National Trends in Wastewater Surveillance

2023 Survey Report

APRIL 2024

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

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INTRODUCTION

Wastewater surveillance—the testing of community wastewater for infectious diseases and other targets—is a relatively new and rapidly-evolving field. Practically non-existent in the US before 2020, implementation of wastewater surveillance testing was expedited by the COVID-19 pandemic and the establishment of the CDC National Wastewater Surveillance System (NWSS). With this accelerated growth, the wastewater surveillance community—laboratories, health departments and wastewater utility partners—needs guidance and context for their testing.

In 2023, the Association of Public Health Laboratories (APHL) conducted a national survey to characterize laboratory capability and capacity. The survey results outlined in this report provide a baseline for laboratories to measure against as they grow and hone their methods. This report can also be used by NWSS and APHL's NWSS Laboratory Community of Practice to understand the overall wastewater surveillance landscape and to identify successes, challenges and resource needs.

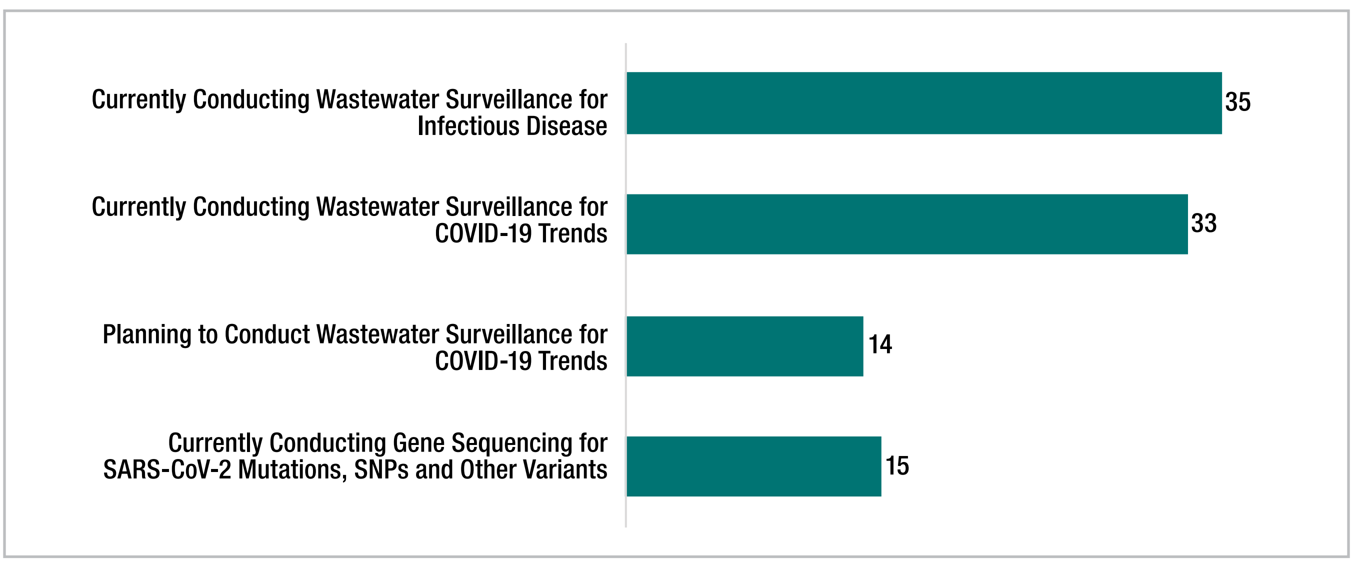
METHODOLOGY

APHL invited 130 state, local and territorial public health, environmental and agricultural laboratories to participate in the APHL 2023 Wastewater Surveillance Survey between January 5 — February 17, 2023. The survey was not sent to other laboratories that might also conduct wastewater surveillance testing for NWSS submission, such as utility, university or private laboratories.

The overall response rate was 98% (127/130); the three non-responders were non-APHL member state environmental laboratories. All 50 states and the District of Columbia were represented by at least one state laboratory response. See **Figure 2** for an overview of the responding laboratories.

Laboratories were designated as a public health, environmental or agricultural laboratory based upon their department affiliation (i.e., department of health, environment or agriculture) or main testing mission. Respondents were routed to different questions based upon their answers and they were not required to answer all questions. **Figure 1** shows the different survey sections and the number of laboratories that responded to those sections based upon their wastewater surveillance activities.

Figure 1. Summary of respondents' wastewater surveillance activities



RESULTS

Overview

The following questions were answered by all responding laboratories (N=127).

Which of these best describe your laboratory: state public health, state environmental, state agricultural, territorial public health, local public health, other?

Figure 2. Respondents by type of laboratory (n=127)

Laboratory Type	Number of Respondents	% Respondents
State Public Health	50	39%
Local Public Health	49	39%
State Environmental	23	18%
Territorial Public Health	3	2%
State Agricultural	1	1%
Other	1	1%
Total	**127**	**100%**

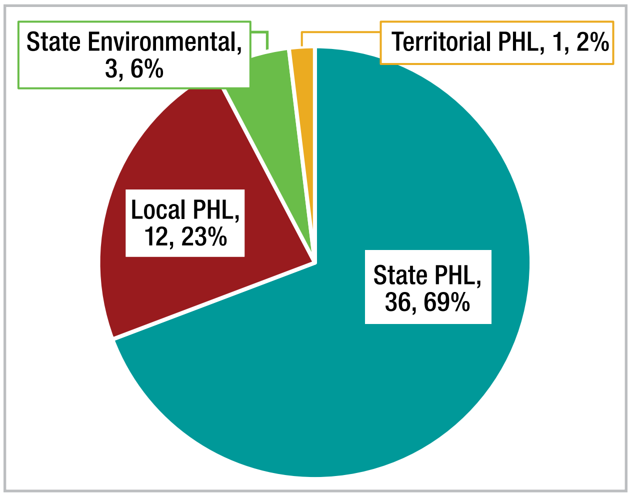
Roughly 80% of respondents were reporting from some type of public health laboratory, with state and local laboratories accounting for 39% each. An additional 18% of respondents were from state environmental laboratories.

Besides the designated laboratory categories, survey respondents could also select "other" and further identify themselves. Reasons for selecting "other" included being a consolidated laboratory that tests agricultural, clinical and environmental samples at the same facility, a department of natural resources, a contracted partner or not having a laboratory. During analysis, most respondents were re-grouped based on standardized definitions of the different laboratory types. For example, one laboratory that identified as "other" performs the state's health testing, so they were grouped with the state public health laboratories. One state and one local public health laboratory use a contracted university laboratory to perform their testing; this will be denoted with an asterisk on the graphs.

Does your laboratory currently perform testing for wastewater surveillance, is your laboratory planning to in the future, and/or did your laboratory do this testing in the past?

Forty-one percent of responding laboratories (52/127) are either currently, planning to or have previously performed wastewater surveillance testing. This encompassed 36 state public health, 12 local public health, three state environmental and one territorial public health laboratory (**Figure 3**). In this question, wastewater surveillance was interpreted broadly as surveillance for any analyte, whether it was an infectious disease target or an environmental contaminant, potentially for regulatory purposes.

Figure 3. Laboratories currently, planning or previously conducting wastewater surveillance (n=52)



For those who have or are planning to conduct wastewater testing, please indicate if your laboratory [currently / planning / previously / have no plans to perform]:

1. **Testing for SARS-CoV-2 to identify COVID-19 trends;**
2. **Using PCR technologies to test for one or more known SARS-CoV-2 mutations found in a variant of interest or concern to evaluate presence and trends;**
3. **Using gene sequencing technologies to identify SARS-CoV-2 mutations, SNPs, and variants; or**
4. **Testing other pathogens or targets besides SARS-CoV-2 using PCR and/or gene sequencing technologies?**

Laboratories that were either currently or had previously conducted any of the listed testing options—35 of the 127 total survey responders (28%)—were deemed to be laboratories performing wastewater surveillance for infectious disease (**Figure 4**). All in this category were public health laboratories, approximately three quarters (77%) were state and one quarter (23%) were local laboratories; this included the one state and one local public health laboratory using university-contracted laboratories.

Of these 35 laboratories that tested wastewater surveillance for infectious disease targets, the vast majority (94%) were testing for SARS-CoV-2 trends. Of those, 29% utilized PCR to test for one or more SARS-CoV-2 mutations and 43% were using gene sequencing technologies to identify SARS-CoV-2 mutations, SNPs and variants. In addition to tracking SARS-CoV-2 trends, 34% were also testing other pathogens or targets using PCR and/or gene sequencing technologies. A higher percentage of state versus local laboratories was testing for SARS-CoV-2 to identify COVID-19 trends (100% vs. 75%) and using gene sequencing technology (48% vs. 25%), but the differences between the remaining types of testing were negligible.

Figure 4. Laboratories currently conducting wastewater surveillance for infectious diseases (n=35)

Testing Activities	State PHLs*,[a] (n=27)	Local PHLs* (n=8)	Total (n=35)
Testing for SARS-CoV-2 to identify COVID-19 trends	27	6	**33**
Using PCR technologies to test for one or more known SARS-CoV-2 mutations found in a variant of interest or concern to evaluate presence and trends	7	3	**10**
Using gene sequencing technologies to identify SARS-CoV-2 mutations, SNPs, and variants	13	2	**15**
Testing other pathogens or targets besides SARS-CoV-2 using PCR and/or gene sequencing technologies	9	3	**12**

For those who have or are planning to conduct testing besides SARS-CoV-2, please indicate which pathogen or target categories you have previously tested, are currently testing, or are strongly considering testing using PCR and/or gene sequencing technologies.

There were 44 respondents that indicated their laboratory had previously, were currently or were planning on testing other pathogens or targets besides SARS-CoV-2 using PCR and/or gene sequencing technologies. There were 10 categories of targets respondents could select from, most with additional specific targets within the category: respiratory pathogens, antimicrobial resistance mechanisms, enteric viruses, fungal pathogens, enteroviruses, enteric bacteria, pox viruses, illicit drugs or pharmaceuticals, parasites or “other.” See **Figure 5** for a breakdown of how laboratories are focusing their efforts on these threats.

a One state environmental laboratory had the capability to test wastewater for other pathogens or targets besides SARS-CoV-2 using PCR and/or gene sequencing technologies, however they performed this testing for regulatory purposes and not strictly for infectious disease surveillance.

Of these laboratories, 27% (12/44) were currently conducting wastewater surveillance for at least one non-SARS-CoV-2 pathogen or target of concern. The most commonly tested targets were respiratory pathogens (92%, 11/12), antimicrobial resistance mechanisms (50%, 6/12) and enteric viruses (50%, 6/12).

Most laboratories in this group (98%, 43/44) were still in the planning stages of testing for additional targets, with more than half planning to test for respiratory pathogens (57%, 25/44), 41% for antimicrobial resistance genes (18/44) and 36% for enteric viruses (16/44). These choices align with the CDC's Priority Core Targets for NWSS Panel 2.0 list. Of laboratories planning to test multiple pathogen/target categories, respiratory pathogens, antimicrobial resistance genes, enteric pathogens (both bacteria and viruses) and fungal pathogens were most common. "Other" options included hepatitis A and B and per- and polyfluoroalkyl substances.

Eight of the pathogen/target categories had additional targets that could be selected. For six of these categories, one target from each was chosen by 100% of laboratories planning to test within that category: carbapenem resistance, Norovirus, *Candida auris*, Salmonella, mpox and Cryptosporidium. Additional targets that were chosen by close to 100% of laboratories were influenza A/B (100% of previous and current testers, 96% of planners), *E. coli*/STEC (100% of previous and current testers, 90% of planners) and respiratory syncytial virus (100% of previous testers, 81% of current testers and 80% of planners).

Figure 5. **Non-SARS-CoV-2 pathogens previously tested for (but not currently), currently testing for or planning to test for via wastewater surveillance (n=44, respondents could select more than one target per category)**

Pathogen/Target Category	Category-specific Targets	Previously Tested	Currently Testing	Planning to Test	Total
Respiratory Pathogens	**Total Testing Any**	**1**	**11**	**25**	**37**
	Influenza A/B	1	11	24	**36**
	Respiratory Syncytial Virus	1	9	20	**30**
	Adenovirus	1	3	9	**13**
	Other Coronaviruses E.g., OC43m 229E	1	1	5	**7**
Antimicrobial Resistance Mechanisms	**Total Testing Any**	**1**	**6**	**18**	**25**
	Carbapenem resistance	1	5	18	**24**
	Beta-lactam resistance	0	2	7	**9**
	Fluoroquinolones	0	2	6	**8**
	Colistin resistance	1	1	5	**7**
	Vancomycin	0	2	5	**7**
	Integrase, class I	0	2	4	**6**
	Tetracycline resistance	0	2	4	**6**
	Macrolides	0	2	2	**4**
Enteric viruses	**Total Testing Any**	**0**	**6**	**16**	**22**
	Norovirus	0	6	16	**22**
	Rotavirus	0	2	6	**8**

Pathogen/Target Category	Category-specific Targets	Previously Tested	Currently Testing	Planning to Test	Total
Fungal Pathogens	**Total Testing Any**	**1**	**3**	**15**	**18**
	Candida auris	1	3	15	**19**
	Blastomyces	0	0	1	**1**
	Histoplasma	0	0	1	**1**
Enteroviruses	**Total Testing Any**	**0**	**5**	**13**	**18**
	Polio	0	3	10	**13**
	Hepatitis A	0	4	9	**13**
	Acute Flaccid Myelitis Enteroviruses E.g., EVD-68, EVD-A71	0	3	6	**9**
Enteric Bacteria	**Total Testing Any**	**1**	**1**	**11**	**13**
	Salmonella	0	0	11	**11**
	E. Coli/STEC	1	1	10	**12**
	Campylobacter jejuni	0	0	7	**7**
	Shigella	0	0	7	**7**
	Enterobacter	1	1	5	**7**
	Yersinia enterocolitica	0	0	5	**5**
Pox Viruses	**Total Testing Any**	**0**	**8**	**7**	**14**
	Mpox	0	8	7	**15**
	Non-variola Orthopoxvirus Generic	0	0	2	**2**
Illicit drugs or pharmaceuticals	**Total Testing Any**	**0**	**1**	**5**	**6**
Parasites	**Total Testing Any**	**0**	**2**	**5**	**7**
	Cryptosporidium	0	2	5	**7**
	Cyclospora cayetanensis	0	0	2	**2**
Other	**Total Testing Any**	**0**	**1**	**3**	**4**

For those with current or future wastewater surveillance programs, what is/will be the goal of the program and how will the data be used for public health impact?

The major theme of laboratories currently and planning to on-board wastewater surveillance is to use the data to improve public health decision making, inform public health interventions and epidemiological response (to the pandemic and other pathogens of concern), identify disease occurrence and trends to effectively allocate resources and inform risk assessments. These types of data could complement those generated by other infectious disease surveillance systems and supplement clinical case data.

For those who have no plans to conduct wastewater testing, please indicate all reasons why your laboratory is not currently doing wastewater surveillance testing (select all that apply).

There were 75 responding laboratories that had no prior or current wastewater surveillance testing activities, and no plans to begin such testing. The top two reasons why 59% of total survey respondents were not currently performing wastewater surveillance testing were staffing limitations and funding uncertainties (**Figure 6**). This may be due to the high number of local and state environmental laboratories that answered this question that do not qualify for CDC's Epidemiology and Laboratory Capacity (ELC) funding, the primary funding source for NWSS laboratories (see **Figure 7**).

Another top reason was lack of experience testing wastewater as a matrix. Also, nearly half of all state environmental laboratories cited wastewater surveillance as not being part of their mission. The laboratories who selected the "other" option included bureaucratic delays and issues finding utility partners. Some health departments partnered with other entities such as universities or private/commercial laboratories, which were not represented in this survey except for one state and one local public health laboratory who answered the survey for their university laboratory partners (indicated in figures with an asterisk).

Figure 6. Reasons for not doing wastewater surveillance testing (n=75, could select more than one reason).

Reason	State PHL (n=15)	Local PHL (n=36)	Territorial PHL (n=2)	State Environmental (n=20)	State Agricultural (n=1)	Other (n=1)	Total (n=75)
Staffing limitations	8	22	0	9	0	0	39
Funding uncertainties / limitations	4	22	1	6	0	0	33
Laboratory does not test wastewater	5	19	1	6	0	0	31
No laboratory space available to dedicate to this testing	6	10	0	7	0	0	23
Jurisdiction's health department contracts with another laboratory If yes, please specify	8	8	1	3	0	0	20
Not within laboratory's mission	2	6	1	9	0	0	18
Unfamiliar with the technology	3	5	0	5	0	0	13
Not a priority within jurisdiction	1	6	1	4	1	0	13
There is interest from laboratory's local and/or state agencies and/or other partners, but wastewater surveillance has not been implemented	3	7	0	3	0	0	13
Laboratory is dedicated to testing clinical SARS-CoV-2 samples	5	3	0	0	0	0	8
Safety concerns	0	2	0	1	0	0	3
Other	6	3	1	3	0	1	14

Currently Conducting Wastewater Surveillance for Infectious Disease

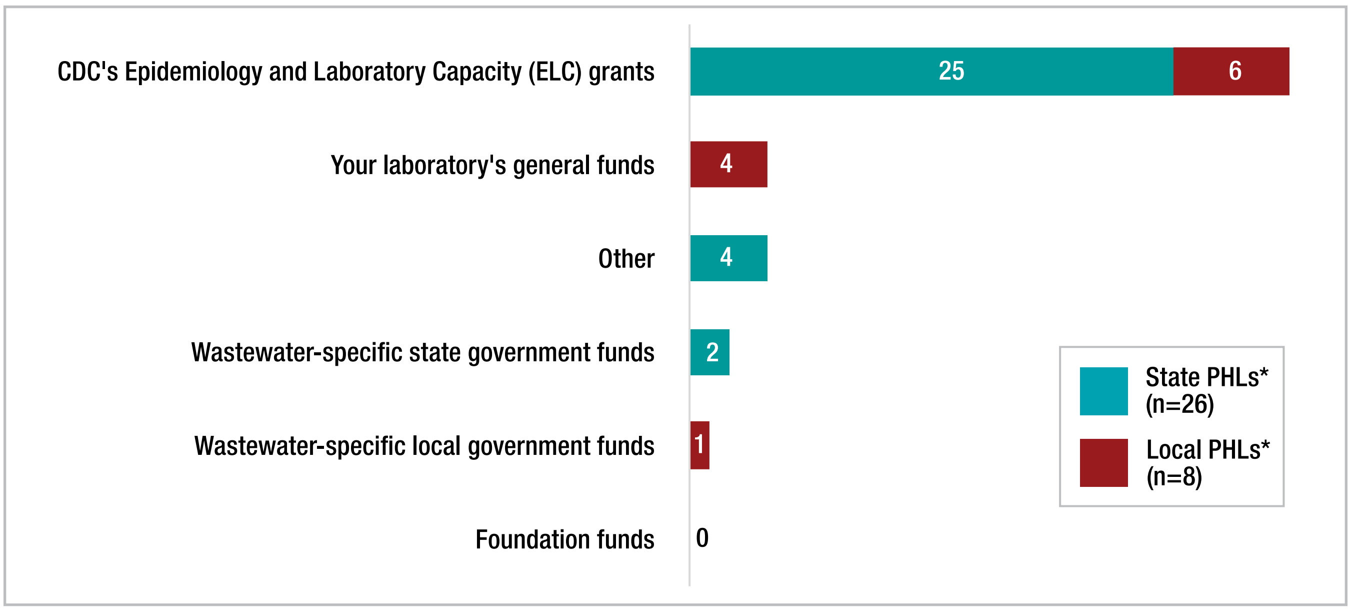
The following questions were answered by 34 laboratories who are currently testing wastewater for any infectious disease; all were either state or local public health laboratories. One state environmental laboratory did answer these questions; however, it was determined that their testing was for regulatory purposes—not infectious disease surveillance—so their data were not included in this report.

Which funding sources are supporting your laboratory's wastewater surveillance testing? (Select all that apply)

A majority of laboratories (91%, 31/34) received their funding support through the CDC's Epidemiology and Laboratory Capacity's grant, including 96% (25/26) of state and 75% (6/8) of local laboratories.

The remaining survey respondents used their laboratories' general funds or another form of funding not listed (12%, 4/34), wastewater-specific state funds (6%, 2/34) or wastewater specific local funds (3%, 1/34). Five laboratories noted that they received funding from multiple sources.

Figure 7. Funding sources supporting wastewater surveillance testing (n=34)



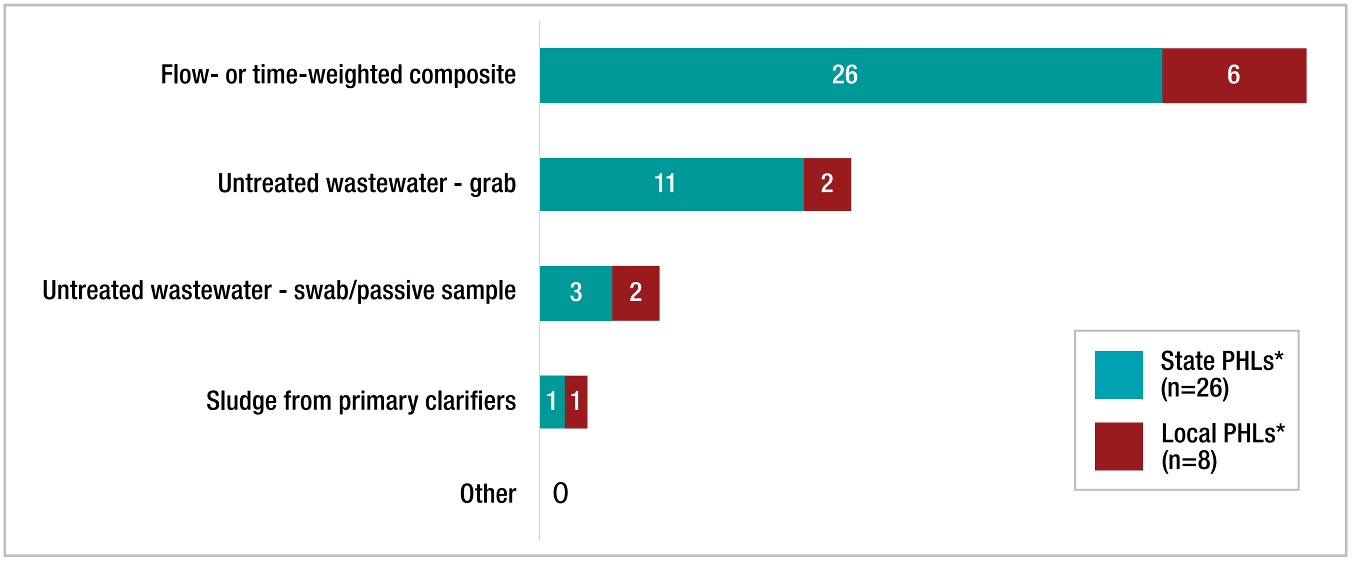
Which types of wastewater samples is your laboratory testing? (Select all that apply)

A majority of laboratories (94%, 32/34) tested flow- or time-weighted composite samples, which were untreated wastewater. All state (26/26) and 75% (6/8) of local laboratories tested flow- or time-weighted composite samples (**Figure 8**).

The remaining survey respondent tested untreated grab samples (38%, 13/34), untreated wastewater from a swab or passive sampler (14%, 5/34) or sludge from primary clarifiers (6%, 2/34). Approximately 47% (16/34) of laboratories noted they collected multiple types of samples, the most common combination being flow- or time-weighted composite and untreated grab samples.

Flow- or time-weighted composite samples best capture the jurisdictions' infectious disease burden because the samples are more representative of all the wastewater coming through the system.

Figure 8. Types of wastewater samples being tested (n=34)

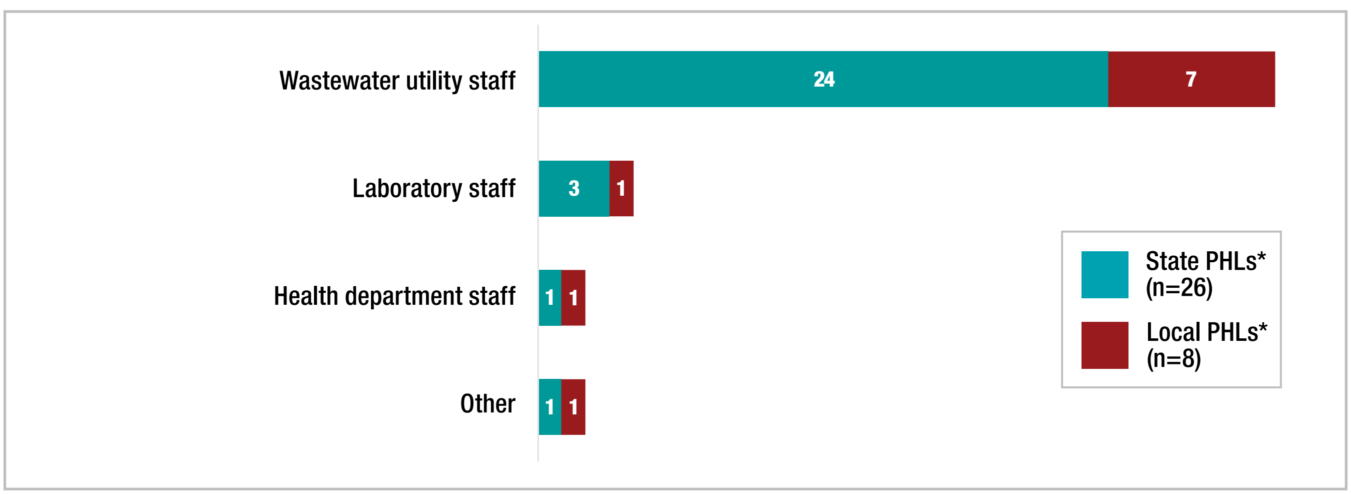


Who is collecting these samples? (Select all that apply)

A majority of laboratory (91%, 31/34) samples were collected by wastewater utility staff. Approximately 92% (24/26) of state and 88% (7/8) of local laboratories relied on wastewater utility staff to collect their samples. Providing utilities stipends and other support can help ensure these collaborations continue.

The remaining survey respondents used laboratory staff (12%, 4/34) or health department staff and other form of personnel (6%, 2/34). Three laboratories (9%, 3/34) used a combination of personnel to collect samples, but a utility staff person was always included.

Figure 9. Entity responsible for sample collection (n=34)



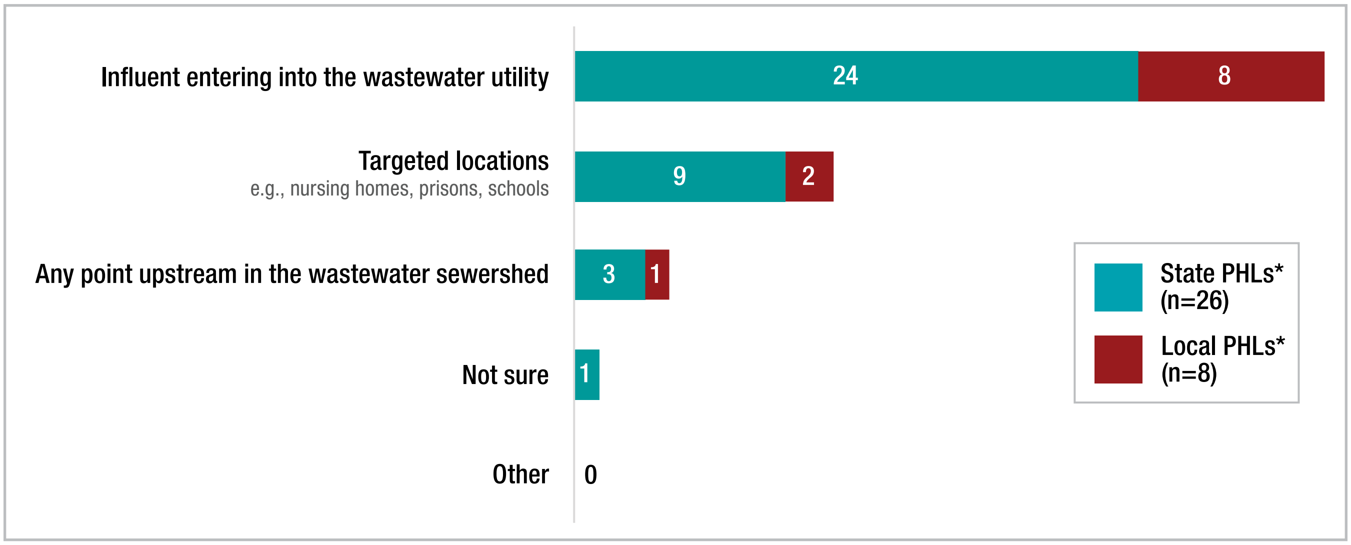
Which parts of the wastewater system are these samples collected from? (Select all that apply)

A majority of laboratory (94%, 32/34) samples were collected from influent entering the wastewater utility. Approximately 92% (24/26) of state and 100% (8/8) of local laboratories collected their samples from influent entering the wastewater utility.

About one-third (11/34) of samples were taken at target locations, one-tenth (4/34) from any point upstream and 3% (1/34) were unsure. About two-fifths of laboratory samples were collected from multiple locations. The most common combination was influent entering the wastewater utility and at target locations such as nursing home and correctional facilities.

Influent samples were the easiest sample to collect and best represent the disease burden within the full wastewater catchment area. There are also fewer privacy and ethical concerns compared to using samples from targeted locations.

Figure 10. Location(s) of wastewater sample collection (n=34)

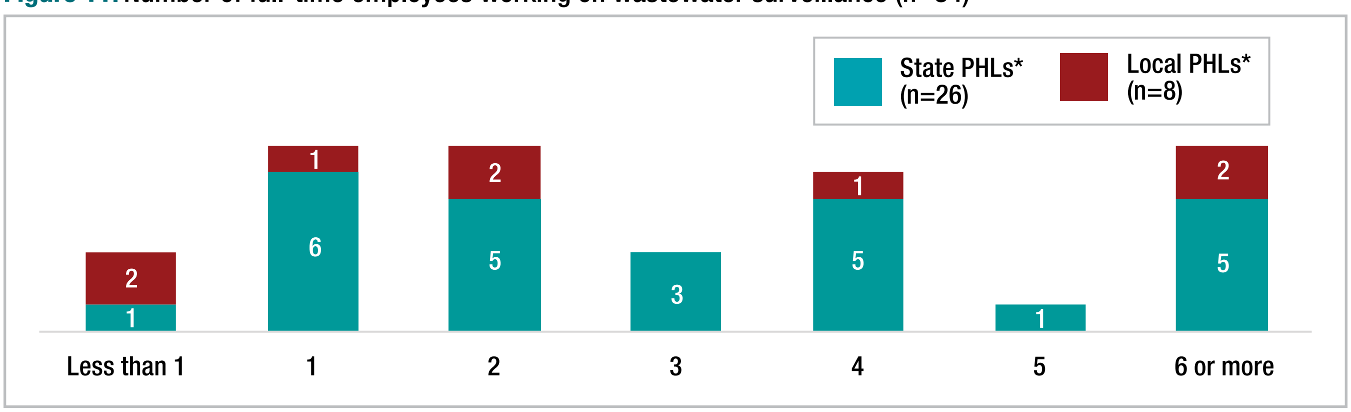


How many full-time employees (FTEs) do you have working on wastewater surveillance?

An even split of 21% (7/34) each for one, two, or six or more FTEs working on wastewater surveillance, while 9% (3/34) had less than one FTE (**Figure 11**). Approximately 42% (11/26) of state and 38% (3/8) of local laboratories had between one and two FTEs.

Laboratories who had six or more FTEs (five states and two locals) had been doing this testing since early on in the pandemic, were funded by the ELC grant and/or are one of four NWSS Centers of Excellence, as of December 2023.

Figure 11. Number of full-time employees working on wastewater surveillance (n=34)



Which biosafety level and practices does your laboratory use to test wastewater for SARS-CoV-2? (Select all that apply)

A majority of testing laboratories (62%, 21/34) used biosafety level-2 (BSL-2) facilities and practices for their SARS-CoV-2 wastewater testing, including 65% (17/26) of state and 50% (4/8) of local laboratories. About half of the BSL-2 laboratories (52%, 11/21) had an inactivation protocol.

The remaining 41% (14/34) had a BSL-2 + safety level, meaning they worked in a BSL-2 environment but used BSL-3 practices. No laboratories used BSL-3 facilities for this testing.

All of the BSL-2 + laboratories had bioaerosol protection on equipment, 86% (12/14) had an approved biosafety protocol for clinical and environmental samples that may have contained SARS-CoV-2, 64% (9/14) had respiratory PPE and 43% (6/14) had separate PPE donning and doffing space.

See **Figure 12** for a breakdown of state and local laboratories biosafety practices. The state laboratory that responded “other” contracted with a university for testing and did not know their safety level.

Figure 12. Biosafety level and practices used for testing wastewater for SARS-CoV-2 (n=34)

Biosafety Level (BSL)	Safety Practices in Place	State PHLs* (n=26)	Local PHLs* (n=8)	Total (n=34)
BSL-2	**Total at this level**	**17**	**4**	**21**
	Inactivation protocols to conduct testing in BSL-2 space	9	2	**11**
BSL-2+ BSL-2 environment with BSL-3 practices	**Total at this level**	**10**	**4**	**14**
	Bioaerosol protection on equipment Caps for centrifuge rotors, dedicated biosafety cabinet, directional airflow	10	4	**14**
	An approved biosafety protocol for clinical and environmental samples with suspected SARS- CoV-2 presence	9	3	**12**
	Respiratory PPE for personnel regardless of space biosafety designation	6	3	**9**
	Separate personal protective equipment (PPE) donning and doffing space from wastewater testing space	3	3	**6**
BSL-3	**Total at this level**	**0**	**0**	**0**
	Ability to culture live SARS-CoV-2 virus	1[b]	0	**1**
Other	**Total at this level**	**1**	**0**	**1**

b One BSL-2+ laboratory answered the survey by hand; therefore, the survey logic did not show. They had the capabilities to culture live virus in other parts of their laboratory, but not for in their wastewater surveillance BSL laboratory.

Please rank the following resources that your laboratory needs to meet your jurisdiction's current and upcoming wastewater surveillance testing needs from most important (1) to least important (16).

The top three most needed resources among laboratories were funding, staffing and laboratory space and facilities. This held true for state laboratories, but local laboratories identified funding, staffing and standardized methods as the top three most needed resources.

Figure 13. Resources needed to support current and upcoming wastewater surveillance testing (n=15), ranked: most important (1) to least important (16)

Resources	n	Median Rank	Mean Rank	Standard Deviation
Funding	34	1.0	2.7	3.1
Staffing	34	2.0	2.7	2.7
Laboratory space and facilities	33	5.0	6.5	4.9
Standardized methods	34	5.5	6.1	2.9
Instrumentation to support high-throughput processing	33	7.0	7.5	3.9
Methodology information sharing	33	7.0	7.1	2.8
Guidance documents E.g., on-boarding new targets, sequencing	33	7.0	7.4	3.4
Instrumentation for sequencing	33	8.0	8.9	3.6
Computing capacity for sequencing	33	8.0	8.0	2.7
Formal standard operating procedures	33	9.0	8.6	2.5
Verification (e.g., proficiency testing) materials	33	11.0	9.4	3.0
Reference materials E.g., surrogates, spike-ins, materials for normalization or recovery calculations	33	11.0	9.3	3.6
Training, in-person or remote	33	13.0	11.7	3.2
Mentor laboratory programs	33	14.0	13.0	2.6
Examples of successful use of wastewater surveillance data/resources to address epidemiologist challenges in using wastewater surveillance data	33	14.0	12.0	4.1
Other needs	33	16.0	14.8	3.8

Please write a brief statement summarizing the barriers that are limiting your laboratory's and/or jurisdiction's ability to fully implement and use wastewater surveillance for public health purposes.

The overarching barrier for fully implementing wastewater surveillance was understanding the utility of the data. Addressing this barrier could help to address others, such as funding, staffing, capacity (of the laboratories and utilities) and space.

Wastewater Surveillance for COVID-19 Trends

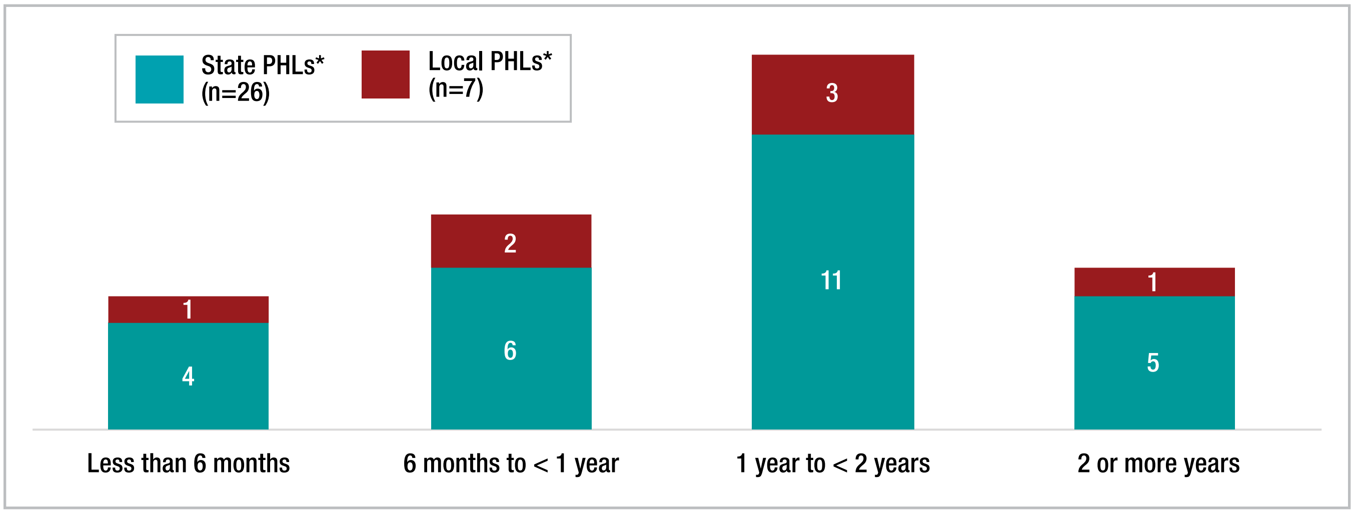
Currently Testing for COVID-19 Trends

The following questions were answered by the 33 laboratories who had used wastewater surveillance to test for SARS-CoV-2 to identify COVID-19 trends. All 33 of these laboratories were state and local public health laboratories.

How long has your laboratory been testing wastewater surveillance samples to identify COVID-19 trends?

Virtually all wastewater surveillance by public health laboratories in the US started after the COVID-19 pandemic began in early 2020, with the first adopters starting in summer/fall of 2020. This indicates a steady growth of five to eight state or local laboratories on-boarding testing every six months through to when this survey closed, in February 2023. At the time of the survey, almost half (42%, 14/33) of the responding laboratories had been testing between one and two years, so their surveillance efforts would have begun between the second half of 2021 and early 2022.

Figure 14. Length of time laboratories have been testing wastewater surveillance samples for SARS-CoV-2 (n=33)



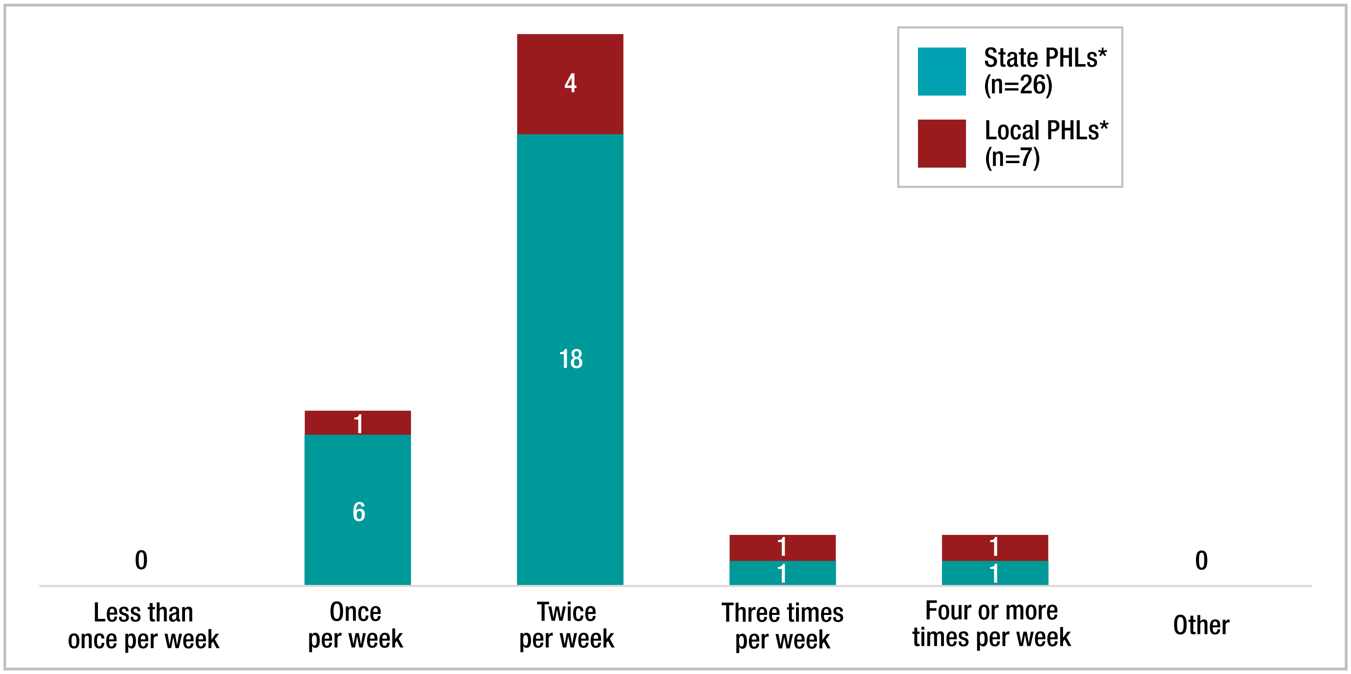
What is the typical frequency of your wastewater sample collection?

A minimum of three samples are required to detect a trend over time,[c] so laboratories must decide how often to sample based on how long they can take to establish a trend and other factors, such as laboratory and utility capacity and utility/sample site access.

A majority of responding laboratories (67%, 22/33) sampled wastewater twice per week, which allows a laboratory to establish a trend within eight days. The second highest sampling frequency (21%, 7/33) was once per week, with trends established in 15 days. No responding laboratories sampled less than once per week, while 6% (2/33) sampled three, four or more times per week (**Figure 15**).

c CDC NWSS, Developing a Wastewater Surveillance Sampling Strategy

Figure 15. Wastewater sample collection frequency (n=33)

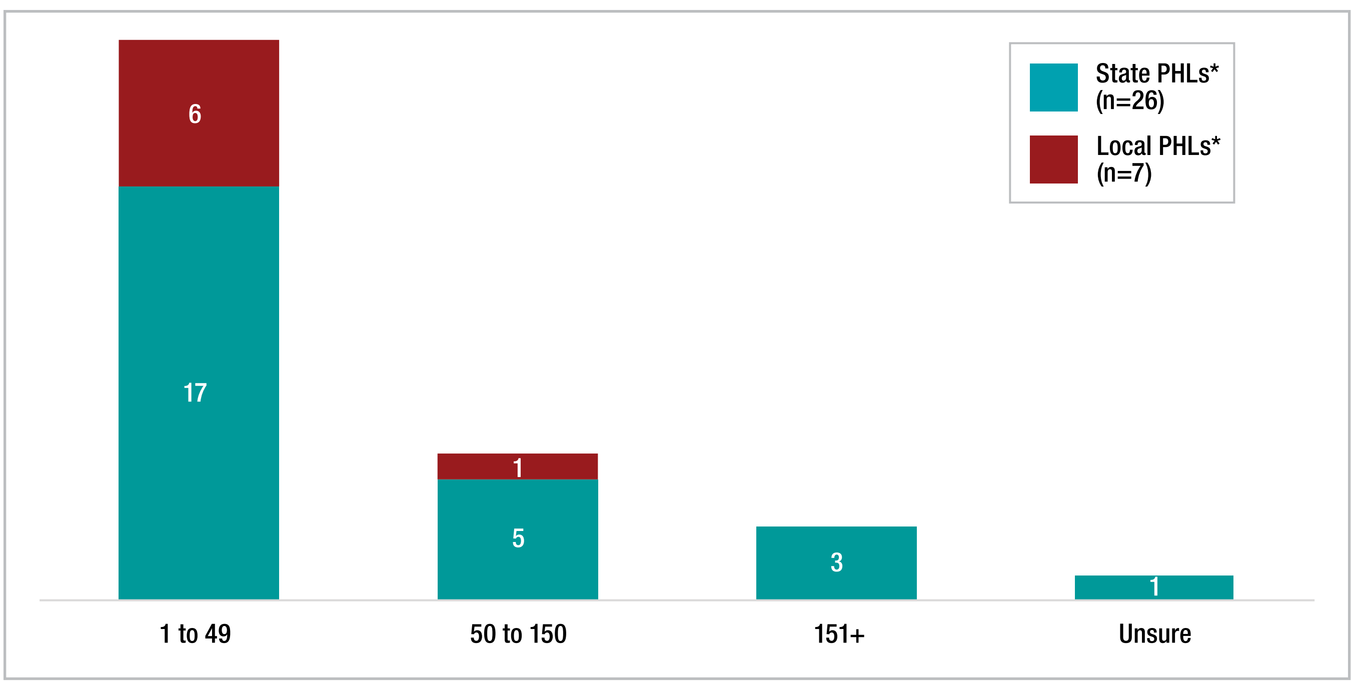


How many samples does your laboratory process per week on average?

A majority of laboratories (70%, 23/33) processed under 49 samples per week; 65% (17/26) of the state and 86% (6/7) of local laboratories fell into this category.

For those processing 50 or more samples a week, 18% (6/33) were processing 50-150 samples and 9% (3/33) were processing over 150 samples per week. Only one of the nine respondents processing 50 or more samples per week was a local laboratory, and one state laboratory was unsure how many samples they were processing (**Figure 16**).

Figure 16. Wastewater samples processed on average per week (n=33)

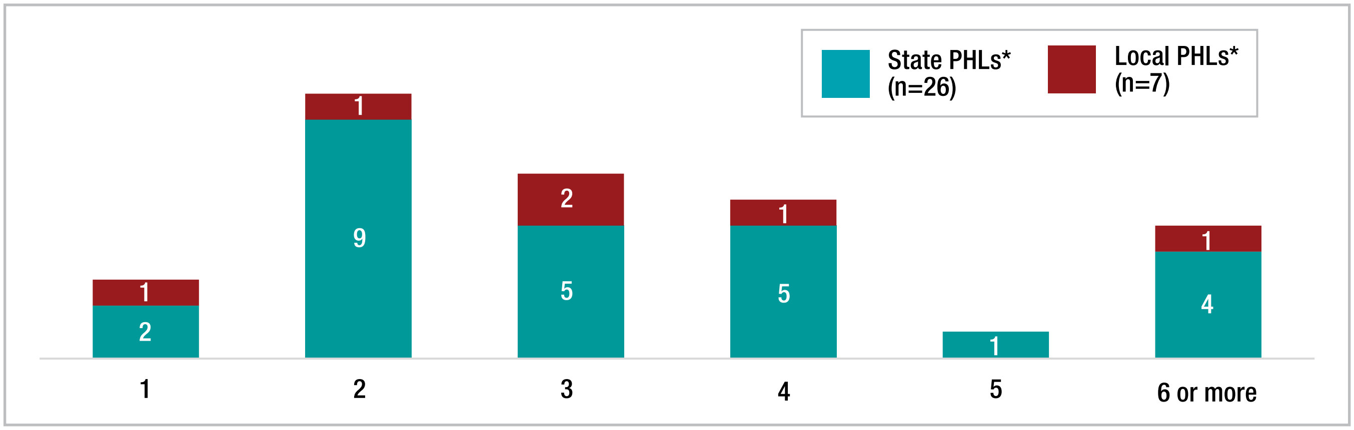


In a non-surge situation, how many days is your average sample turnaround time, from sample receipt to reporting to state public health?

In non-surge situations, 61% of responding laboratories had an average sample turnaround time (TAT) of under three days (**Figure 17**). A two day TAT was the most common (30%, 10/30) response, accounting for 35% (9/26) of state and 14% (1/7) of local laboratories.

Of the 15% (5/33) of laboratories with an average TAT of six or more days, all were processing an average of 49 samples or fewer per week, mostly performed manual extractions and had two or fewer full-time employees dedicated to wastewater surveillance.

Figure 17. Average non-surge sample TAT for SARS-CoV-2 wastewater surveillance (n=33)



What are you using to concentrate your samples? (Select all that apply)

The vast majority of laboratories used either CeresNano nano beads (52%, 17/33) and/or InnovaPrep filter pipette (36%, 12/33) to concentrate samples (**Figure 18**). CeresNano nano bead usage was fairly even between state (54%) and local (43%) laboratories, while a higher number of state laboratories (42%) use InnovaPrep filter pipettes compared to only 14% of local laboratories. Approximately 30% (2/7) of local laboratories use membrane filtration as a concentration method.

Six laboratories (18%) used more than one concentration method but, notably, all used InnovaPrep filter pipettes paired with another method (most often Polyethylene glycol (PEG) or nano beads). One laboratory used four methods: PEG, CeresNano nano beads, InnovaPrep filter pipettes and ultracentrifugation.

Laboratories likely used these methods because of their high sensitivity and throughput, lower comparative cost (specifically CeresNano), existing protocols and availability of peers already using these methods to provide guidance.

The state laboratory that selected the “other” option used centrifugation with a water concentrating buffer as their concentration method.

Figure 18. Sample concentration methods for SARS-CoV-2 wastewater surveillance (n=33, respondents could select more than one option)

Sample Concentration Methods	State PHLs* (n=26)	Local PHLs* (n=7)	Total (n=33)
CeresNano nano beads	14	3	17
InnovaPrep filter pipette	11	1	12
Ultracentrifugation	2	1	3
We are not concentrating our samples, our laboratory is using direct extraction	2	1	3
Membrane filtration	1	2	3
Polyethylene glycol (PEG) - centrifuge	2	0	2
Invitrogen Dynabeads nano beads	1	0	1
Other	1	0	1
Unsure	1	0	1
Ultrafiltration	0	0	0
Skim milk flocculation	0	0	0

What extraction method is your laboratory using? (Select all that apply)

Respondents identified their extraction methods from within three categories: automated, manual or kit-based. Kit-based extractions were defined as methods that do not require phenol or chloroform, whereas manual extraction "kits" do use these reagents.[d] See **Figure 19** for a breakdown of method selection.

A majority of laboratories were conducting automated extractions (70%, 23/33) compared to manual (24%, 8/33) and kit-based (36%, 12/33). A higher percentage of state (73%, 19/26) versus local (57%, 4/7) laboratories were conducting automated extractions.

KingFisher was the most used extraction platform overall, with almost 70% of automated extraction users utilizing it (16/23), and approximately half of all state (50%, 13/26) and local laboratories (43%, 3/7). Many laboratories were likely already using KingFisher for their clinical microbiological work, making it an easy option to implement for wastewater surveillance.

Laboratories who selected "other" in any of the three extraction categories identified the following as their extraction methods: PerkinElmer Chemagic, Promega Maxwell, Geneses Orleans, the EZ1 extraction, Zymo Research Quick-RNA Viral Kit and the 96 well kit, Quadrant, Imhoff cone, Monarch total RNA miniprep kit, MagMax Viral Pathogen Nucleic Acid Isolation Kit, Qiagen QiAmp Viral RNA mini Kit and Stonybrook, Genese - Orleans, Quadran.

d LabCE.com: Automated Vs. Manual Extraction

Figure 19. Extraction methods used for wastewater surveillance (n=33, respondents could select more than one option)

Extraction Category	Extraction Method	State PHLs* (n=26)	Local PHLs* (n=7)	Total (n=33)
Automated Extractions	**Total Using Automated Extractions**	**19**	**4**	**23**
	KingFisher	13	3	**16**
	Other	4	1	**5**
	QIAcube	5	0	**5**
	eMAG	0	0	**0**
	QIAxcel	0	0	**0**
Manual Extractions	**Total Using Manual Extractions**	**7**	**1**	**8**
	MagMAX Viral Pathogen Nucleic Acid Isolation Kit	5	0	**5**
	Other	**3**	1	**4**
	QIAamp Viral RNA Kit	3	0	**3**
Kit-based Extractions	**Total Using Kit-based Extractions**	**9**	**3**	**12**
	ThermoFisher MagMax Wastewater	5	0	**5**
	Other	3	2	**5**
	Promega Wizard Enviro	0	1	**1**
	QIAGEN PowerViral	1	0	**1**
	IDEXX Magnetic Bead	1	0	**1**
	QIAGEN PowerSoil (sludge)	0	0	**0**
	Trizole	0	0	**0**

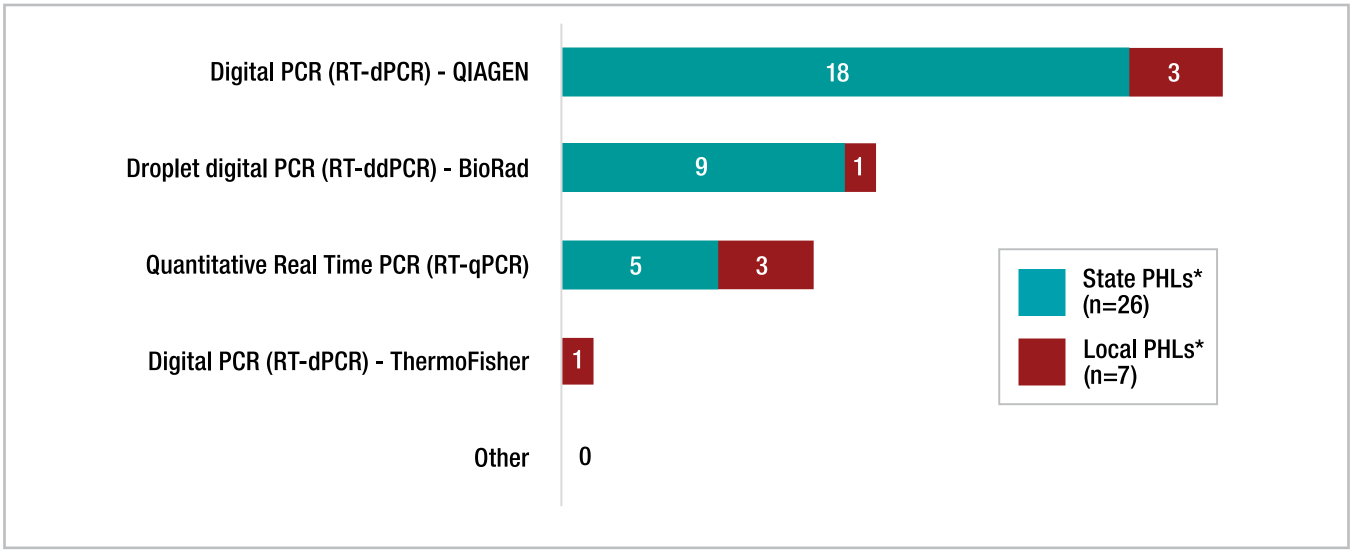
What analysis platform is your laboratory using? (Select all that apply)

To conduct analysis on their samples, most laboratories (64%, 21/33) were using QIAGEN's digital PCR (dPCR) platform; breaking down to approximately 69% (18/26) of state and 43% (3/7) of local laboratories. The remaining respondents used Bio-Rad's ddPCR (30%, 10/33), RT-qPCR (24% (8/33) and ThermoFisher's dPCR 3% (1/33) (**Figure 20**).

Seven laboratories used more than one analysis platform—one laboratory used both qPCR and Bio-Rad ddPCR, two laboratories used both qPCR and QIAGEN dPCR— and four laboratories used both QIAGEN dPCR and Bio-Rad ddPCR.

QIAGEN was likely such a popular choice because laboratories had participated in the QIAGEN pilot project and many laboratories likely already had it (or other QIAGEN instruments) in their workflow.

Figure 20. Analysis platforms used for wastewater surveillance (n=33, respondents could select more than one option)

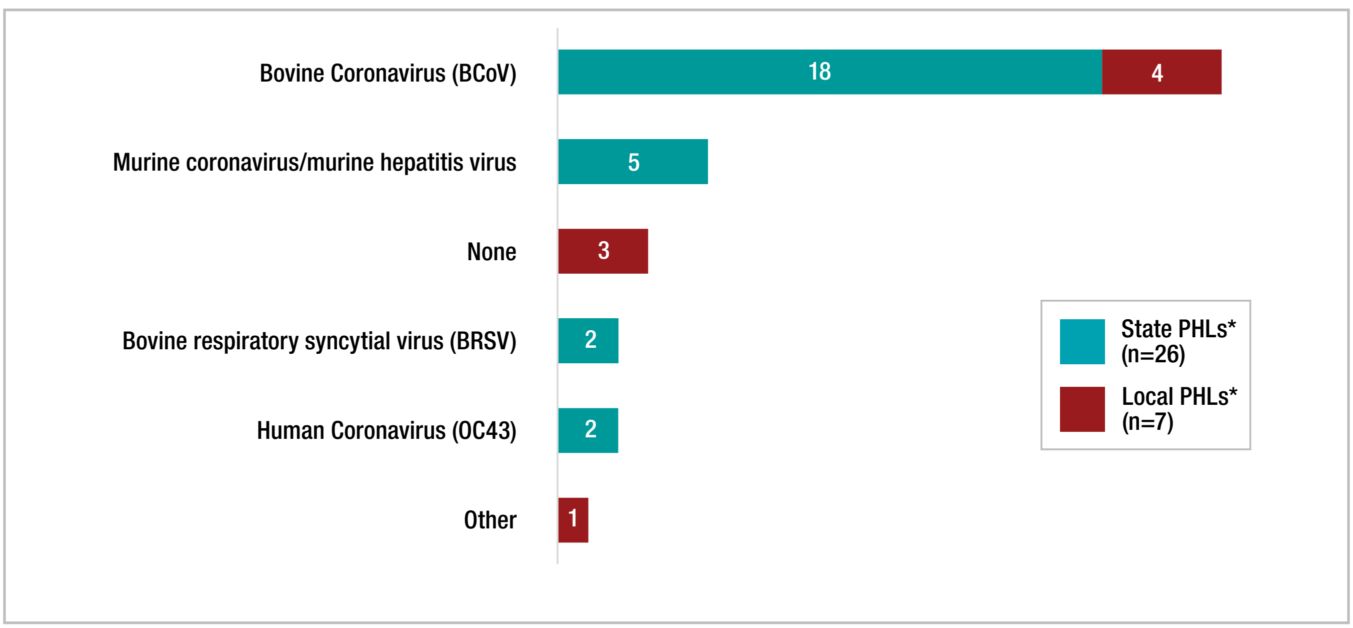


Which recovery control(s) is your laboratory using? (Select all that apply)

More than half the testing laboratories (67%, 22/33) used bovine coronavirus (BCoV) as their recovery control. Approximately 69% (18/26) of state and 57% (4/7) of local laboratories used this method (**Figure 21**). Local laboratories were either using BCoV or did not use a recovery control at all (9%, 3/33). BCoV was likely popular because it was readily available at the beginning of the COVID-19 pandemic and was part of an early GT Molecular protocol. It was thought to be the ideal control for SARS-CoV-2 because it shared similar characteristics; both were positive-sense, enveloped, single-stranded RNA coronaviruses.

The remaining survey respondents that used a recovery control utilized murine coronavirus/hepatitis virus (15%, 5/33), bovine respiratory syncytial virus (BRSV; 6%, 2/33), human coronaviruses (6%, 2/33) or another form of recovery control (3%, 1/33). No survey respondents used more than one recovery control.

Figure 21. Recovery control methods for SARS-CoV-2 wastewater surveillance (n=33, respondents could select more than one option)



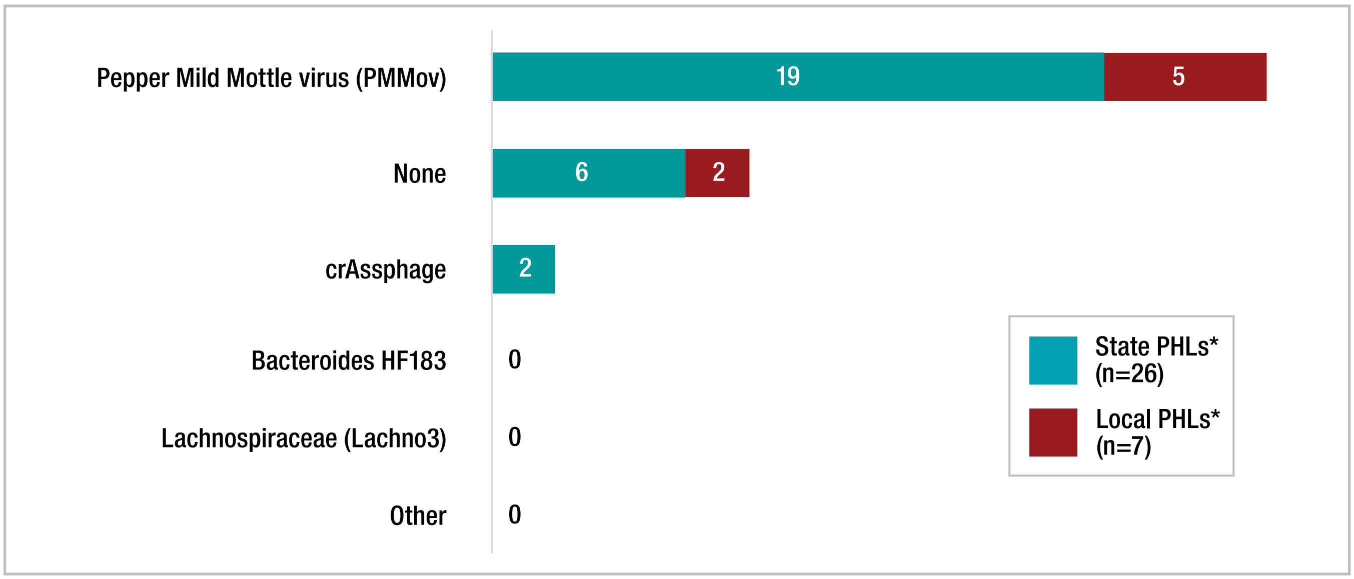
Which fecal marker(s) is your laboratory using for quantification? (Select all that apply)

A majority of laboratories (73%, 24/33) used pepper mild mottle virus (PMMoV) as a fecal marker (**Figure 22**). PMMoV is the dominant RNA virus in human feces, stable in wastewater and comes as the marker in detection kits. Approximately 73% (19/26) of state and 71% (5/7) of local laboratories used PMMoV, and local laboratories either used PMMoV or did not use a fecal marker (29%, 2/7).

The remaining survey respondents either did not use a fecal marker (24%, 8/33) or used crAssphage (6%, 2/33), which has been demonstrated to work well in a variety of environmental applications.

One state laboratory used two fecal markers (PMMoV and crAssphage). In that case, PMMoV was used for normalization and crAssphage is an alternative fecal marker to give them flexibility in case they decided to switch.

Figure 22. Fecal marker usage for quantification during SARS-CoV-2 wastewater surveillance (n=33, respondents could select more than one option)

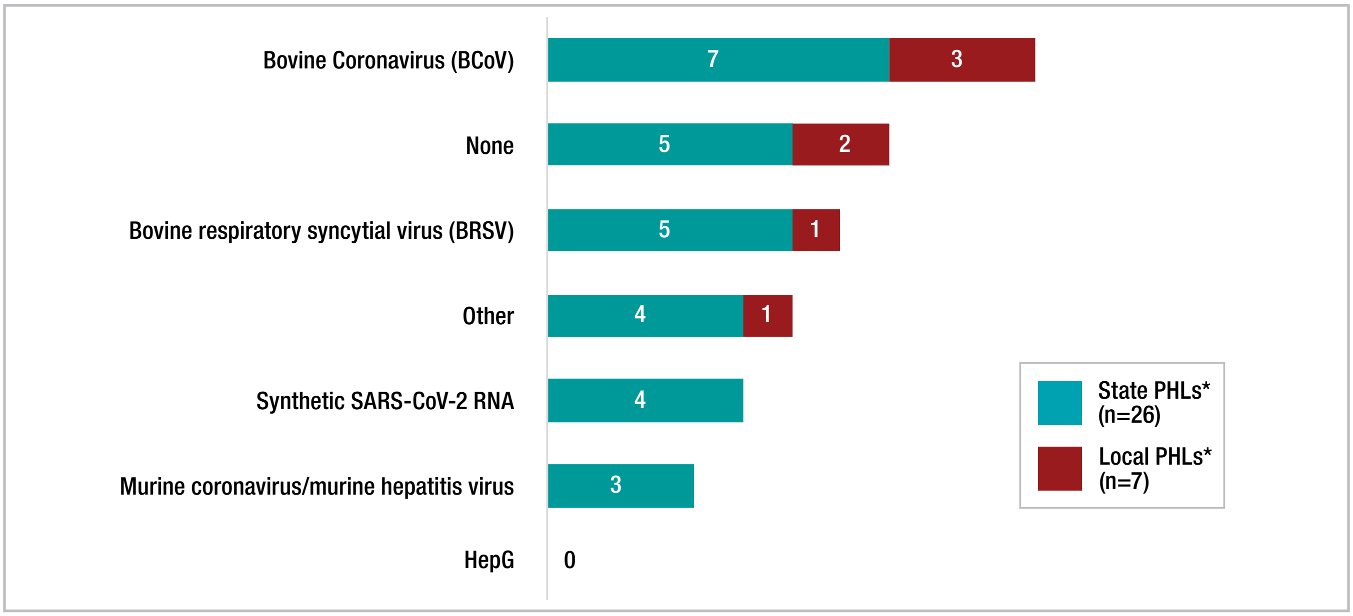


Which inhibition control(s) is your laboratory using? (Select all that apply)

BCoV was the most common inhibition control (30%, 10/33) for the survey respondents—used by approximately 27% (7/26) of state and 43% (3/7) of local laboratories—but it was also common for laboratories not to use an inhibition control (21%, 7/33 overall, 19% for state and 29% for local) (**Figure 23**).

The remaining respondents that did use inhibition control(s) used BRSV (18%, 6/33), human coronaviruses (18%, 6/33), another form of inhibition control (15%, 5/33), synthetic SARS-CoV-2 RNA (12%, 4/33) or murine coronavirus/hepatitis virus (9%, 3/33). No survey respondents used more than one inhibition control.

Figure 23. Inhibition control methods for SARS-CoV-2 wastewater surveillance (n=33, respondents could select more than one option)

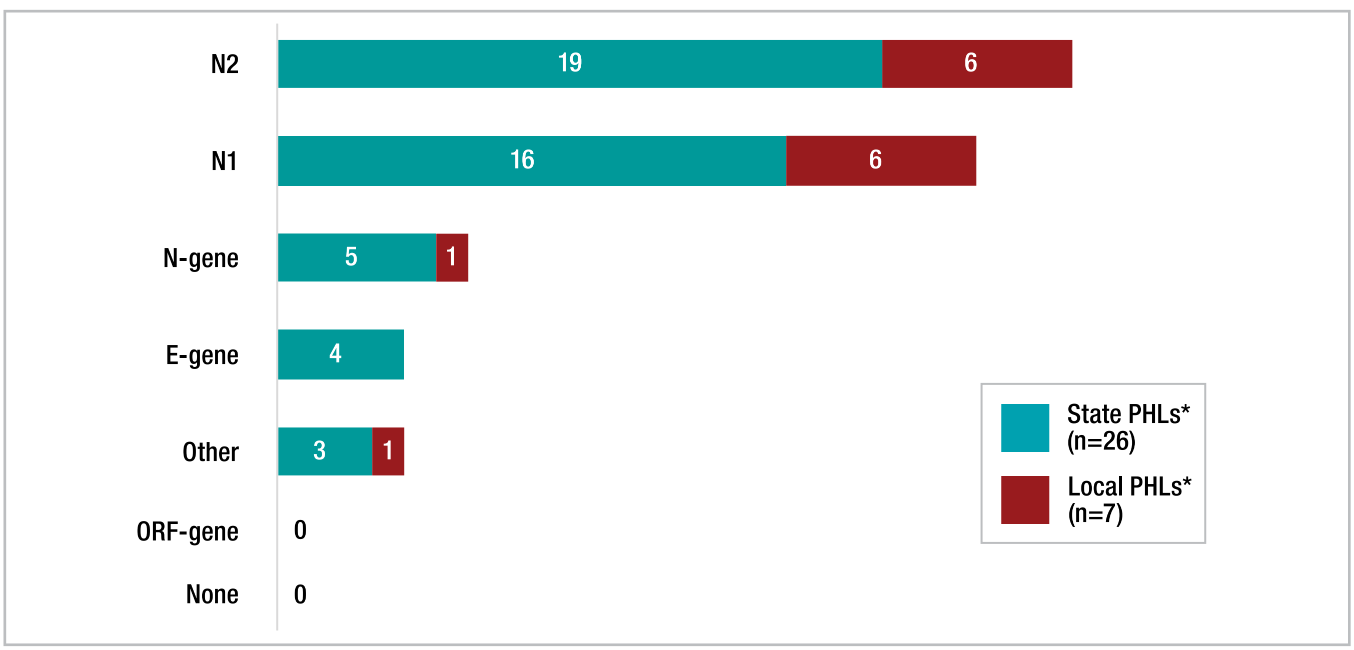


Which genetic markers are used by your laboratory to test wastewater for SARS-CoV-2? (Select all that apply)

A majority of laboratories used the N2 (76%, 25/33) and N1 (67%, 22/33) genes as their SARS-CoV-2 genetic marker. The other respondents used the N-gene (18%, 6/33), the E-gene (12%, 4/33) or other markers for SARS-CoV-2 (**Figure 24**).

The N1 and N2 genes are in a stable region of the virus and were established by CDC as primers/probe targets early in the pandemic for clinical testing. Approximately 12% (3/26) of state laboratories used only the N1 gene as a SARS-CoV-2 marker, and 19% (5/26) used only the N2 gene; no local laboratories used either maker exclusively. More than half (60%, 20/33) of laboratories used both the N1 and N2 genes as SARS-CoV-2 markers, with 54% (14/26) of state and 86% (6/7) of local laboratories using this combination.

Figure 24. Genetic markers used for SARS-CoV-2 wastewater surveillance (n=33, respondents could select more than one option)



Planning to Test for COVID-19 Trends

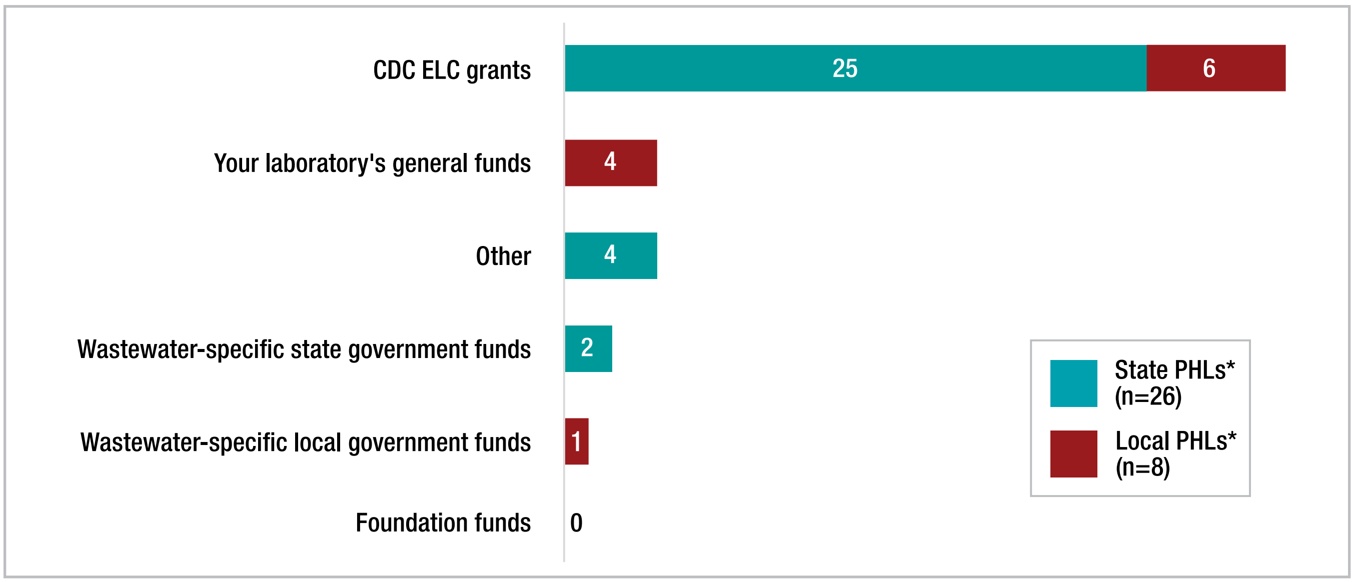
The following questions were answered by 14 laboratories who were planning to test SARS-CoV-2 to identify COVID-19 trends; this group included only public health laboratories (nine state, four local and one territorial).

Which funding sources are or will be used to support your laboratory's wastewater surveillance testing? (Select all that apply)

A majority of laboratories (79%, 11/14) will be using CDC's Epidemiology and Laboratory Capacity (ELC) grant to support wastewater surveillance testing. Approximately 89% (8/9) of state, 50% (2/4) of local and 100% (1/1) of the territorial laboratories will be using CDC's ELC grant to support wastewater surveillance testing. The remaining survey respondents will be using wastewater-specific state government funds (21%, 3/14), their laboratory's general funds (14%, 2/14), wastewater-specific local government funds or other funds not listed (7%, 1/14).

A majority of laboratories plan to use only one form of support, but two laboratories plan to use a combination of wastewater-specific state and general laboratory funds. The ELC grant is the only funding the territorial laboratory plans to use to support this testing.

Figure 25. Funding sources that are/will support wastewater surveillance testing? (n=14)



What other resources does your laboratory need to meet your jurisdiction’s current and upcoming wastewater surveillance testing needs? (Select all that apply)

The top three resource needs for state laboratories planning to test for SARS-CoV-2 to identify COVID-19 trends are funding, staffing and instrumentation to support high-throughput processing. Local laboratories also noted funding and staffing as their top two resource needs, but standardized methods, methodology information sharing, and formal standard operating procedures all ranked equally for third. The territorial laboratory noted that every resource besides sequencing instrumentation would be needed.

Figure 26. Additional resources needed to meet current and upcoming wastewater surveillance testing needs (n=14)

Additional Resources Needed	State PHLs (n=9)	Local PHLs (n=4)	Territory PHL (n=1)	Total (n=14)
Funding	8	4	1	**13**
Staffing	7	4	1	**12**
Standardized methods	5	3	1	**9**
Instrumentation to support high-throughput processing	6	1	1	**8**
Methodology information sharing	3	3	1	**7**
Formal standard operating procedures	3	3	1	**7**
Laboratory space and facilities	5	0	1	**6**
Verification materials E.g., proficiency testing	4	1	1	**6**
Guidance documents E.g., on-boarding new targets, sequencing	3	2	1	**6**
Reference materials E.g., surrogates, spike-ins, materials for normalization or recovery calculations	3	2	1	**6**
Training, in-person or remote	3	2	1	**6**
Examples of successful use of wastewater surveillance data/resources to address epidemiologist challenges in using wastewater surveillance data	3	2	1	**6**
Mentor laboratory programs	2	2	1	**5**
Computing capacity for sequencing	2	0	1	**3**
Other needs	1	1	1	**3**
Instrumentation for sequencing	0	0	0	**0**

Currently Conducting Gene Sequencing for SARS-CoV-2 Mutations, SNPs and Other Variants

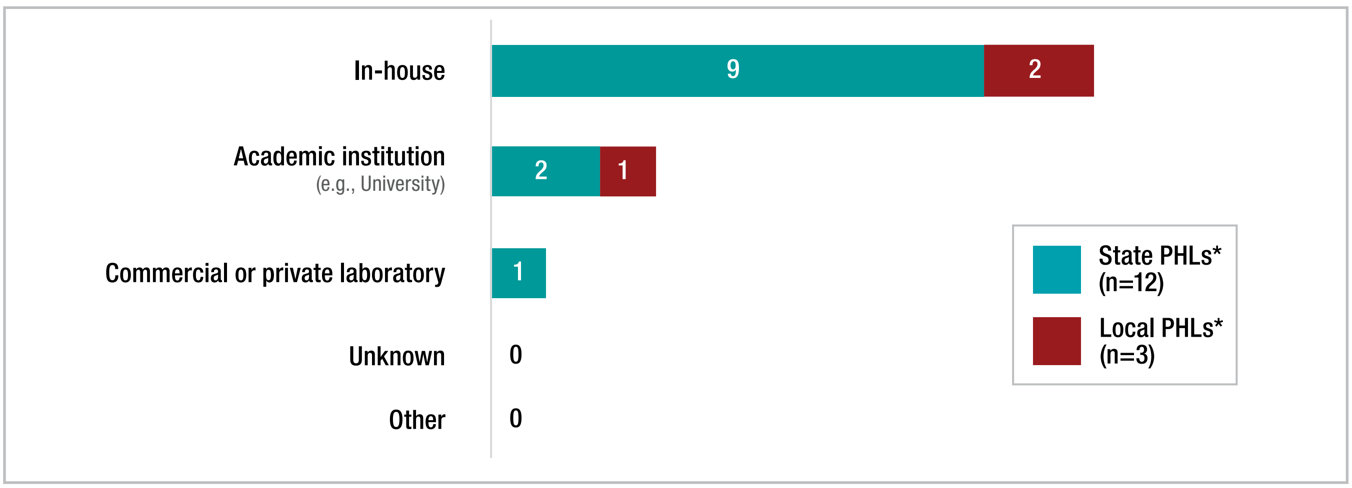
The following questions were answered by the 15 laboratories who are currently using gene sequencing technologies to identify SARS-CoV-2 mutations, SNPs and variants. Gene sequencing is an example of how wastewater testing can be utilized for active infectious disease surveillance because of its ability to detect specific, possibly new, strains in the wastewater catchment area. Only state and local public health laboratories were part of this group.

Where is your SARS-CoV-2 viral mutation and variant detection work being performed?

A majority of laboratories (73%, 10/15) performed SARS-CoV-2 viral mutation and variant detection in house. This breaks down to 75% (9/12) of state and 67% (2/3) of local laboratories.

For the remaining survey respondents, 20% (3/15) used academic institutions and 7% (1/15) used a commercial or privately-owned laboratory.

Figure 27. SARS-CoV-2 viral mutation and variant detection method location (n=15)

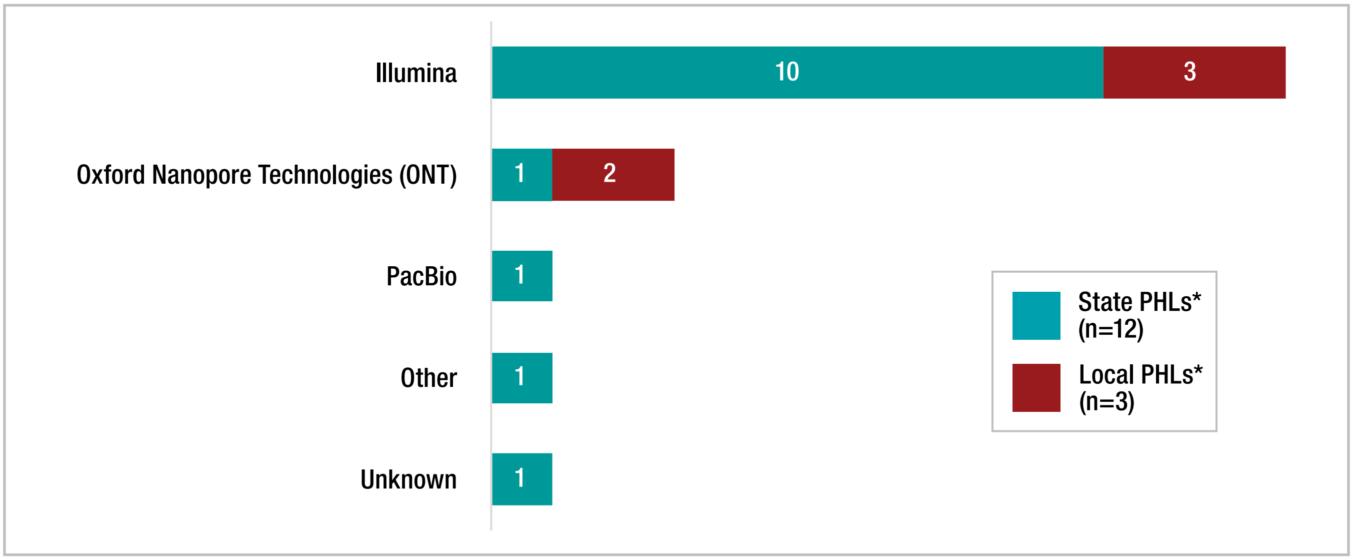


What types of next generation sequencing (NGS) have you (or, if applicable, your sequencing collaborator) used for wastewater sequencing? (Select all that apply)

A majority of laboratories (87%, 13/15) used the Illumina platform for sequencing. This breaks down to approximately 83% (10/12) of state and 100% (3/3) of local laboratories. Two thirds of local laboratories (67%) were also using Oxford Nanopore Technologies. Four laboratories (two state and two local) used a combination of platforms, but one of them was always Illumina.

The remaining survey respondents used Oxford Nanopore Technologies (20%, 3/15), PacBio, other platforms or their platform was unknown (7% each, 1/15).

Figure 28. Types of Next Generation Sequencing (NGS) used for wastewater sequencing (n=15)



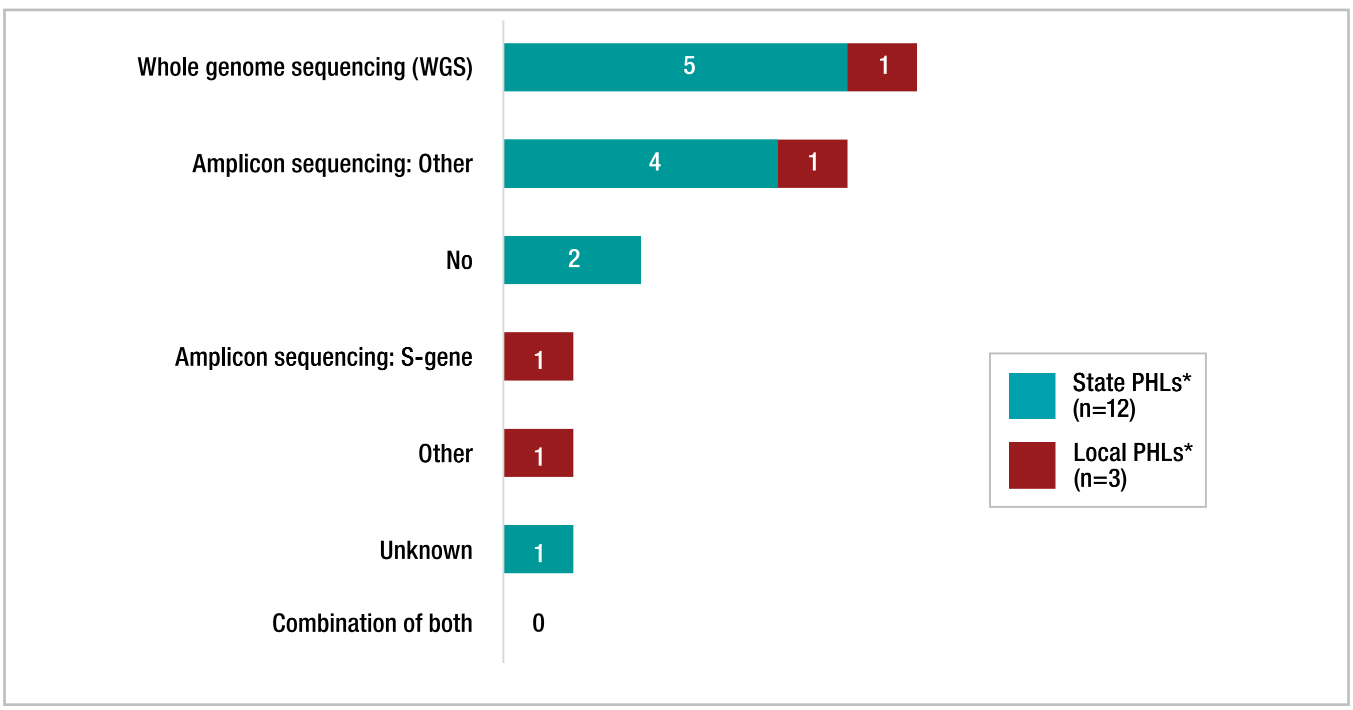
Have you conducted or are you conducting targeted S-gene or other marker gene or whole genome sequencing on wastewater samples? (Select all that apply)

For the purpose of this question, whole genome sequencing refers to sequencing the entire genome and amplicon sequencing is a more targeted approach of specific genes.

Whole genome sequencing (40%, 6/15) and amplicon sequencing (other than S-gene) (33%, 5/15) were the most commonly used sequencing techniques. Approximately 42% (5/12) of state and 33% (1/3) of local laboratories used whole genome sequencing while approximately 33% (4/12) of state and 33% (1/3) of local laboratories used amplicon-based sequencing.

For the remaining survey respondents, 13% (2/15) did not conduct sequencing and 7% (1/15) conducted amplicon sequencing of the S-gene, other sequencing techniques or their laboratory's sequencing technique was unknown.

Figure 29. Methods of sequencing on wastewater samples (n=15, respondents could select more than one option)

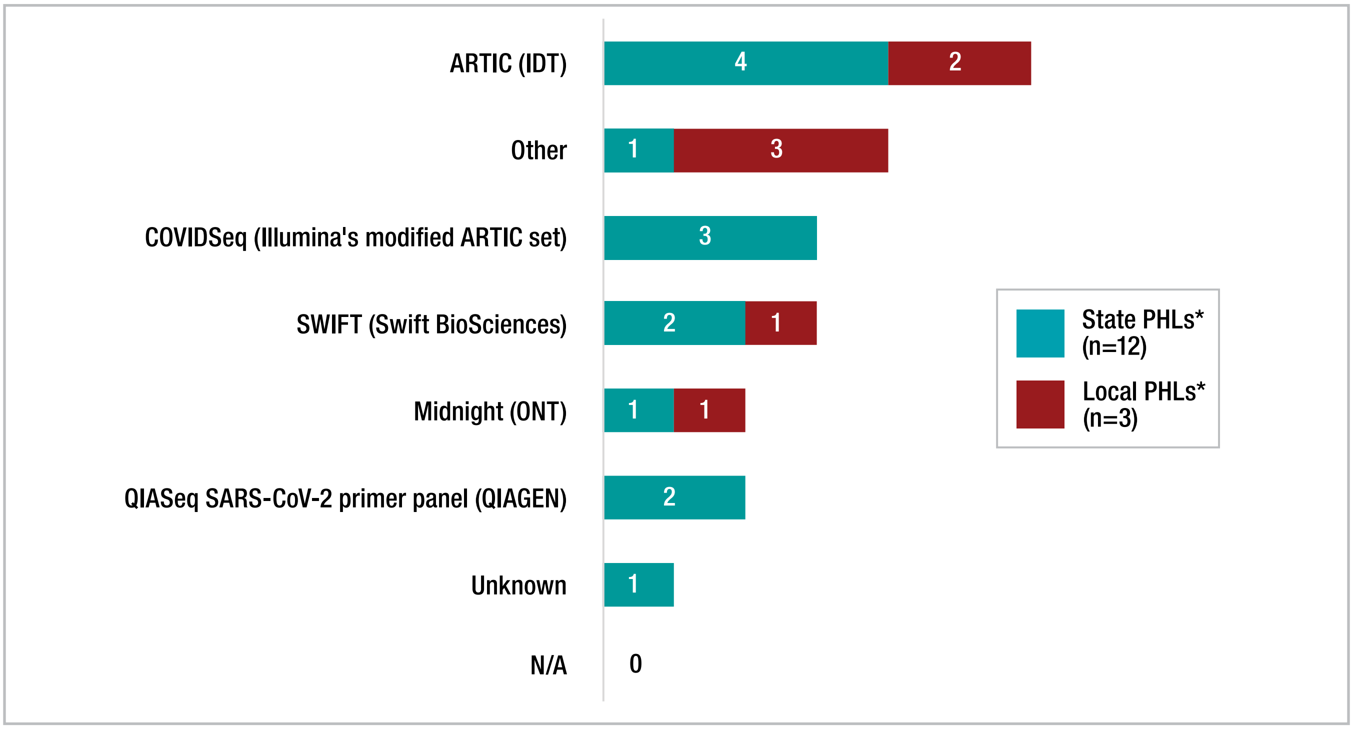


If applicable, what amplicon-based sequencing protocol for SARS-CoV-2 have you or your sequencing collaborator used? (Select all that apply)

The most commonly used amplicon-based sequencing protocol for SARS -CoV-2 was ARTIC (IDT) (40%, 6/15). Approximately 33% (4/12) of state and 67% (2/3) of local laboratories used ARTIC (IDT) for the amplicon-based sequencing protocol. The remaining survey respondents used other protocols (27%,4/15), COVIDSeq and SWIFT (20%, 3/15), Midnight and QIASeq SARS-CoV-2 Primer Panel (13%, 2/15) or did not know their sequencing protocol for SARS-CoV-2 (7%, 1/15).

One third of the survey respondents used two or more protocols. Both laboratories that used Midnight used it in combination with ARTIC (IDT). One hundred percent (3/3) of local laboratories also noted they used other protocols. These responses included NEBNext Varskip 2 short protocol, QIAseq DIRECT SARS-CoV-2 Kit, single amplicon and Genexus AmpliSea.

Figure 30. Amplicon-based sequencing protocols for SARS-CoV-2 (n=15, respondents could select more than one option)

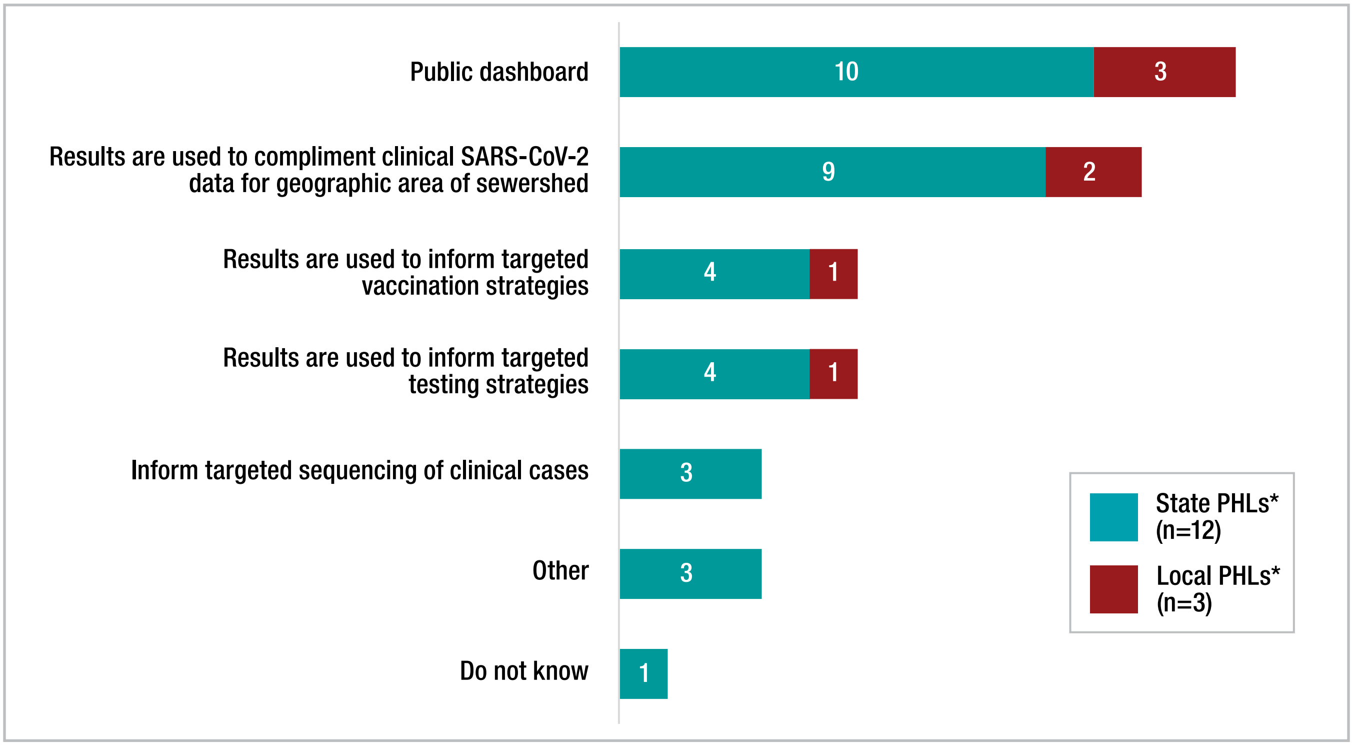


How does your jurisdiction intend to use SARS-CoV-2 wastewater sequencing data for public health action? (Select all that apply)

A majority of laboratories (87%, 13/15) intend to use a public dashboard for their SARS-CoV-2 wastewater sequencing data so that it can inform public health action; this was true for approximately 83% (10/12) of state and 100% (3/3) of local laboratories.

The remaining survey respondents intend to use results to complement clinical data (73%, 11/15), to inform targeted vaccination strategies and targeted testing strategies (33%, 5/15), to inform targeted sequencing of clinical cases (20%. 3/15) or do not know (7%, 1/15). Twenty percent (3/15) selected other, which were not specified. However, all laboratories selected more than one option and plan to use their sequencing data for public health action in more than one way.

Figure 31. Intended uses of SARS-CoV-2 wastewater sequencing data for public health action (n=15, respondents could select more than one option)

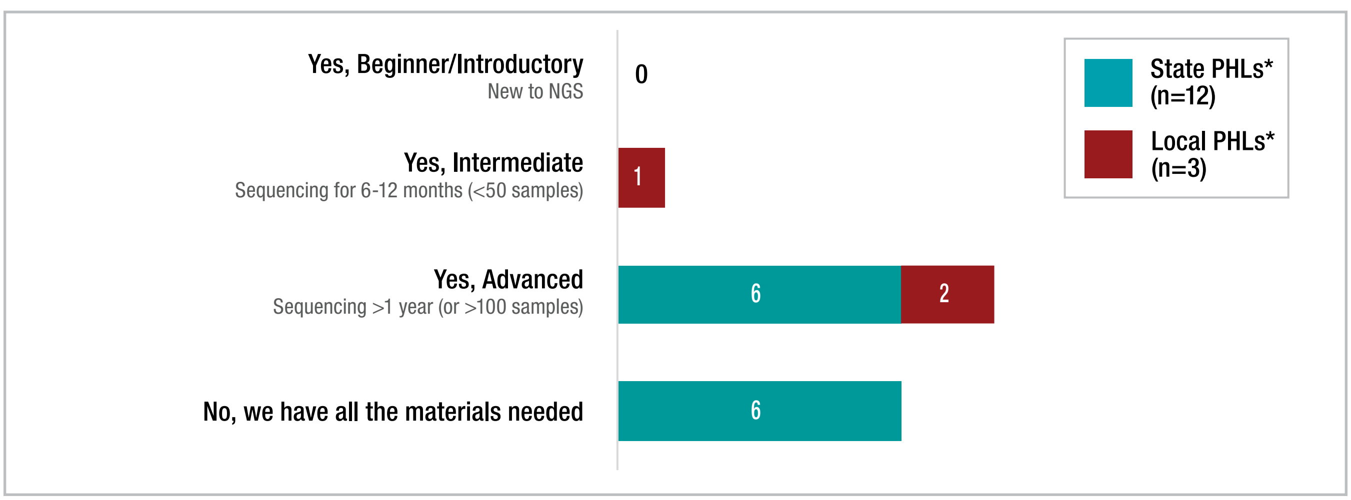


Would training materials for wastewater SARS-CoV-2 sequencing be of interest to your program? Examples of training and support focus areas could include library prep, safety, QC/QA, bioinformatics, etc.

A majority of laboratories (53%, 8/15) indicated they would benefit from advanced sequencing training, as they had been performing this testing for more than one year (starting approximately in late 2021). Half of state (6/12) and 67% (2/3) of local laboratories would benefit from this training as they have also been performing this testing for more than one year.

Forty percent (6/15) noted they have all the materials they need, all of which were state laboratories, and 7% (1/15) would benefit from an intermediate level training. No laboratories needed beginner-level trainings.

Figure 32. Training materials needed for wastewater SARS-CoV-2 sequencing (n=15)

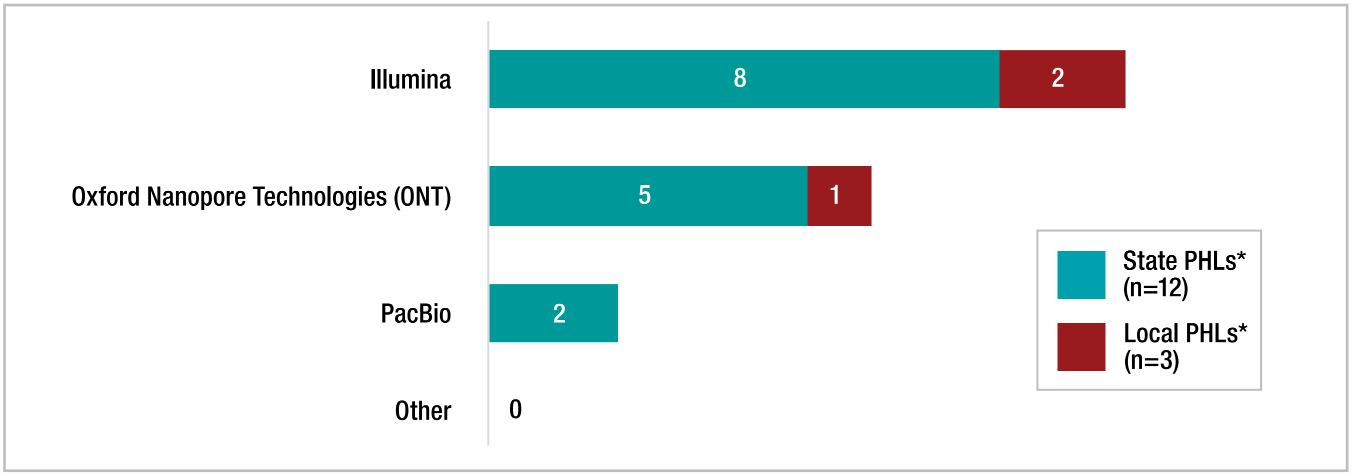


What NGS technologies are you considering for generating SARS-CoV-2 wastewater sequence data? (Select all that apply)

A majority of laboratories (67%, 10/15) considered the Illumina platform to generate sequencing data. Approximately 67% (8/12) of state and 67% (2/3) of local laboratories also considered this platform.

The remaining survey respondents considered Oxford Nanopore Technologies (40%, 6/15) or PacBio (13%, 2/15). One third of survey respondents considered more than one platform for generating sequencing data.

Figure 33. NGS technologies considered for generating SARS-CoV-2 wastewater sequencing data (n=15, respondents could select more than one option)

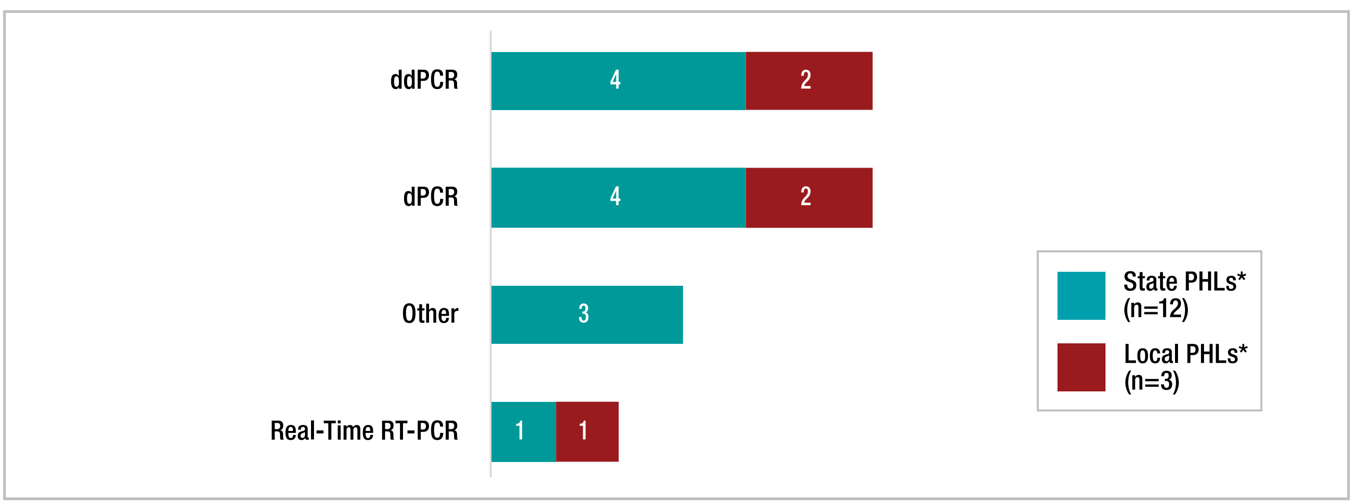


What non-sequencing based assays are you considering for mutation and variant detection in wastewater samples? (Select all that apply)

A majority of laboratories (80%, 12/15) considered using some form of digital (digital (40%, 6/15) or droplet digital (40%, 6/15)) PCR for mutation and variant detection. Approximately 33% (4/12) of state and 67% (2/3) of local laboratories considered droplet digital PCR (ddPCR) for mutation and variant detection while approximately 33% (4/12) of state and 67% (2/3) of local laboratories also considered digital PCR (dPCR).

The remain survey respondents considered other methods (20%, 3/15) or real-time RT-qPCR (13%, 2/15). Laboratories who are newer to wastewater surveillance were still considering all options, including qPCR.

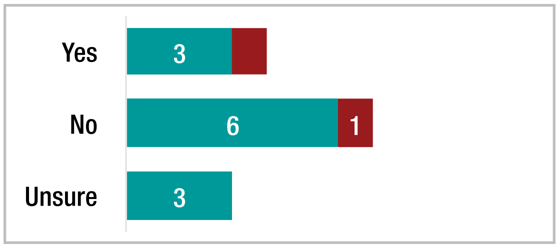
Figure 34. Non-sequencing based assays considered for mutation and variant detection in wastewater samples (n=15, respondents could select more than one option)



Have you conducted or are you conducting shotgun metagenomic sequencing on wastewater samples?

Only about a quarter of the laboratories (27%, 4/15) have conducted shotgun metagenomics sequencing, defined as the method that captures all genetic material in a sample. Forty-six percent (7/15) of laboratories were/have not conducted shotgun metagenomics sequencing. Half (6/12) of state and 33% (1/3) of local laboratories have not used this method, while 25% (3/12) of state laboratories are unsure if they have (**Figure 35**).

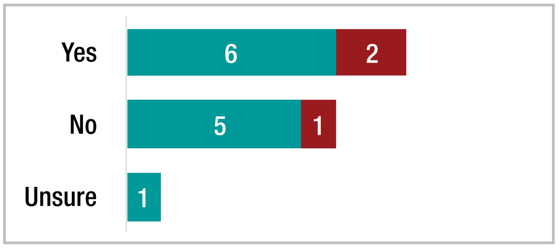
Figure 35. Use of shotgun metagenomic sequencing on wastewater samples (n=15)



Do you use a real-time reverse-transcription PCR, dPCR or ddPCR screen and employ a cycle threshold (Ct) value cut-off when determining which wastewater samples to sequence?

Sixty-seven percent (8/15) of laboratories were using a real-time reverse-transcription PCR (RT PCR), dPCR, or ddPCR screen and Ct value threshold to determine which samples to sequence, including half of state (6/12) and two-thirds of local laboratories (2/3). Forty percent (6/15) were not using this method and 7% (1/15) are unsure (**Figure 36**).

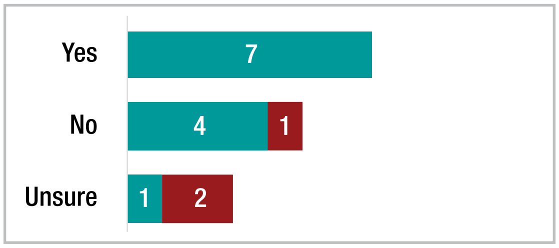
Figure 36. Use of real-time RT PCR, dPCR, or ddPCR screen and Ct value cut-off to determine what wastewater samples to sequence (n=15)



Have you submitted sequence data from wastewater sample(s) to a public repository?

Forty-seven percent of laboratories have submitted wastewater data to a public repository—such as the National Center for Biotechnology Information (NCBI)—including 58% (7/12) of state and zero of three local laboratories. One-third (5/15) had not and 20% (3/15) were unsure (**Figure 37**).

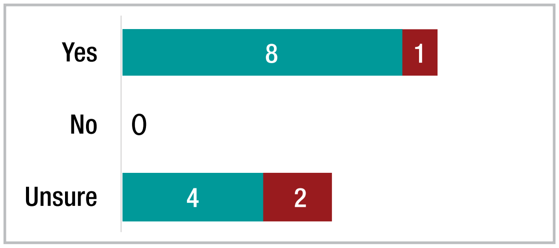
Figure 37. Sequencing data from wastewater samples submitted to a public repository (e.g., NCBI) (n=15)



Does your laboratory plan on releasing data using the NCBI wastewater BioSample surveillance metadata package to submit data to NCBI?

Sixty percent (9/15) of laboratories planned to release data using the NCBI, including two thirds (8/12) of state and one third (1/3) of local laboratories. No laboratories explicitly planned not to release data to NCBI, though 40% (6/15) were unsure (**Figure 38**).

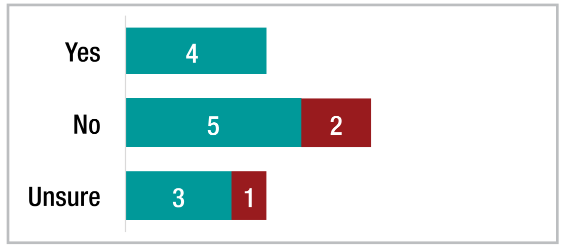
Figure 38. Plans to release data to NCBI using their wastewater BioSample surveillance metadata package (n=15)



Have you used *in silico* datasets to evaluate/ validate your analysis pipelines for mutation and variant detection in wastewater samples?

Forty-seven percent (7/15) of laboratories did not use *in silico* datasets, including 42% (5/12) of state and 67% (2/3) of local laboratories. About a quarter of the laboratories (4/15)—all state—had used *in silico* datasets, and another quarter were unsure if they had (**Figure 39**).

Figure 39. Use of *in silico* datasets to evaluate/ validate analysis pipelines for mutation and variant detection in wastewater samples (n=15)

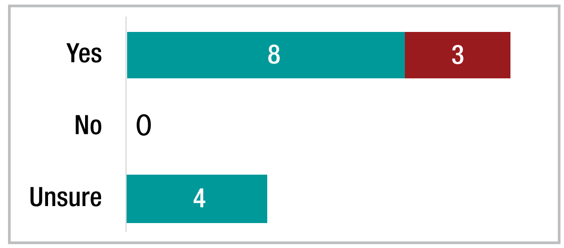


Has your laboratory identified a bioinformatics tool, pipeline, platform or application to analyze SARS-CoV-2 wastewater sequencing data? If yes, please list primary tools.

A bioinformatics platform had been identified by 73% (11/15) of laboratories to analyze SARS-CoV-2 sequencing data, including 67% (8/12) of state and 100% (3/3) of local laboratories. The remaining 27% (4/15) were unsure (**Figure 40**).

Primary tools identified included iVar (v.1.3.1), BCFtools (v.1.14), SnpEff (v.5.0e), SnpSift (v.4.3), Freyja, Kallisto and viralrecon.

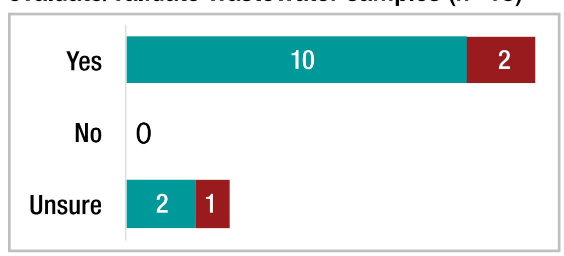
Figure 40. Identification of a bioinformatics platform to analyze SARS-CoV-2 wastewater sequencing data (n=15)



Would publicly available model datasets be of value for mutation and variant detection evaluate/ validate of wastewater samples?

Eighty percent (12/15) of laboratories would find publicly available model datasets of value—such as *in silico* datasets or well-characterized actual wastewater sequencing datasets—including 83% (10/12) of state and 67% (2/3) local laboratories. No laboratories would not find this of value and 20% (3/15) were unsure (**Figure 41**).

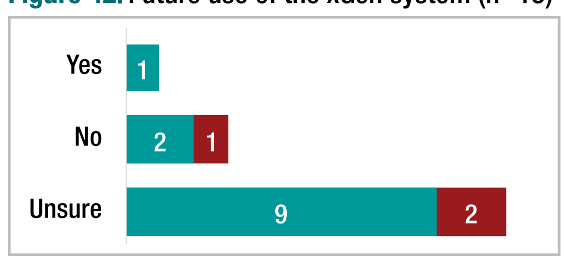
Figure 41. The value of publicly available model datasets for mutation and variant detection to evaluate/validate wastewater samples (n=15)



Are you using or planning to use the xGen system?

Only one state laboratory (7%, 1/15) was using or planned to use the xGen system. Nearly three quarters of the respondents (73%, 11/15) were unsure about their laboratory's plans for using the system—75% (9/12) of state and 67% (2/3) of local laboratories—while 20% (3/15) were not or were not planning to use it (**Figure 42**).

Figure 42. Future use of the xGen system (n=15)



GLOSSARY

Acronyms

AG .... Agricultural laboratory
APHL .... Association of Public Health Laboratories
BCoV .... Bovine Coronavirus
BRSV .... Bovine Respiratory Syncytial Virus
BSL .... Biological safety level
CDC .... US Centers for Disease Control and Prevention
COVID-19 .... Coronavirus Disease 2019
Ct .... cycle threshold
EL .... Environmental laboratory
ELC .... Epidemiology and Laboratory Capacity (CDC cooperative agreement)
FTE .... Full Time Employee
NCBI .... National Center for Biotechnology Information
NWSS .... National Wastewater Surveillance System
PEG .... Polyethylene glycol
PHL .... Public health laboratory
PMMoV .... Pepper Mild Mottle Virus
PPE .... Personal protective equipment
QC/QA .... Quality control/quality assurance
RNA .... Ribonucleic acid
SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2
TAT .... Turnaround time

Terms

in silico: Computation and/or experimentation performed on a computer.

Kit-based extractions: Manufacturer produced extraction technology.

Mutation: A permanent change to an organism's genetic code.

Polymerase Chain Reaction (PCR):

- **Digital polymerase chain reaction (dPCR):** This technique detects and quantifies nucleic acids in genetic material after partitioning (dividing) the sample. Single templates are amplified, resulting in exact quantification using statistical analysis.
- **Droplet digital polymerase chain reaction (ddPCR):** In droplet digital PCR, a sample is partitioned into thousands of droplets. Each droplet goes through the amplification (PCR) reaction, and then genetic material is measured in each droplet as positive or negative based on a fluorescent output. The output is then processed using Poisson statistical analysis to provide exact quantification.
- **Reverse transcription quantitative polymerase chain reaction (RT-qPCR):** This laboratory technique quantifies an RNA target by first converting mRNA to cDNA, and then amplifying and quantifying the cDNA using primers and fluorescently labeled probes. This method requires standard curves for quantification.

Sequencing: Determining the order of nucleotides (building blocks or bases) in a DNA molecule.

- **Amplicon Sequencing:** A targeted approach of sequencing for specific genes, such as the 16S gene.
- **Next-Generation Sequencing (NGS):** Refers to sequencing various lengths of DNA and RNA genes and genomes.
- **Shotgun Metagenomics:** A sequencing method that captures all genetic material in a sample.
- **Whole Genome Sequencing (WGS):** Refers to sequencing the entire genome.

Single nucleotide polymorphism (SNP): A single mutation in the virus's genetic code, which may or may not result in an observable phenotype.

Variant: A variant is a viral genome (genetic code) that may contain one or more mutations.

Variant of concern: A variant for which there is evidence of an increase in transmissibility, more severe disease (e.g., increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures.

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7700 Wisconsin Avenue, Suite 1000 Bethesda, MD 20814 | www.aphl.org | 240.485.2745

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