challenging steps in the sample

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collection and analysis process. The trainer should offer "hacks" for organizing the lab for the purpose of the wastewater analysis (such as, color coding reagents and materials with each step).
- The trainer should make "how-to" videos and documents available to the trainees so they can be reviewed before the initial training. These videos and documents can also be helpful to have on during program startup.
- The lag between the completion of the initial training and the startup of the testing program should be minimized.
- The initial, in-person training should be followed up with individual visits by the trainer to each facility if possible. If individual visits are not possible, follow-up trainings could be performed virtually.
- Ongoing check-in meetings should be used throughout the program duration as a forum for the testing team to bring up any quality control issues or testing challenges.
- The technology provider should be available to provide prompt answers to any questions from the testing team within the first 6 months after the initial training.

In addition to training the CF staff on the testing method, it may be helpful for clinical and public health staff to participate in a workshop that covers examples of how the wastewater data can be used to make decisions about resident care.

Above all, it is important to adapt the training and resources as needed during implementation of the testing program.

Lesson 5: Communications should be frequent, inclusive, and adaptive

Optimal program communication should start with sharing the sampling plan, laboratory analysis protocol, data processing and sharing protocol, and public health/clinical action protocol with the entire program team before initiating testing. Regular check-in meetings with the entire WBS program team are recommended, along with more frequent meetings between the program manager and the laboratory staff/testing technicians. The WBS program manager should adapt the meetings as needed to ensure their frequency is sufficient to address program issues as they arise. Further, laboratory, public health, and clinical staff should all be given a chance to contribute during team meetings.

Lesson 6: The correctional facility's sewer system should be understood

Many sewer system factors can affect wastewater testing results for disease surveillance. Flow variation due to stormwater or infiltration/influences or the use of industrial or large-scale cleaning processes can affect measured gene copy concentrations. Having good flow data can help correct for sewer flow variations that affect testing results. Certain chemicals (such as some detergents) can inhibit PCR reactions. If used intermittently rather than continuously, these PCR inhibitors can affect some sample results but not all, thereby confounding wastewater trends.

It is helpful, then, to answer the following questions when developing a wastewater surveillance program:

- Where is flow metered within the sewer system and, therefore, where does it make sense to collect samples so that the relevant flow data can be used?

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- Is stormwater conveyed to the sewer and, if so, where are those tie-in points relative to the sampling location(s)?
- Who is connected to which sewer and, therefore, which residents are captured in the wastewater at the program sampling location(s)?
- What disinfectants and other chemicals are used at the facility and in what quantities? Are any of these known to cause PCR inhibition?

If possible, trial wastewater samples should be collected before startup of the wastewater surveillance program and sent to a commercial laboratory with wastewater PCR testing capabilities to evaluate the presence of PCR inhibition. These samples should be collected from the locations and at the approximate times planned for the facility's program. If PCR inhibition is present, technicians should work with the supplier of the analytical supplies to develop a protocol for overcoming inhibition, if possible.

Lesson 7: Challenges and delays should be expected

Challenges faced during the pilot were numerous and due to a variety of factors, including overcommitted staff, repeated COVID-19 outbreaks, shipping delays, and barriers to getting supplies into, and data out of, CFs. Challenges should be expected as part of any full-scale wastewater surveillance program. Recommendations for anticipating and managing challenges and delay include the following:

- Consider starting the program with a small-scale, short-term pilot, consisting of testing at two or three CFs for 6 to 8 weeks. This will give the program team an opportunity to work out issues not anticipated during the program planning phase.
- Develop robust contingency plans by building in more time than expected for program elements and more budget than anticipated for supplies.
- Maintain open lines of communication with all program participants, ideally through regular meetings with all relevant stakeholders.

6. CONCLUSIONS: Were the pilot objectives achieved?

The pilot objectives were achieved because

- The state testing teams, with support from CDC and WEF, were able to demonstrate that field test kits can be successfully used by CF staff without prior public health laboratory experience; and
- Onsite wastewater testing has the potential to provide high quality, timely COVID-19 surveillance data and assist with early identification of COVID-19 outbreaks.

Further, the lessons learned and protocol developed from this pilot effort can be used to optimize the design other onsite wastewater testing programs.

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Appendix A: List of Supplies Provided to Each Participating State

qPCR SYSTEM AND ASSOCIATED CONSUMABLES
GeneCount Q-16 qPCR device
120-mL sample vials
Pipets and pipet tips for 1.0-10 mL
GeneCount SARS-CoV-2 Wastewater RT-qPCR Kit
GeneCount COVID-19 Positive Control
Isopropyl alcohol
Ethanol, undenatured
AUTOSAMPLER AND ASSOCIATED SUPPLIES
Portable compact sampler bundle with 2.5 gallon bottle
12 Volt lead acid battery
Battery charger assembly
OTHER SUPPLIES
Laptop (to run GeneCount software)
Compact refrigerator with freezer
Nitrile gloves
Safety glasses
Laboratory notebook

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Appendix B: LuminUltra GeneCount SARS-CoV-2 RT-qPCR Detection Workflow

Copy of instructions provided by Hach Company and LuminUltra to testing teams

LUMINULTRA®
microbial monitoring

Test Kit Instructions: Wastewater Testing Using GeneCount™ SARS-CoV-2 RT- qPCR Detection Workflow

SECTION 1: RNA Concentration & Extraction

SECTION 2: qPCR Assay Preparation

SECTION 3: Analysis on Q-Series Device (Q-8 / Q-16)

SECTION 4: Result Interpretation

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SECTION 1: RNA Concentration & Extraction

PROVIDED

- 15 mL Sterile Conical Tubes
- Lysis Buffer Concentrate
- Lysis Supplement 1A
- Wash Solution 1 Concentrate (Store at 25°C)
- Wash Solution 2 Concentrate (Store at 25°C)
- Elution Buffer NA (Store at 25°C)
- Nuclease Free Water
- Magnetic Beads (Store at 4°C)
- 1000 µL and 200 µL Filtered pipette Tips
- Wide mouth 1mL pipette tips (only for sample addition)
- 1.5 mL sterile disposable transfer pipettes

REQUIRED BUT NOT PROVIDED

- Tube Rack for 2 mL and 15 mL Tubes
- Permanent Marker
- Isopropanol* (95-100%)
- Ethanol (95-100%)
- Adjustable Volume 1000 µL and 200 µL Pipets

*Ethanol may be used as a replacement for isopropanol if it is not available.

GETTING STARTED

- Wear safety glasses and disposable exam gloves.
- **Please read all reagent SDSs for instructions, hazards, and material safety.**
- Clean and set up a work area to process samples.
- Create "Wash Buffer 1" by adding **60 mL** of **Isopropanol** to **120 mL** of **Wash Solution 1 Concentrate**, per label instructions.
- Create "Wash Buffer 2" by adding **160 mL** of **Ethanol** to **40 mL** of **Wash Solution 2 Concentrate**, per label instructions.
- Rehydrate vial of **Lysis Supplement 1A** with **6.6 mL of Nuclease-Free Water**. Mix intermittently for 1 minute by swirling. Do not invert bottle.

  Note: Solution may not fully dissolve.

RNA CONCENTRATION & EXTRACTION

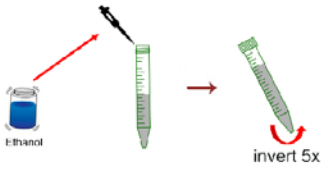
- Label a sterile 15 mL conical tube with identifying information for each sample.
- Add **6 mL** of Lysis Buffer Concentrate, **250 µL** of rehydrated **Lysis Supplement 1A**, and **1 mL** of **wastewater sample** using wide mouth 1mL pipette tips to each 15 mL sterile conical tube.

  Note: Use wide mouth 1mL pipette tips only for addition of sample.

  Note: Store unused Lysis Supplement 1A frozen at -20°C for up to 1 month in single-use aliquots to minimize freeze-thaws. Thaw completely before use.
- Close cap and invert **5 times** to mix the sample solution, then incubate for **10 minutes**.

  Note: If an incubator is not available room temperature can be used.

- After incubation, add **3.5 mL** of **Ethanol** and mix thoroughly by gently inverting the tube **5 times**.



- Add **40 µL** of **Magnetic Beads** to the sample mixture. Invert the sample mixture 5 times to mix, then incubate again for **10 minutes**.

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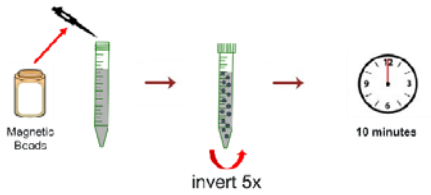
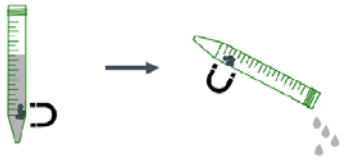
Note: Ensure that Magnetic Beads are fully resuspended by inverting 5 times before adding to the sample mixture. If an incubator is not available, room temperature can be used.



- After incubation, place in the magnetic rack to precipitate the Magnetic Beads on the side of the tube. Discard the supernatant.



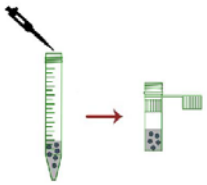
- Add **1 mL** of **Wash Buffer 1**. Cap and swirl the tube 10 times to mix.
- Place the tube in the magnetic rack to precipitate the Magnetic Beads. Discard the supernatant.
- Repeat the previous two steps with **Wash Buffer 1** twice more.

Note: Wash Buffer 1 is added and discarded a total of three times.

- Add **1 mL** of **Wash Buffer 2** to the Magnetic Beads. Cap and swirl the tube 10 times to mix.
- Place the tube in the magnetic rack to precipitate the beads and discard the supernatant.
- Repeat the above two steps with **Wash Buffer 2**.

Note: Wash Buffer 2 is added and discarded a total of two times.

- Add **1 mL** of **Ethanol** to the Magnetic Beads. Cap and swirl the tube 10 times to mix then transfer the mixture to a new sterile 2 mL tube using a sterile disposable transfer pipette.



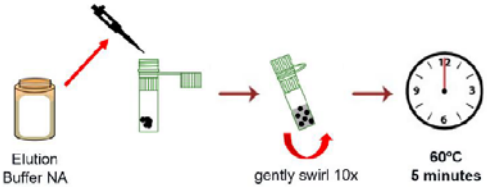
- Place the tube in the magnetic rack to precipitate the beads and discard the supernatant.

- Remove the tube from the magnet rack and allow the remaining pellet to pool in the bottom of the tube.
- Place the tube in magnet rack and allow the Magnetic Beads to accumulate to the side of the tube for 2 minutes. Remove the remaining pooled liquid at the bottom of the tube with a sterile 200 uL pipette without agitating the Magnetic Beads.

- Add **50 µL** of **Elution Buffer NA** to the **2 mL tube**. Cap and **gently** swirl the tube 10 times to mix.

Note: Ensure that the beads are resuspended in the elution buffer.

- Incubate the **Magnetic Beads** at **60°C** for 5



minutes.

- After incubation, apply the magnet to separate the **Magnetic Beads** and use a pipette to collect eluted RNA for analysis.

- Eluted RNA can be stored at -20°C

Note: If biobanking samples for longer than 2 weeks, store the eluted RNA at -80°C.

Note: Please read all reagent SDSs for instructions, hazards, and material safety

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SECTION 2: qPCR Preparation

PROVIDED

- SARS-CoV-2 Advanced RT-qPCR Master Mix (Store at 25°C)
- PCR Strip Tubes
- Nuclease-Free Water (Store at 25°C)
- 20 µL Filtered Pipet Tips

REQUIRED BUT NOT PROVIDED

- Tube Racks for 1.5 / 2.0 mL and PCR Strip Tubes
- Adjustable Volume 20 µL Pipet

OPTIONAL BUT NOT PROVIDED

- Positive Control DNA (Lyophilized: Store at Ambient)

GETTING STARTED

- Wear safety glasses and disposable exam gloves.
- Clean and set up a work area to process samples.

INITIAL SETUP

- Gently tap **RT-qPCR Master Mix** bottle on a hard surface to collect contents at the bottom of the bottle.

  Note: Each bottle of RT-qPCR Master Mix contains enough for 48 samples.

- Remove and discard rubber stopper, and then transfer 825 µL of **Nuclease-Free Water** into the **RT-qPCR Master Mix** bottle.
- Recap and let **RT-qPCR Master Mix** rehydrate for 3 minutes. Mix occasionally by swirling. Do not invert bottle.

  Note: Rehydrated RT-qPCR Master Mix should be used immediately or frozen at – 20°C for up to 12 months. Avoid freeze-thawing the mix more than 3 times for best results.

- **Optional**: If using a positive control, transfer 50 µL of Nuclease Free Water into the Positive Control DNA tube. Recap tube tightly. Allow Positive Control to rehydrate for 5 minutes. Mix tube occasionally by inverting. Centrifuge for 5-10 seconds before use.

  Note: Rehydrated Positive Control should be used immediately or frozen at – 20°C for up to 12 months in single use aliquots to minimize freeze-thaws. Thaw completely before use.

ASSAY SETUP

- Thaw rehydrated **RT-qPCR Master Mix** if necessary.

  Note: Thaw on ice or on the benchtop. Avoid trying to speed up thawing by warming bottle in your hands.

- Dispense 15 µL of the Master Mix into each PCR Strip Tube.

- Transfer 5 µL *of Nuclease-Free Water into the first PCR tube*. This is the **Negative Control**. Close tube.

  Note: Only one set of controls is needed per instrument run not per strip tube (if more than one is being used)

- Transfer 5 µL of each sample RNA into individual PCR tubes. Close each tube as sample is added. Leave the last PCR tube with no RNA if adding an optional positive control.
- **Optional**: Transfer 5 µL of the Positive Control DNA into the **last** PCR tube. This is the **Positive Control**. Close tube.

  Note: The Positive Control DNA is highly concentrated so care should be taken to not contaminate other samples or your work area.

- Gently mix each qPCR reagent tube.
- Using a robust downward motion, shake the contents of qPCR tubes to the bottom of tube.

Note: Be careful to note the correct orientation of the tubes to prevent accidentally reversing the tubes when inserting into the qPCR device. A small mark with a permanent marker on the side of the first tube can help prevent mis-orienting.

- Samples are now ready for analysis in a GeneCount™ Q-8, Q-16, or Q-96 device.

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SECTION 3: Analysis on Q-Series Device

REQUIRED

- Q-8 or Q-16 qPCR Device
- GeneCount™ Software

OVERVIEW

The below procedure is based on using a GeneCount Q-8 device to run up to 7 samples plus a negative control or a GeneCount Q-16 to run up to 15 samples plus a negative control.

If using a GeneCount Q-96, please contact LuminUltra for alternative instructions.

GETTING STARTED

- Please visit https://www.luminultra.com/genecount/software to download the latest GeneCount™ instrument software
- Plug in qPCR Device to power outlet.
- Connect qPCR Device to computer via USB cable.
- Power on qPCR Device
- Open GeneCount™ software

Warning ⚠

When operating or performing work on the device, all relevant PPE guidelines should be followed taking special care to protect oneself and others from potentially contagious material.

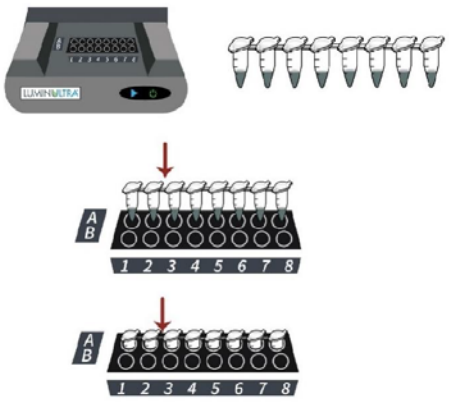
Note: Please read the GeneCount Q8 or Q-18 equipment manual for more instructions on safety and how to operate the qPCR Device.

qPCR Device precautionary Labels ⚠

	**This symbol indicates a safety alert.** Obey all safety messages that follow this symbol to avoid potential injury. If on the instrument, refer to the instruction manual for operation or safety information.
	**This symbol indicates an aperture from which potentially harmful light is emitted.** Do not peer into the LED light aperture/s.
	**This symbol indicates a potentially hot surface.** Avoid touching the marked surface when putting samples in or when lid is open

INITIAL SETUP

- Open latch on the front of the device and lift up lid gently.
- Place PCR strip tubes inside device, noting the coordinates of each sample.



- Close lid firmly until the latch is engaged.

SOFTWARE SETUP

- Chose "New Experiment" to start a new experiment or "Choose Template" if a template file of the experiment is already saved.

  Note: Check the top of the screen to confirm that the qPCR Device is connected (indicator = green). If it is not, close software, reconnect the qPCR Device, and reopen software.

- Enter in experiment name and all sample data in the corresponding sections by double clicking the appropriate box.

Field	Description
Name	Identity of the sample you are testing
Type	**Unknown:** The environmental sample you are testing **Negative control:** The assay mixed with **Nuclease-Free Water** **Positive control:** The assay mixed with the optional **Positive Control**
Quantity	Approximate volume of sample processed – for this method 1 mL. For the positive and negative control enter "20 µL"
Units	Use mL for liquid samples.
Extraction Method	This method is classified as a Lab Extraction.
Assay	Choose 'SARS-CoV-2 Advanced'

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- Click "Continue setup..." to view the program parameters.

  Note: These parameters have been pre-calibrated to suit the qPCR assay being run and do not need to be adjusted.

- Click "Start" and the qPCR program will begin.
- Upon completion of qPCR run, analyze data according to manufacturer's instructions to determine Ct values. Interpret data as outlined in below two tables.

SECTION 4: Result Interpretation

- Upon completion of qPCR run, analyze data to determine Ct values. Interpret data as outlined in below two tables.

Expected Performance of Controls

Control Type	SARS-CoV-2	Internal Control
Positive (Optional)	PASS	PASS or FAIL*
Negative	PASS	PASS

*Positive controls or positive samples may result in the internal control not amplifying properly. This is normal. The internal control is only important for negative controls and negative samples.

Interpreting Sample Results

SARS-CoV-2	Internal Control	Result
DETECTED	PASS or FAIL*	SARS-CoV-2 Detected
BELOW DETECTION LIMIT	PASS	SARS-CoV-2 Not Detected
BELOW DETECTION LIMIT	FAIL	Invalid

Quantitative results will be provided in test results and expressed as units of copies/mL.

TROUBLESHOOTING

Issue	Recommendation
I would like to process a different sample type than that recommended for this test kit.	Please contact LuminUltra to discuss your sample type. Additional procedures and test kits are available.
The negative control came up positive for SARS-CoV-2.	Try re-running the assay in a cleaner location and keep all qPCR reagents separate from the extraction process.
The positive control was not detected.	1. Check the assay file to see that the correct microbe was chosen from the "Assay" drop down menu. 2. Ensure that the positive control is being stored properly 3. Check to see if the positive control has expired.
A sample that came up negative for SARS-CoV-2 also showed a failed internal control.	The sample may contain residual inhibitors: 1. Verify that the RNA extraction procedure was followed correctly 2. Dilute extracted RNA with water 1:5 or 1:10 and rerun the qPCR..

Note: All reagents used in this test have a 12-month shelf life.

Note: For Research Use Only. Not for use in human or veterinary diagnostic procedures.

ORDERING INFORMATION

- **LuminUltra Technologies Ltd.**
  520 King Street, Fredericton, NB,
  Canada, E3B 6G3
- **LuminUltra Technologies Inc.**
  1448 South Rolling Road, Suite 018,
  Baltimore, MD, USA, 21227
- **LuminUltra Technologies SAS**
  Paris Montparnasse Business Centre
  140 bis rue de Rennes,75006 Paris

  Tel: +1 (506) 459 8777

LUMINULTRA microbial monitoring

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Appendix C: Syllabus for Initial Training Sessions

Hach SARS-CoV-2 Training

Overview: Good Lab Practice (GLP) / Sampler / qPCR Analysis

GLP:

1. Use of PPE
2. Clean the environment
3. Use care when handling Influent wastewater
    a. COVID may be present
    b. Influent contains many pathogens

Sampler (further discussions on programming during qPCR analysis):

1. Where to collect the most representable sample
2. How many samples going to be analyzed a week?

qPCR:

1. Unpack reagents and accessories
    a. Discuss how each item is used and its intent
    b. Prepare reagents needed for the analysis
        i. Master Mix (add 825 µL nuclease-free water)
        ii. Supplement 1A (add 6.6 mL nuclease-free water)
        iii. Cleaning Solution 1 (add 60 mL isopropanol)
        iv. Cleaning Solution 2 (add 160 mL of ethanol)
2. Qualify system
    a. Review GeneCount software
    b. Analyze negative and positive control without extraction to qualify system
3. Perform sample extraction with blank / sample / spikes
    a. Trainees perform all steps for multiple samples
    b. Trainees setup GeneCount for analysis
4. Review results from negative and positive controls
5. During the qPCR analysis: Sampler programming configuration and operation
6. Review GeneCount results from sample analysis

Recap & questions

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Appendix D: Summary of Quality Controls for GeneCount Analysis of SARS-CoV-2 in Wastewater

Copy of instructions provided to testing staff in CA in response to uncertainty about the GeneCount quality control metrics

Quality Control	Purpose	How to Implement	How to Label Sample in GeneCount	Expected Performance
Negative control	Verify no contamination is present and prevent reporting of false positives	Dispense 5 µL of *Nuclease-Free Water* into a PCR tube with the Master Mix already in it. Usually, the negative control is placed into the first PCR tube of the strip of 8 tubes.	Name: NC Assay: SARS-CoV-2 Advanced/AUTO Type: "Unknown" (or "NC") Extraction Method: Lab Method Quantity: 1 mL (or 20 uL if using "NC")	SARS-CoV-2: PASS Internal Control: PASS
Positive control	Confirm that reverse transcription and/or PCR reactions are proceeding normally and prevent reporting of false negatives	Dispense 5 µL of the *Positive Control DNA** into a PCR tube with Master Mix already in it. Usually, the positive control is placed into the last PCR tube in a strip of 8 tubes.	Name: PC Assay: SARS-CoV-2 Advanced/AUTO Type: "Unknown" (or "PC") Extraction Method: Lab Method Quantity: 1 mL (or 20 uL if using "PC")	SARS-CoV-2: PASS Internal Control: PASS or FAIL**
Matrix spike	Confirm that there is no interference from the wastewater matrix with reverse transcription and/or PCR reactions	Dispense 10 µL of the *Positive Control DNA* into 1 mL of unconcentrated wastewater already in a 15 mL conical tube. Add 6 mL of the rehydrated *Lysis Buffer* and 250 µL of the rehydrated *Lysis Supplement 1A,* and then proceed with treating this spiked sample as a regular sample and take it through all the concentration and extraction steps. One matrix spike can be run with each set of samples per week.	Name: Spike Assay: SARS-CoV-2 Advanced/AUTO Type: "Unknown" Extraction Method: Lab Method Quantity: 1	SARS-CoV-2: PASS Internal Control: PASS or FAIL**

Notes:

* To make the *Positive Control DNA*, open a Positive Control pouch and remove the vial. Add 50 µL of *Nuclease Free Water* to the Positive Control vial and mix well and let sit for 15 minutes until it's turned to a clear color (instead of orange). This rehydrated Positive Control can be stored in the refrigerator or freezer for use in subsequent weeks.

** Positive controls or positive samples may result in the internal control not amplifying properly. This is normal. The internal control is only important for negative controls and negative samples.

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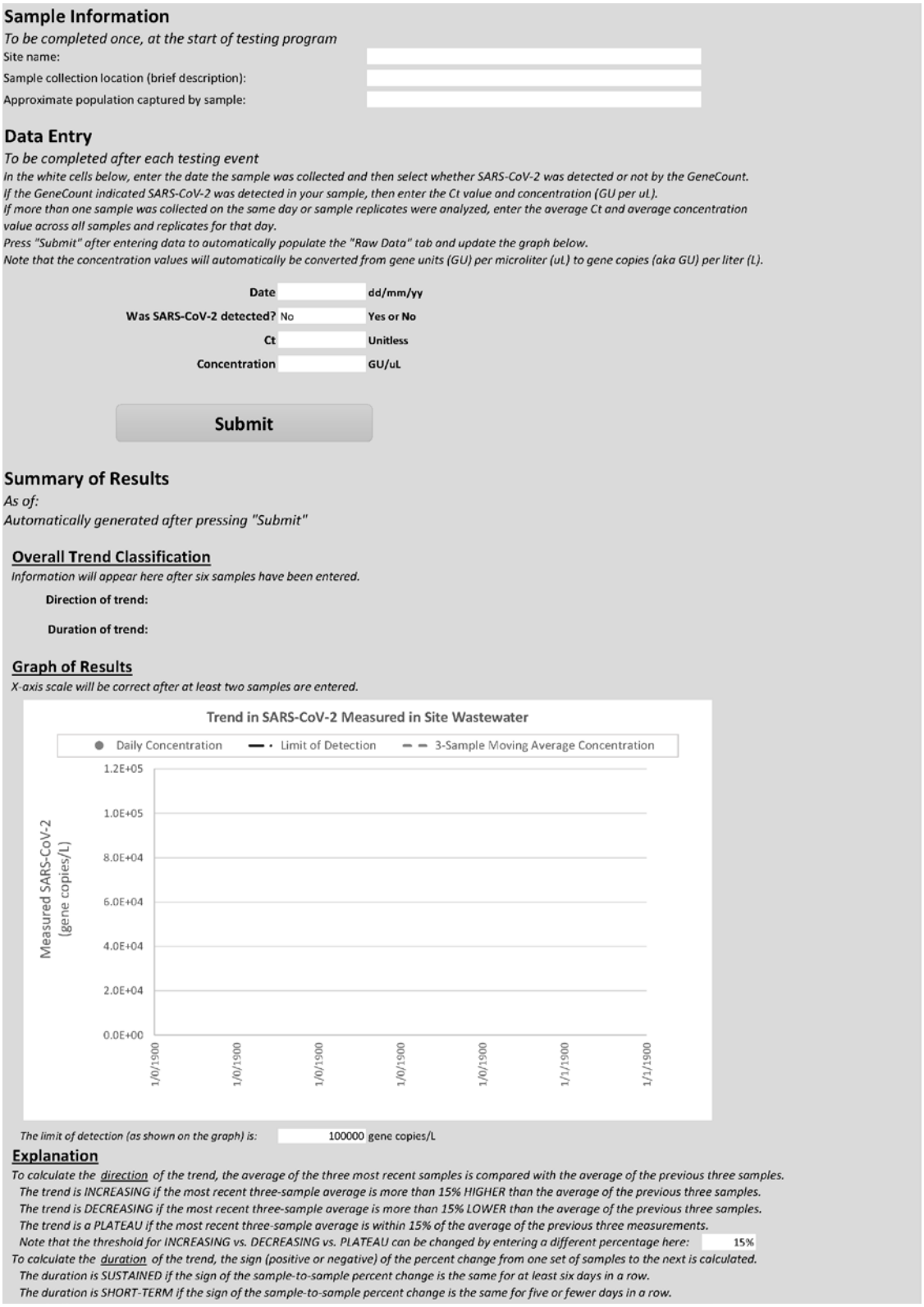
Appendix E: Facility Data Tracking Tools

Screenshot of the file input spreadsheet



Sample Information

To be completed once, at the start of testing program

Site name:

Sample collection location (brief description):

Approximate population captured by sample:

Data Entry

To be completed after each testing event

In the white cells below, enter the date the sample was collected and then select whether SARS-CoV-2 was detected or not by the GeneCount.
If the GeneCount indicated SARS-CoV-2 was detected in your sample, then enter the Ct value and concentration (GU per uL).
If more than one sample was collected on the same day or sample replicates were analyzed, enter the average Ct and average concentration value across all samples and replicates for that day.
Press "Submit" after entering data to automatically populate the "Raw Data" tab and update the graph below.
Note that the concentration values will automatically be converted from gene units (GU) per microliter (uL) to gene copies (aka GU) per liter (L).

Date		**dd/mm/yy**
Was SARS-CoV-2 detected?	No	**Yes or No**
Ct		**Unitless**
Concentration		**GU/uL**

Submit

Summary of Results

As of:
Automatically generated after pressing "Submit"

Overall Trend Classification

Information will appear here after six samples have been entered.

Direction of trend:

Duration of trend:

Graph of Results

X-axis scale will be correct after at least two samples are entered.

The limit of detection (as shown on the graph) is: 100000 gene copies/L

Explanation

To calculate the direction of the trend, the average of the three most recent samples is compared with the average of the previous three samples.
The trend is INCREASING if the most recent three-sample average is more than 15% HIGHER than the average of the previous three samples.
The trend is DECREASING if the most recent three-sample average is more than 15% LOWER than the average of the previous three samples.
The trend is a PLATEAU if the most recent three-sample average is within 15% of the average of the previous three measurements.
Note that the threshold for INCREASING vs. DECREASING vs. PLATEAU can be changed by entering a different percentage here: 15%
To calculate the duration of the trend, the sign (positive or negative) of the percent change from one set of samples to the next is calculated.
The duration is SUSTAINED if the sign of the sample-to-sample percent change is the same for at least six days in a row.
The duration is SHORT-TERM if the sign of the sample-to-sample percent change is the same for five or fewer days in a row.

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Manual data logging sheet

Oklahoma Correctional Facilities COVID-19 Wastewater Surveillance Pilot Data Log

Facility: _____

Instructions:

For each day of sample collection and analysis, enter the date in Column 1 and the measured SARS-CoV-2 concentration in Column 2. If no SARS-CoV-2 was detected, record "Below LOD". If more than one sample was tested for the same day, record the average of the results for all samples tested for the same day of collection.

Calculate the percent change in SARS-CoV-2 concentration for the new day of sampling relative to the previous day of sampling.

If the answer to either of the questions in the last two columns is "Yes" for a given day of sampling, this indicates a new or worsening COVID-19 outbreak in the facility.

1	2	3	4	5	6
Date	**SARS-CoV-2 Concentration Measured in Wastewater (gene copies per mL)**	**Did All QC Pass?**	**Percent Change in SARS-CoV-2 Concentration from Previous Sample (calculated)**	**Is this the first sample with detectable SARS-CoV-2 out of the previous three samples?**	**Is this sample the third sample in a row showing a positive percent change in the SARS-CoV-2 concentration?**

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Guide on How to Interpret GeneCount Output for Wastewater SARS-CoV-2 Testing

Prepared by Anna Mehrotra, WEF

General important notes to remember:

1. The GeneCount uses "GU" (or gene units). This is the same as "gene copies", which is more commonly used. We use "gene copies" below.
2. The limit of detection for the method is 50,000 gene copies per L.
3. The limit of quantification for the method is 100,000 gene copies per L.

Steps to extracting the wastewater concentration data:

Leave the GeneCount software open after the PCR run, navigate to the "Results" tab and follow the steps below. The screenshot on the next page shows an example from a recent run.

1. For the Positive Control (PC) and Negative Control (NC), look at the "Result" column to make sure that:
   (a) The PC results show that SARS-CoV-2 was detected (in Channel 1) and the internal control passed (in Channel 4)
      - The example below shows that the PC sample in well B6 was detected and that the internal control passed. *Therefore, the PC passed!*

   (b) And the NC results show that no SARS-CoV-2 was detected and the NC internal control passed
      - The example below shows that the NC sample in well A1 (which is probably just mislabeled as PC) was a non-detect for SARS-CoV-2 and the internal control passed. *Therefore, the NC passed!*
2. If both the PC and NC passed, then for each site wastewater sampled:
   (a) Check the "Result" column of the sample spike (if any) to make sure SARS-CoV-2 was detected and the spike internal control passed
      - The example below shows that SARS-CoV-2 was detected in both WA-1 spikes in wells A4 and A5, and that both spikes passed the internal quality control; the concentration for the first WA-1 spike was 3.13 x 10^1 gene copies per mL (or 31,300 gene copies per L) and the concentration for the second WA-1 spike was 4.60 x 10^1 gene copies per mL (or 46,000 gene copies per L). Both internal controls for the spikes passed. *Therefore, the spike control passed!*

   (b) Check the "Result" column of the sample itself to see if SARS-CoV-2 was detected and, if so, what the concentration is.
      i. If a number is reported in the "Concentration" column for both sample duplicates, multiple each number by 1,000 (to convert to gene copies per liter), take the average of the two values, and report the result (in gene copies per liter) over the phone to E—explaining that both duplicates were above the limit of detection.
      ii. If both samples are reported as "< LOD" in the "Concentration" column, then there is no need to alert E.
      iii. If one sample is reported as "< LOD" and one has a concentration in the "Concentration" column, then multiply the one number by 1,000 (to convert to gene copies per liter), and average that number with 50,000 and report the averaged number to E over the phone, explaining that one duplicate was below the limit of detection and one was detected.
      - The example below shows that one of the duplicate WA-1 samples for 11/29/21 was "< LOD" and one was reported as 5.33 x 10^1 GU/mL. If we convert the numerical value to 53,300 gene copies per L and average that with 50,000 gene copies per L, we get an overall result for WA-1 of 51,650 gene copies per L on 11/29/21. Also, SARS-CoV-2 was not detected in the 11/18/21 WA-2 or 11/18/21 WA-5 samples. *Therefore, the results for WA-1 should be reported to E over the phone as 51,650 gene copies per L for WA-1 on 11/29/21, with an explanation that only one of the duplicate results was above the limit of detection. Results for WA-2 and WA-5 do not need to be reported verbally to E, although you can explain that they were all below the limit of detection because you'll need to call anyway.*
3. If there were issues with quality control (the PC and/or NC controls didn't pass and/or the spike was not detected), it's probably a good idea to report any detectable sample results to E anyway – but explain that not all the quantity control metrics were met.

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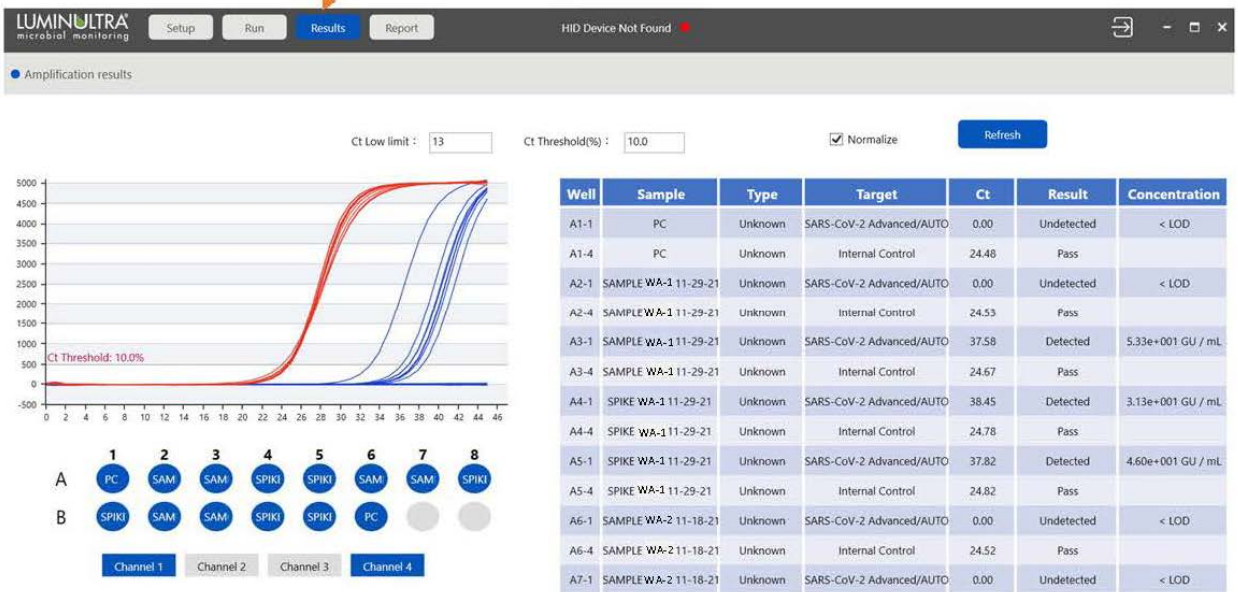
Example from "Experiment WA-1,WA-2,WA-5_11-30-2021_105541.json" file

Go to "Results" tab if you aren't already there and follow the steps on the first page



Well	Sample	Type	Target	Ct	Result	Concentration
A1-1	PC	Unknown	SARS-CoV-2 Advanced/AUTO	0.00	Undetected	< LOD
A1-4	PC	Unknown	Internal Control	24.48	Pass	
A2-1	SAMPLE WA-1 11-29-21	Unknown	SARS-CoV-2 Advanced/AUTO	0.00	Undetected	< LOD
A2-4	SAMPLE WA-1 11-29-21	Unknown	Internal Control	24.53	Pass	
A3-1	SAMPLE WA-1 11-29-21	Unknown	SARS-CoV-2 Advanced/AUTO	37.58	Detected	5.33e+001 GU / mL
A3-4	SAMPLE WA-1 11-29-21	Unknown	Internal Control	24.67	Pass	
A4-1	SPIKE WA-1 11-29-21	Unknown	SARS-CoV-2 Advanced/AUTO	38.45	Detected	3.13e+001 GU / mL
A4-4	SPIKE WA-1 11-29-21	Unknown	Internal Control	24.78	Pass	
A5-1	SPIKE WA-1 11-29-21	Unknown	SARS-CoV-2 Advanced/AUTO	37.82	Detected	4.60e+001 GU / mL
A5-4	SPIKE WA-1 11-29-21	Unknown	Internal Control	24.82	Pass	
A6-1	SAMPLE WA-2 11-18-21	Unknown	SARS-CoV-2 Advanced/AUTO	0.00	Undetected	< LOD
A6-4	SAMPLE WA-2 11-18-21	Unknown	Internal Control	24.52	Pass	
A7-1	SAMPLE WA-2 11-18-21	Unknown	SARS-CoV-2 Advanced/AUTO	0.00	Undetected	< LOD
A7-4	SAMPLE WA-2 11-18-21	Unknown	Internal Control	24.07	Pass	
A8-1	SPIKE WA-2 11-18-21	Unknown	SARS-CoV-2 Advanced/AUTO	37.06	Detected	7.33e+001 GU / mL
A8-4	SPIKE WA-2 11-18-21	Unknown	Internal Control	24.74	Pass	
B1-1	SPIKE WA-2 11-18-21	Unknown	SARS-CoV-2 Advanced/AUTO	37.09	Detected	7.23e+001 GU / mL
B1-4	SPIKE WA-2 11-18-21	Unknown	Internal Control	24.76	Pass	
B2-1	SAMPLE WA-5 11-18-21	Unknown	SARS-CoV-2 Advanced/AUTO	0.00	Undetected	< LOD
B2-4	SAMPLE WA-5 11-18-21	Unknown	Internal Control	24.77	Pass	
B3-1	SAMPLE WA-5 11-18-21	Unknown	SARS-CoV-2 Advanced/AUTO	0.00	Undetected	< LOD
B3-4	SAMPLE WA-5 11-18-21	Unknown	Internal Control	24.83	Pass	
B4-1	SPIKE WA-5 11-18-21	Unknown	SARS-CoV-2 Advanced/AUTO	36.35	Detected	1.14e+002 GU / mL
B4-4	SPIKE WA-5 11-18-21	Unknown	Internal Control	24.80	Pass	
B5-1	SPIKE WA-5 11-18-21	Unknown	SARS-CoV-2 Advanced/AUTO	37.55	Detected	5.45e+001 GU / mL
B5-4	SPIKE WA-5 11-18-21	Unknown	Internal Control	24.87	Pass	
B6-1	PC	Unknown	SARS-CoV-2 Advanced/AUTO	33.18	Detected	8.00e+002 GU / mL
B6-4	PC	Unknown	Internal Control	24.70	Pass	

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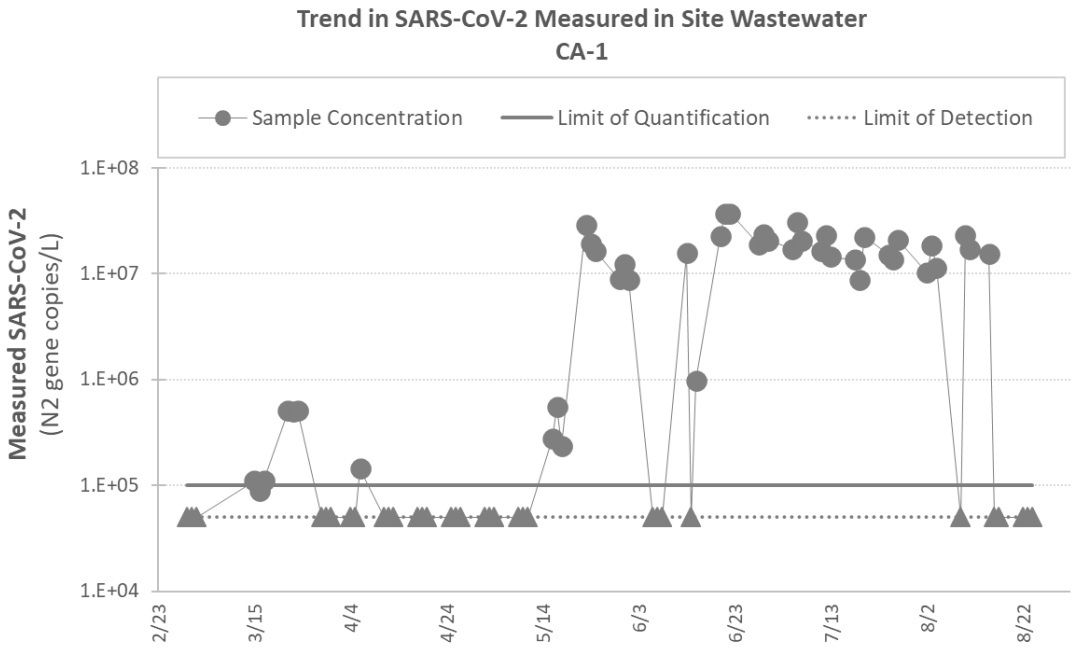
Appendix F: Time Series Plots for SARS-CoV-2 RNA Concentrations at 18 Pilot Facilities

Note that the y-axis scale is identical for all plots except OK-2



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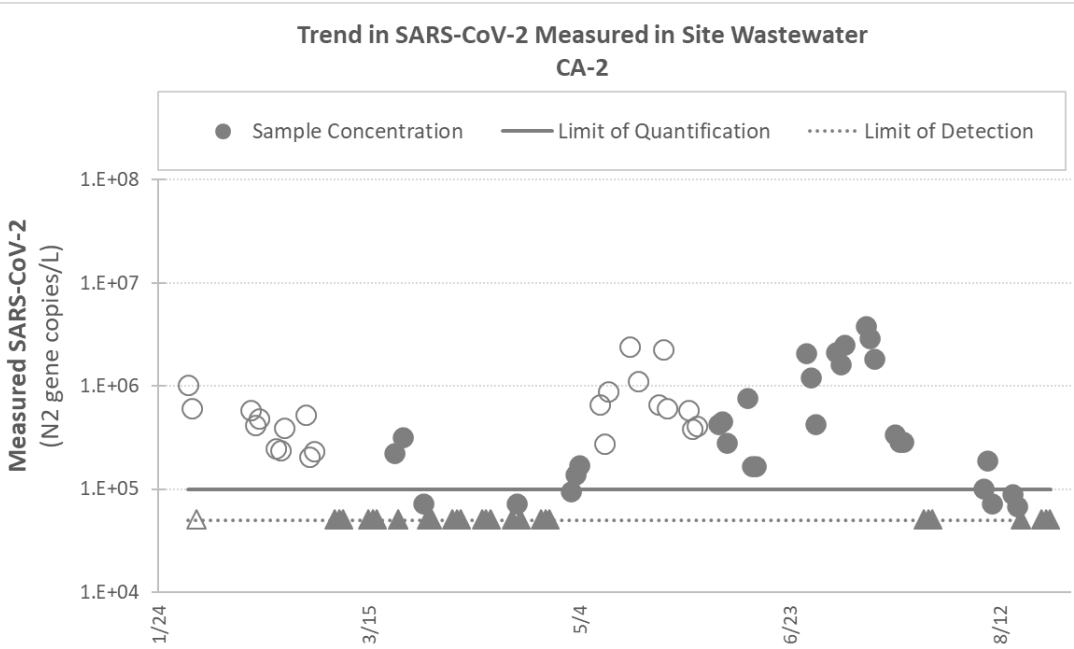

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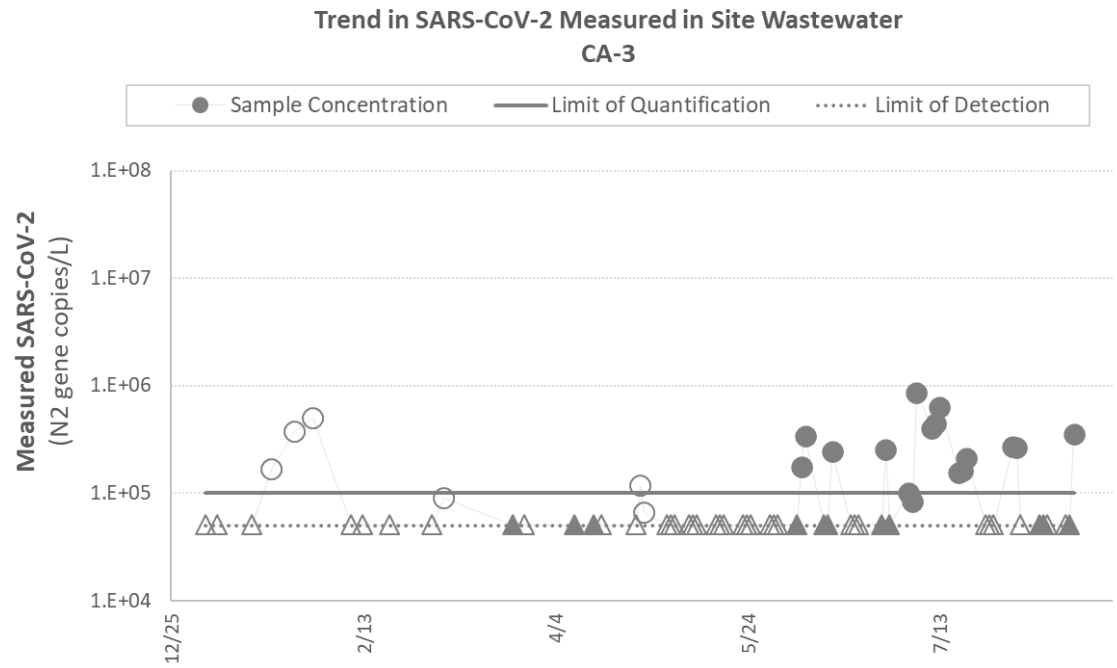

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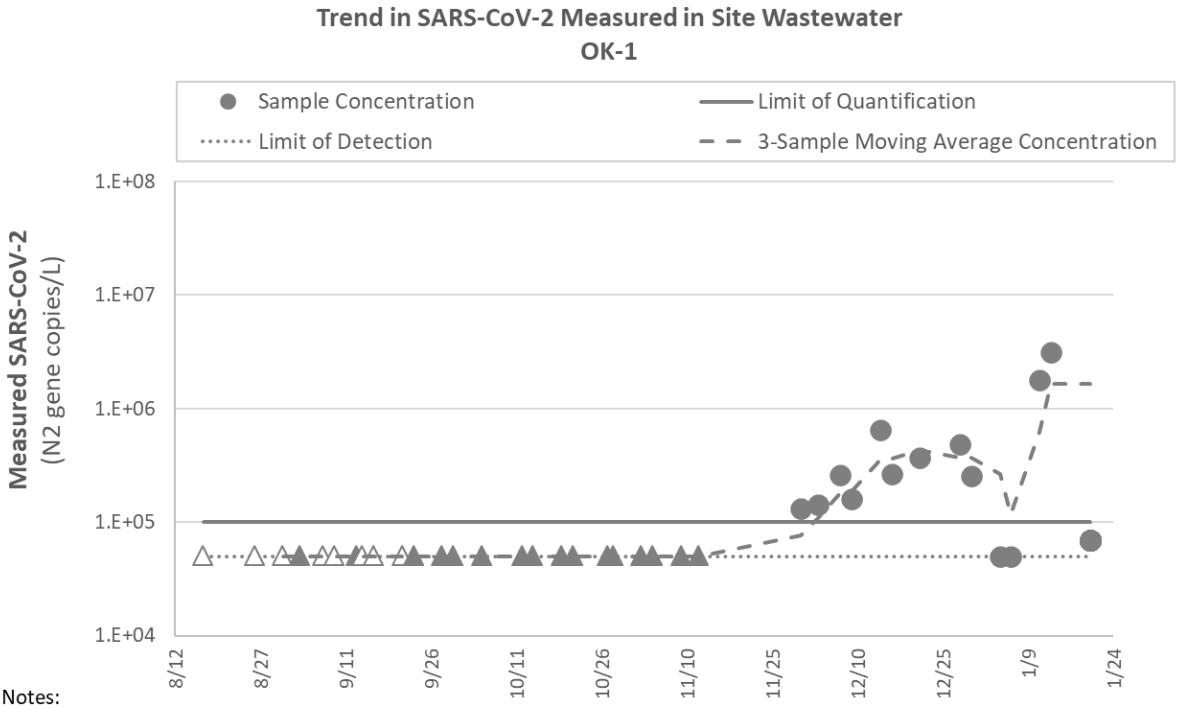

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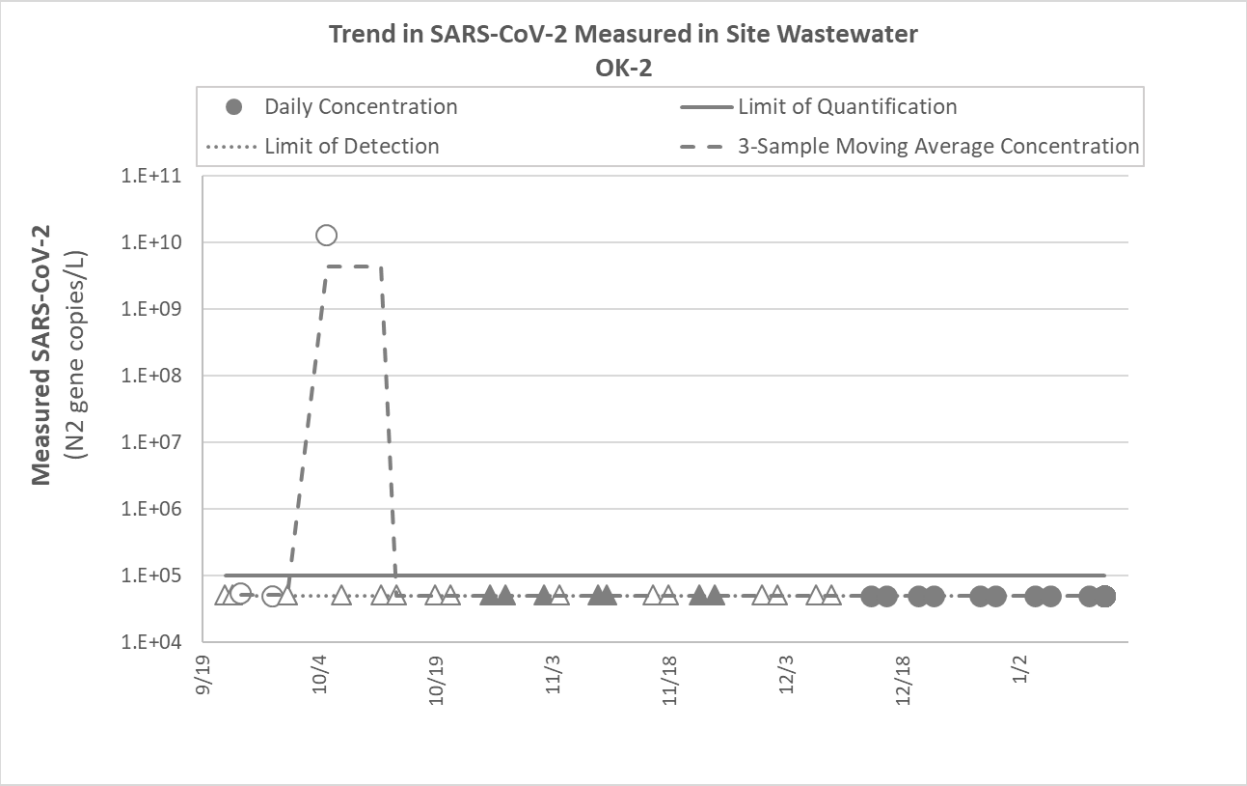

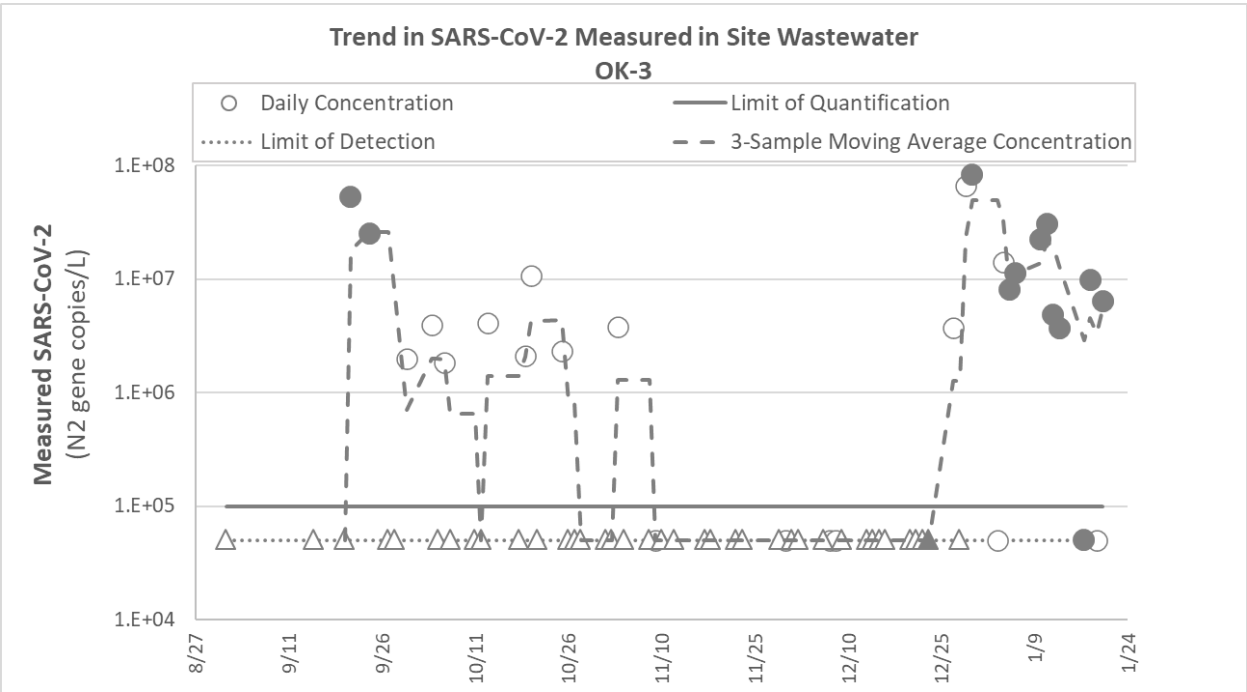

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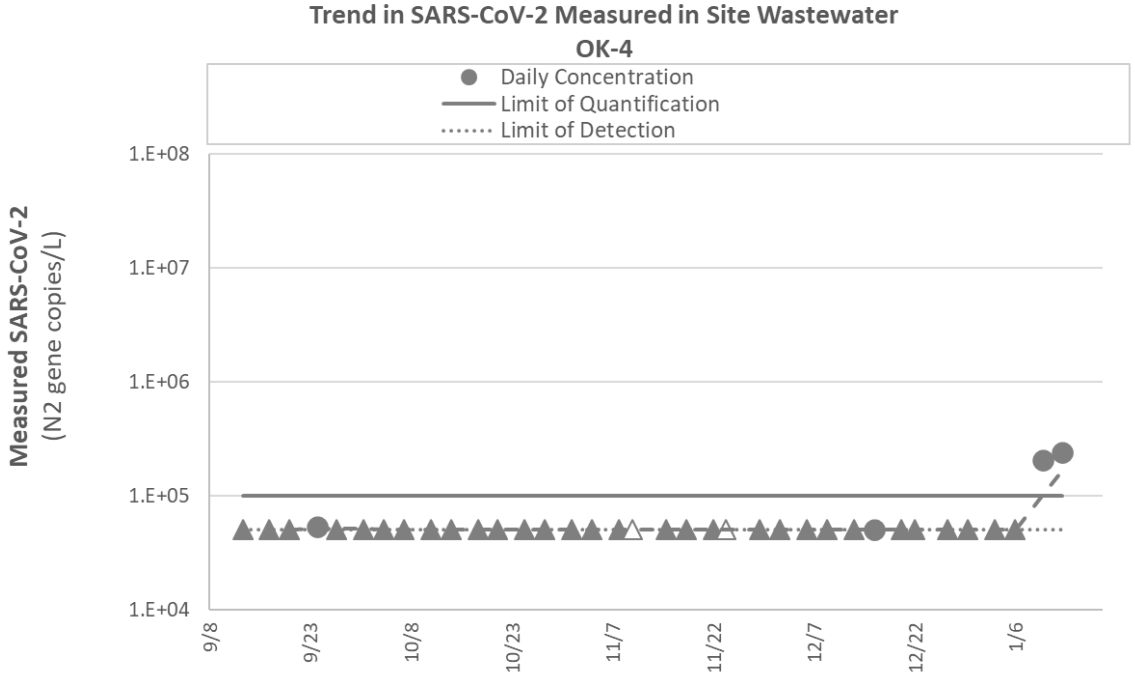

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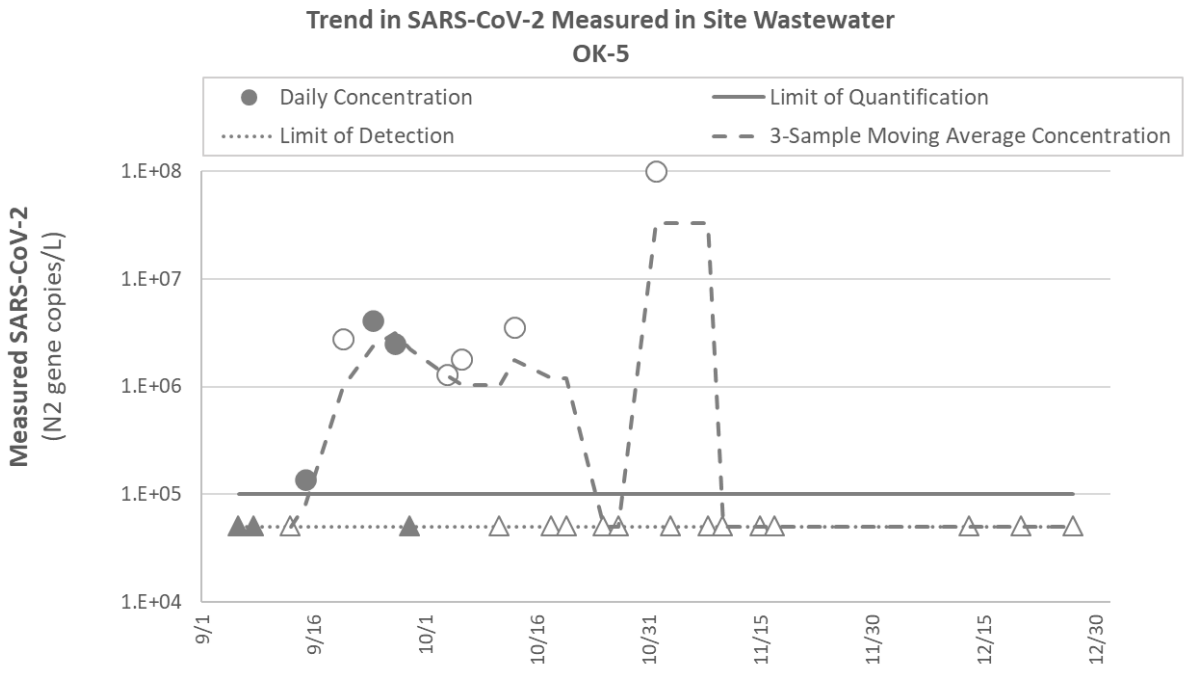

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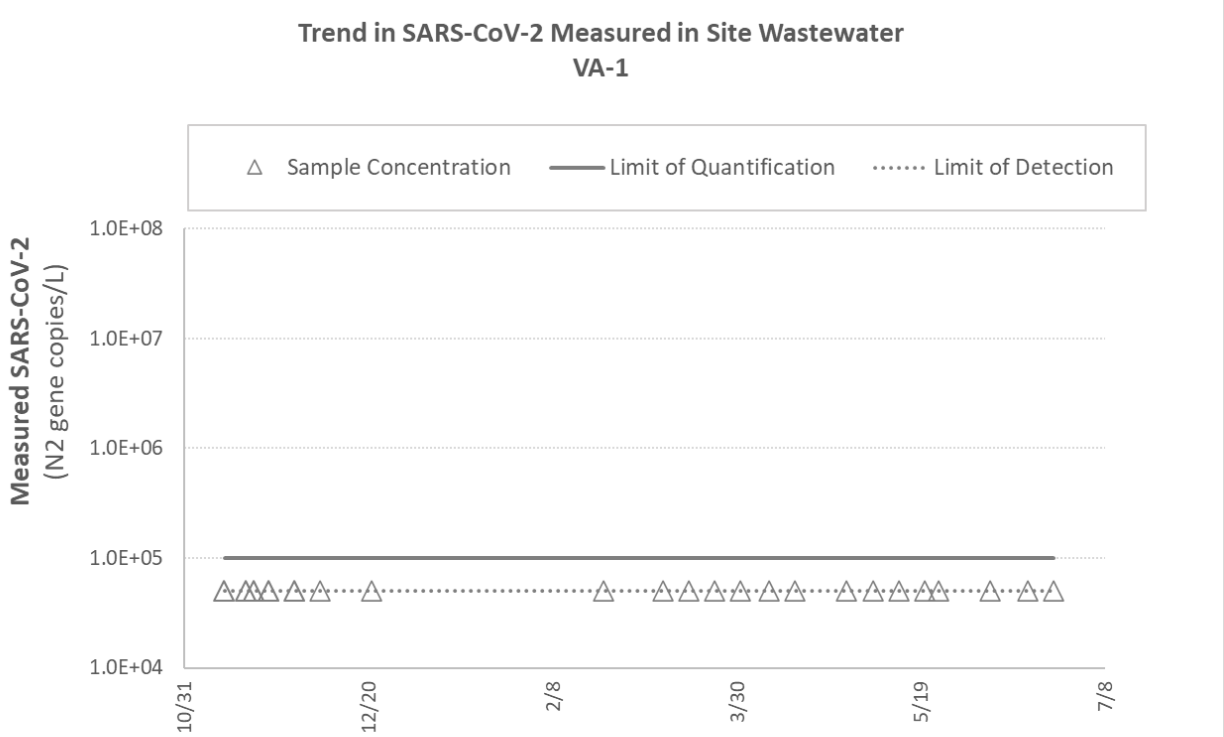

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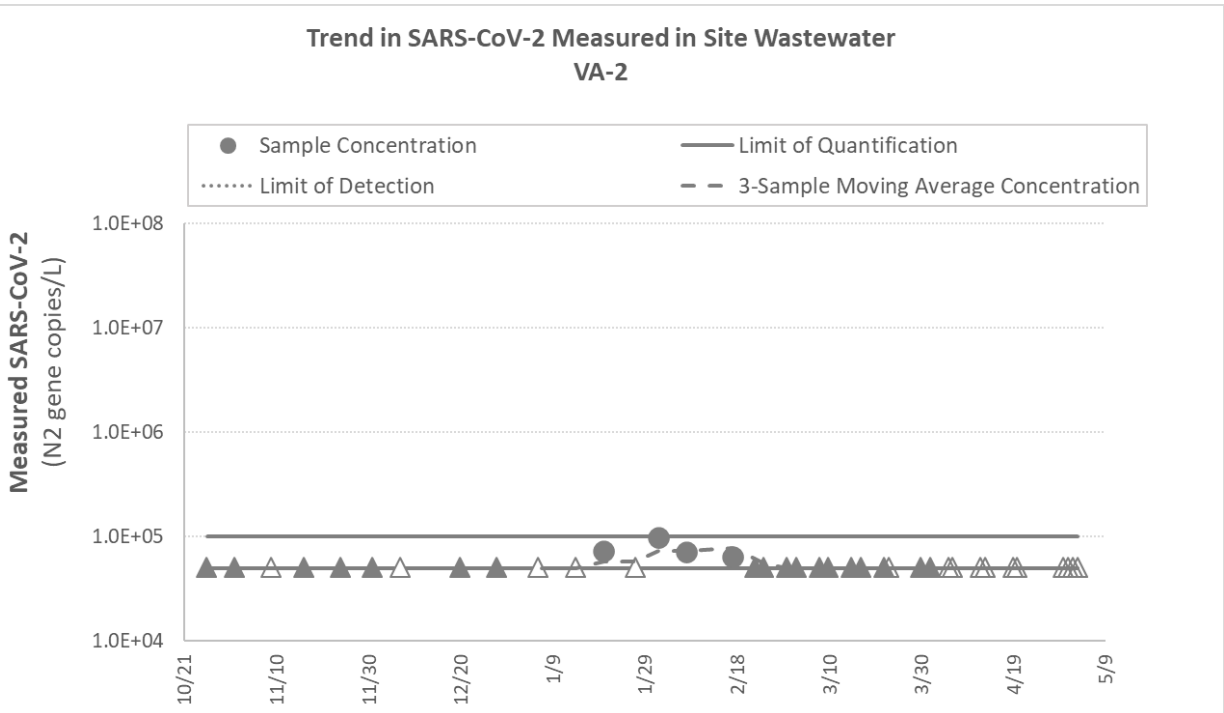

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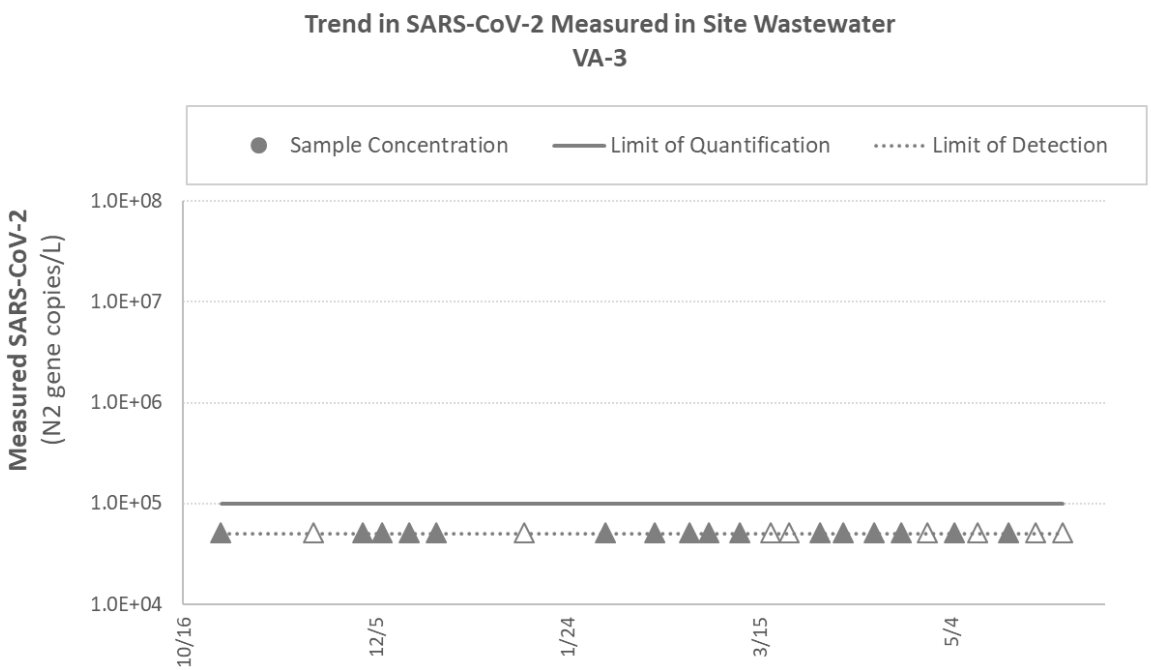

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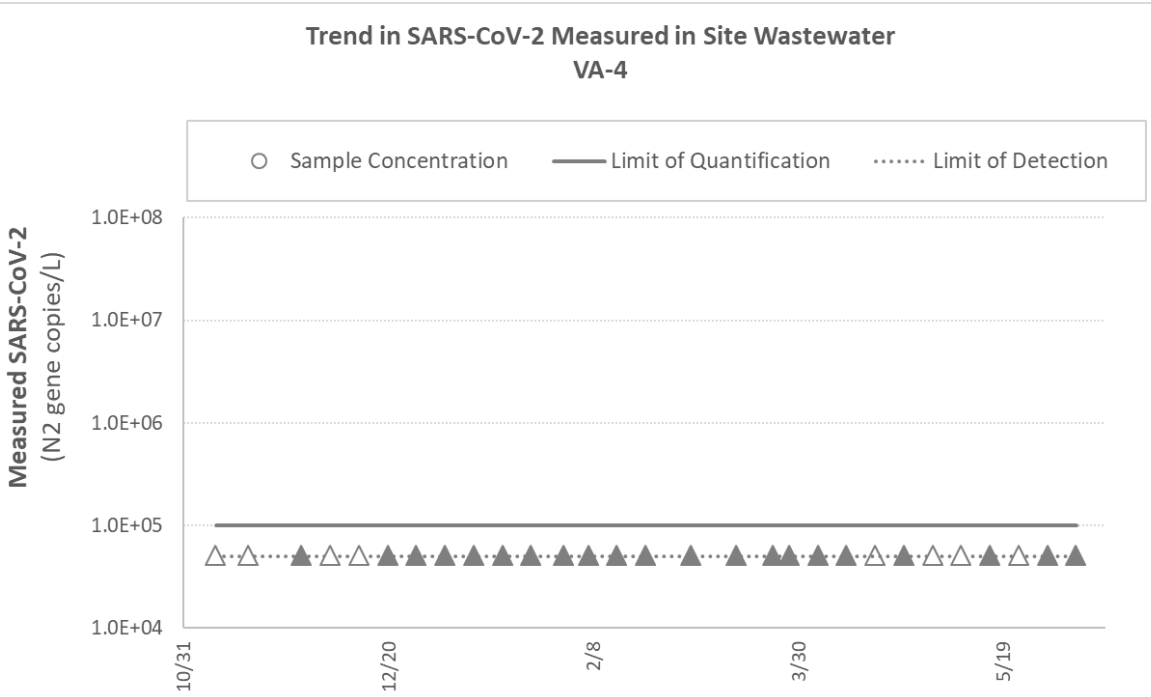

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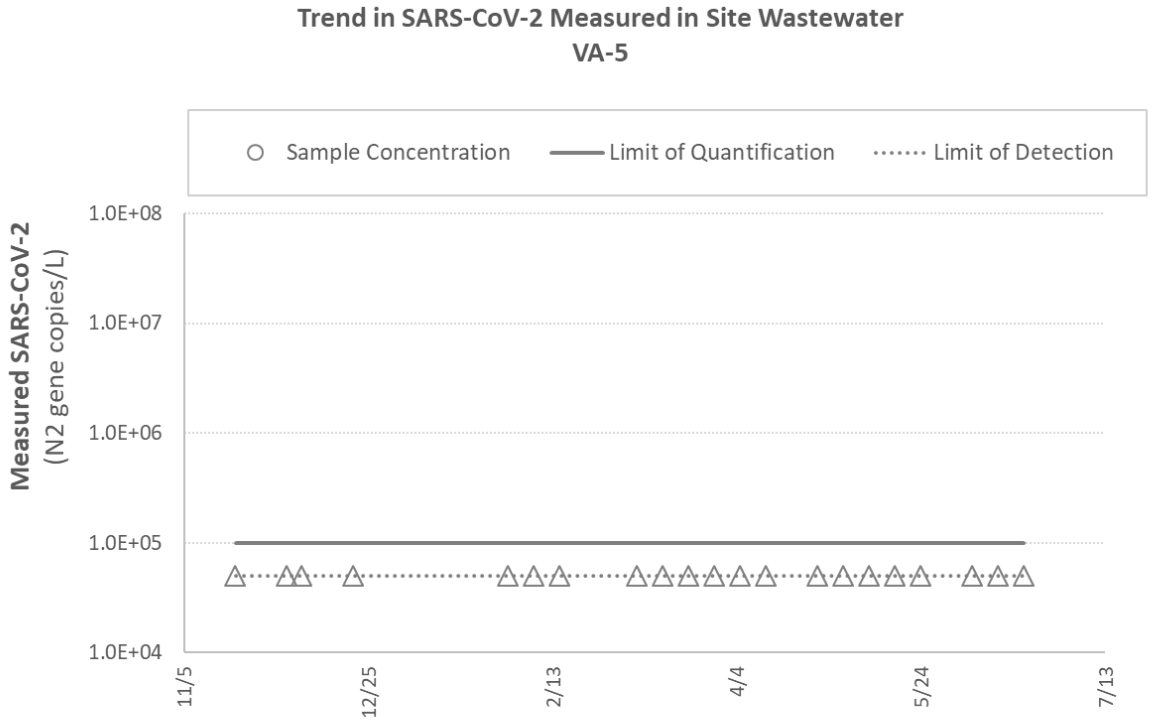

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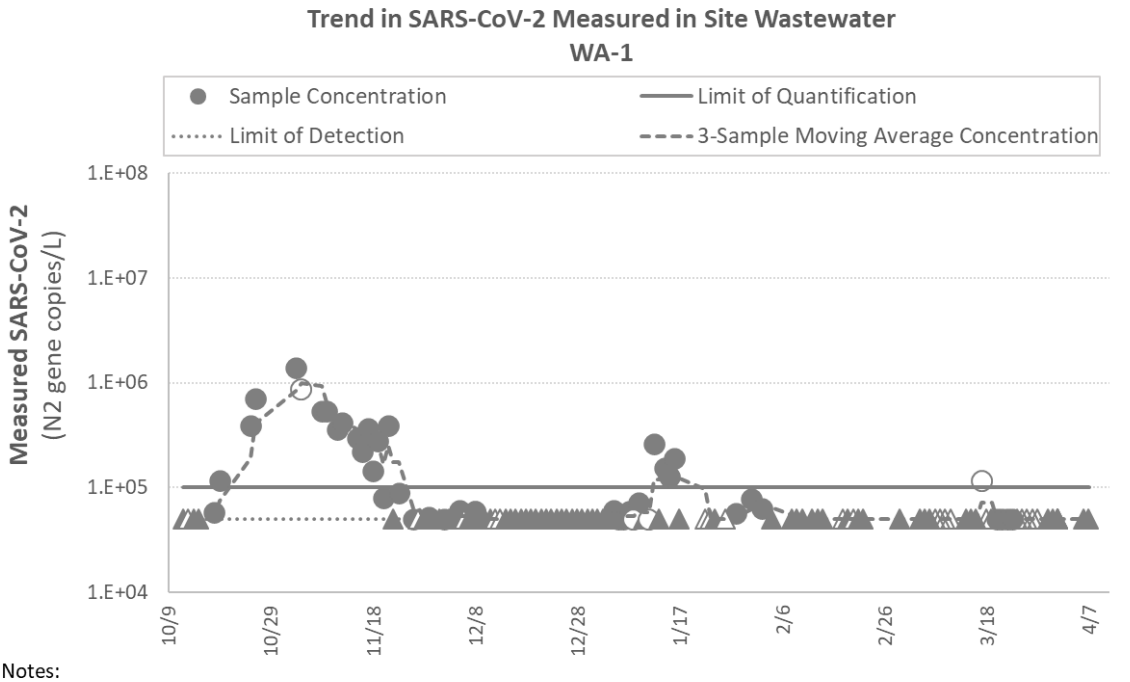

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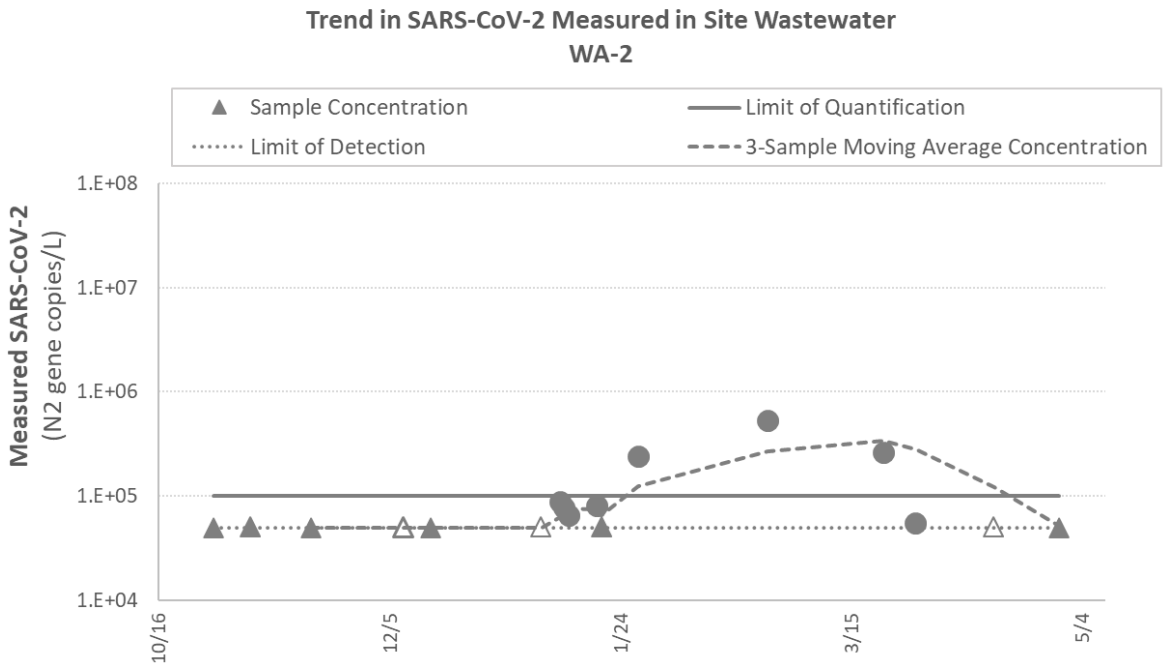

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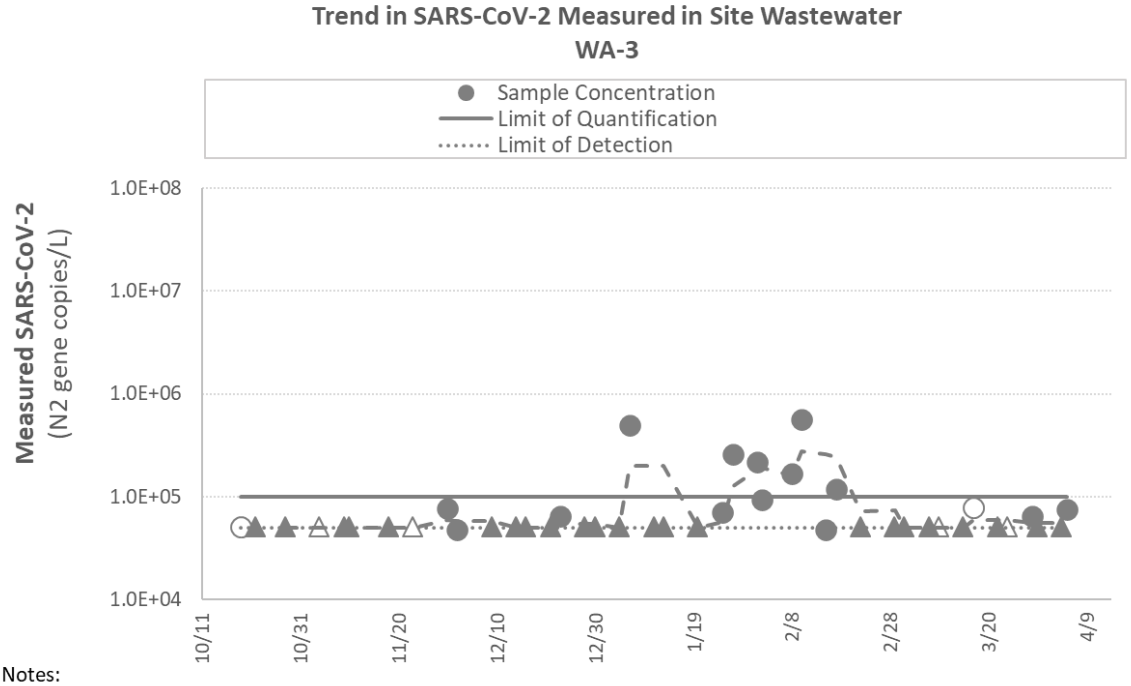

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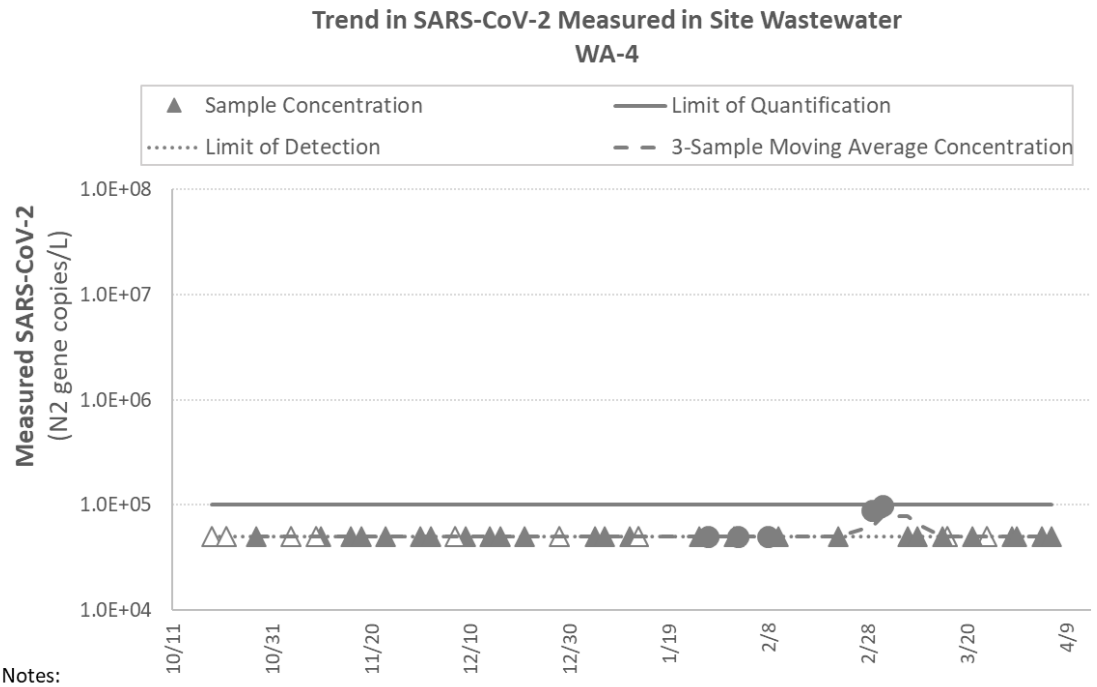

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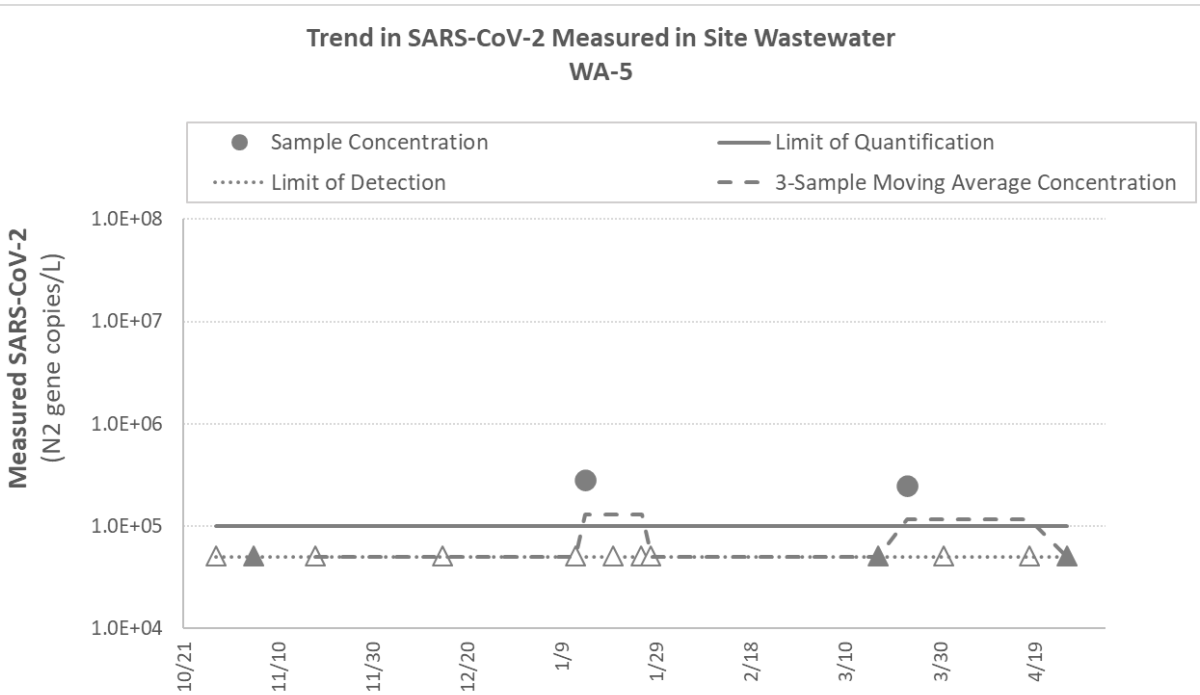

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Appendix G: Protocol for Institutional Wastewater Surveillance Using Onsite Testing

The protocol is provided on the following four pages. The Excel version of the file may be obtained by emailing nwbe@wef.org or going to https://bit.ly/CFProtocol.

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PROTOCOL FOR INITIATING AN INSTITUTIONAL ONSITE WASTEWATER SURVEILLANCE PROGRAM

Version 1.0

Originally published October 26, 2022

Please email nwbe@wef.org with any comments or questions

ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
MoU	Memorandum of Understanding
PCR	polymerase chain reaction
PPE	personal protective equipment
NWSS	National Wastewater Surveillance System
WRRF	water resource recovery facility

PROTOCOL CONTENTS

1. IDENTIFY THE MULTIDISCIPLINARY TEAM AND DEFINE TEAM MEMBER ROLES

1.1 ☐	Consider the different disciplines that should be involved in an onsite wastewater surveillance program, both internally and externally, which will vary by institution and may include some or all of the following:
1.1.1 ☐	Institutional administrative staff
1.1.2 ☐	Utilities staff, including WRRF operators if applicable
1.1.3 ☐	Those conducting the sampling (if different than WRRF operators)
1.1.4 ☐	Those conducting the analysis (if different than WRRF operators)
1.1.5 ☐	Public health and clinical staff
1.1.6 ☐	Local and/or state health department representatives
1.1.7 ☐	Technical support, possibly from the testing technology supplier (note: testing technology will be determined in step 3 below)
1.1.8 ☐	Other: _____
1.2 ☐	Identify the following team members, noting that one person may serve as the sampling, analytical testing, and data lead but that the project manager and health lead will likely be distinct people:
1.2.1 ☐	Project manager: responsible for overall program management, overseeing procurement of equipment and supplies, coordinating regular check-in meetings, and monitoring conformance with plans and protocols
1.2.2 ☐	Sampling lead: responsible for leading development of the sampling plan, identifying individuals responsible for ongoing sample collection, and ensuring samples are collected on a regular basis
1.2.3 ☐	Analytical testing lead: response for leading testing the technology selection and lab analysis protocol development, identifying individuals responsible for ongoing sample analyis, and ensuring sample testing conforms with the protocol
1.2.4 ☐	Data lead: responsible for leading the development of the data processing and sharing protocol and ensuring data are shared in a timely manner with the project team
1.2.5 ☐	Health lead: responsible for leading the development of the public health and clinical action protocols and ensuring wastewater data are used for public health action throughout the program
1.3 ☐	Document roles and responsibilities of each of the team members
1.4 ☐	Consider writing MoUs for interorganizational cooperation so that all partners understand their responsibilities

2. IDENTIFY PUBLIC HEALTH DATA NEEDS AND ESTABLISH PUBLIC HEALTH ACTION PROTOCOL

2.1 ☐	Determine which of the following wastewater surveillance will be used for (note that the program can be designed to achieve both):
2.1.1 ☐	Detect the presence of disease within the community
2.1.2 ☐	Monitor trends in disease wtihin the community
2.2 ☐	Identify public health actions that can be taken in response to positive wastewater detections and/or increasing wastewater trends
2.2.1 ☐	Communication and outreach to the community
2.2.2 ☐	Instituting masking requirements

Click on this link to read the CDC NWSS program's description of appropriate wastewater surveillance response objectives

2.2.3	☐	Initiating quarantine or isolation
2.2.4	☐	Changing visitation policies
2.2.5	☐	Changing the clinical testing strategy (increase or expand testing or use a faster clinical testing method)
2.2.6	☐	Other: _____
2.3	☐	Identify public health actions that can be taken in response to decreasing wastewater trends (when consistent with clinical data)
2.3.1	☐	Communication and outreach to the community
2.3.2	☐	Removing masking requirements
2.3.3	☐	Lifting quarantine or isolation
2.3.4	☐	Changing visitation policies
2.3.5	☐	Changing the clinical testing strategy (decrease testing frequency)
2.3.6	☐	Other: _____
2.4	☐	Document planned public health actions, or criteria for determining public health actions, in public health action protocol

3. IDENTIFY THE ANALYTICAL TESTING LOCATION

3.1	☐	Is the location safe, secure, and protected from the elements?
3.2	☐	Does the location have a reliable power source?
3.3	☐	Does the location have a sanitizable lab bench with sufficient space to house any benchtop equipment and provide a workspace to conduct the testing?
3.4	☐	Does the location have adequate storage for supplies?
3.5	☐	Does the location have a refrigerator and freezer, if needed? Note: reagent storage temperature requirements will depend on the testing method selected, but a refrigerator is recommended at a minimum for sample storage
3.6	☐	Does the location have a computer with internet access?
3.7	☐	Are there any other location requirements?

4. SELECT THE ONSITE ANALYTICAL TESTING TECHNOLOGY

4.1	☐	Identify constraints that impact testing, including but not limited to:
4.1.1	☐	Budget available for testing
4.1.2	☐	Time availability of those conducting the testing
4.1.3	☐	Space available for testing
4.2	☐	Consider the factors specific to each candidate technology, including but not limited to:
4.1.1	☐	Complexity of method and extent of training required
4.1.2	☐	Availability of technical support
4.1.3	☐	Lead time for equipment and supplies
4.1.4	☐	Sensitivity and reliability of method
4.3	☐	Based on constraints and factors identified above, select onsite testing technology

5. DEVELOP SAMPLING PLAN

5.1	☐	Determine how wastewater samples will be collected, keeping in mind that only composite and grab samples can give quantitative results
5.1.1	☐	Composite samples - mixtures of individual samples collected over a period of time (often 24 hours), facilitated by use of an autosampler
5.1.2	☐	Grab samples - discrete samples collected manually at a single point in time
5.1.3	☐	Passive samples - absoprtive material immersed in the wastewater stream for a predetermined period of time
5.2	☐	Determine where samples will be collected
5.2.1	☐	If you want to capture data from the entire population, then sampling at the WRRF or a single downstream location is suitable
5.2.2	☐	If you want to capture a particular subset of the entire population, then sampling at a building cleanout, manhole or lift station is needed
5.2.3	☐	Ensure there is safe access to the sampling location(s)
5.2.4	☐	Ensure samples will be collected upstream of any chemical addition points at the institution or WRRF

Click on this link to read the CDC NWSS program's description of grab and composite samples
Click on this link to read a description of passive sampling
Click on this link to read the CDC NWSS program's discussion of sampling locations

5.2.5	❑	If quantitative results are desired, then ensure that wastewater flow can be measured or estimated for the sampling location(s)
5.3	❑	If collecting composite samples, select type of autosampler(s) needed
5.3.1	❑	If electricity and adequate space available for secure installation, consider using a refrigerated autosampler
5.3.2	❑	If electricity is unavailable and the autosampler needs to be installed in a challenging location (such as a manhole), consider using a portable autosampler
5.4	❑	Determine sampling frequency
5.4.1	❑	Once per week is considered the minimum useful frequency
5.4.2	❑	Two times per week yields more useful information than once per week
5.4.3	❑	Three times per week enables higher confidence in week-over-week wastewater trends
5.4.4	❑	Other: _____
5.5	❑	Determine where samples will be stored once collected, noting the following sample storage guidance:
5.5.1	❑	Samples should be stored at temperatures no higher than 4°C
5.5.2	❑	Process (that is, concentration and/or analyze) samples within 24-hours, if possible, to avoid sample degradation
5.5.3	❑	Remaining samples can be frozen at -70°C for archiving, but avoid more than one freeze-thaw cycle
5.6	❑	Will samples be archived?
5.7	❑	Ensure those collecting samples are trained to safely handle sewage and have received the recommended vaccinations
5.8	❑	Ensure those collecting samples have access to proper PPE and are trained to use it correctly
5.9	❑	Will metadata be collected? If so, what metadata?
5.10	❑	Document sampling approach in sampling plan

Click on this link to read the CDC NWSS program's discussion of sampling frequency

Click on this link to read the CDC NWSS program's discussion of sampling safety and storage

Click on this link to read the CDC's Guidance for Reducing Health Risks to Workers Handling Human Waste or Sewage

6. DEVELOP LAB ANALYSIS PROTOCOL

6.1	❑	Develop a health and safety plan for the laboratory analyses
6.1.1	❑	Required PPE
6.1.2	❑	Hazards and hazard mitigation
6.1.3	❑	Training required
6.1.4	❑	Other
6.2	❑	Identify individuals who will conduct the analytical testing (primary and backup)
6.3	❑	Develop a training plan, in conjunction with technology provider selected in section 4 above
6.3.1	❑	Depending on method complexity, consider follow-up trainings
6.3.2	❑	Will those conducting the analytical testing have access to troubleshooting support? How will they receive support?
6.4	❑	Train individuals conducting the testing, including backup(s), to conduct analytical testing. In-person training should be prioritized for complex analytical methods.
6.5	❑	Determine the frequency at which the analytical method will be run, considering the following:
6.5.1	❑	Samples should be tested soon after collection so that results can be reported within an actionable time frame, to be determined by team. As noted in 5.2, samples should be processed within 24-hours if possible
6.5.2	❑	If the testing protocol is time consuming, it may be beneficial to batch test samples from a few days to streamline process, if possible, while still meeting data sharing responsibilities (see section 7 below)
6.6	❑	Identify quality control measures that will be used to evaluate data quality
6.6.1	❑	Negative controls
6.6.2	❑	Positive controls
6.6.3	❑	Matrix spikes
6.6.4	❑	Replicates
6.7	❑	Identify all steps in analytical workflow and approximate time required for each
6.7.1	❑	Setup
6.7.2	❑	All analytical steps
6.7.3	❑	Cleanup
6.7.4	❑	Other
6.8	❑	Document lab analytical approach in lab analysis protocol

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Version 1.0

Originally published October 26, 2022

Please email nwbe@wef.org with any comments or questions

7. DEVELOP DATA PROCESSING AND SHARING PROTOCOL

- **7.1** ❑ Develop template for processing and logging data
- **7.2** ❑ Identify individuals responsible for processing the data (primary and backup)
- **7.3** ❑ Identify individual(s) responsible for sharing the data
- **7.4** ❑ Identify which individuals/groups should receive the data
  - 7.4.1 ❑ Administrative staff
  - 7.4.2 ❑ Clinical staff
  - 7.4.3 ❑ Facilities staff
  - 7.4.4 ❑ Local health department
  - 7.4.5 ❑ Residents
  - 7.4.6 ❑ General public
- **7.5** ❑ Identify what format(s) will be used for data sharing (database, spreadsheet, dashboard etc.)
  - 7.5.1 ❑ For internal team members
  - 7.5.2 ❑ For residents
  - 7.5.3 ❑ For the general public
  - 7.5.4 ❑ If a public dashboard will be used, develop a website
- **7.6** ❑ Identify how frequently and on which days the data should be shared
  - 7.6.1 ❑ Data to be shared with internal team every _____
  - 7.6.2 ❑ Data to be shared with general public every _____
- **7.7** ❑ If desired, schedule regular meetings to share results with key team members

8. PROCURE EQUIPMENT AND SUPPLIES

- **8.1** ❑ Develop a list of all required equipment and supplies
  - 8.1.1 ❑ Sampling equipment, including extra collection bottles and parts that are likely to wear out
  - 8.1.2 ❑ Equpment and supplies for storing samples
  - 8.1.3 ❑ Equipment for analytical testing (PCR machine)
  - 8.1.4 ❑ Consumables for analytical testing
  - 8.1.5 ❑ PPE for those conducting sampling and testing
- **8.2** ❑ Identify suppliers for all equipment and supplies
  - 8.2.1 ❑ Ensure all suppliers of equipment and and reagents are on approved supplier list, if applicable
- **8.3** ❑ Order equipment and supplies identified in 8.1
- **8.4** ❑ Develop a plan for re-ordering consumable supplies, considering availability of supplies, shipping time, and constraints specific to the facility, including security procedures
  - 8.4.1 ❑ Based on testing volume and frequency, conservatively estimate when supplies will run out
  - 8.4.2 ❑ Note expiration dates of critical supplies and plan to re-order supplies accordingly

9. INITIATE TESTING

- **9.1** ❑ Hold a kick-off meeting with core team members and key stakeholders to review Protocol
- **9.2** ❑ Select start date for sampling and analysis, considering training and procurement timelines and other constraints identified above
- **9.3** ❑ Identify a start-up period to allow for troubleshooting, follow-up training, steamlining of data processing and data sharing procedures
- **9.4** ❑ Schedule a follow-up meeting to review Protocol at the conclusion of start-up period. Adjust Protocol as necessary.
- **9.5** ❑ Review protocols on a regular basis and adjust as necessary