SARS-CoV-2 Wastewater Surveillance Testing Guide for Public Health Laboratories

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APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

CONTENTS

All the information contained in this document is based upon current information. APHL will plan to update this document as needed.

OVERVIEW OF WASTEWATER-BASED DISEASE SURVEILLANCE

The purpose of this document is to provide an overview of the SARS-CoV-2 wastewater laboratory setup and analytical process to orient public health laboratories as they implement testing. This guidance provides details on the management system and controls needed to produce quality, robust wastewater testing data that can be reported to health officials and to the US Centers for Disease Control and Prevention (CDC) National Wastewater Surveillance System (NWSS) for public health decision making. Topics covered include the role of the public health laboratory in wastewater surveillance, wastewater surveillance testing methods, laboratory infrastructure needed for wastewater testing and program performance evaluation.

Wastewater surveillance is the strategic sampling and testing of wastewater to detect pathogens or other health targets to better understand disease burden and spread within a community. Wastewater surveillance is a promising tool for the COVID-19 response because the virus is shed in the feces of up to 80% of infected individuals—both symptomatic and asymptomatic. Wastewater data are also unique from case-based surveillance because wastewater surveillance captures subclinical infections and data are independent of healthcare-seeking behavior and testing access. Since a large portion (~75%) of feces in the United States will be flushed into sewers and arrive at a wastewater treatment facility within hours, untreated wastewater can be seen as an efficient pooled fecal sample representing community level or sub-community level infections if strategically sampled from within the piped network. Finally, wastewater data have shown to be a leading indicator of trends in new reported cases by anywhere from four days to even a few weeks. With these benefits, it is important to note wastewater cannot determine the most effective mitigation strategies for reducing transmission within a community. Consequently, wastewater-based disease surveillance is not a solution to be used in isolation, but instead a tool used to complement other public health measures and indicators.

For more information on wastewater surveillance, see the NWSS website, which includes an overview of wastewater surveillance, the advantages of this approach and guidance on engaging with NWSS. The site also provides guidance for implementing wastewater surveillance, including developing sampling strategies, testing methods, data reporting and analytics, public health interpretation and use, targeted wastewater surveillance at facilities, institutions and workplaces, and application in low-resource waste systems. More resources can also be found in Appendix D.

ROLE OF PUBLIC HEALTH LABORATORIES IN WASTEWATER SURVEILLANCE TESTING

Wastewater surveillance for the COVID-19 response started as a grassroots effort by researchers and wastewater utilities to demonstrate the power of this emerging public health tool. As this effort grows into a sustainable, nationwide disease surveillance system, wastewater testing will need to rapidly transition out of the research laboratory and into industry and public health laboratories that can provide the necessary capacity and public health reporting abilities. Public health laboratories are particularly well-suited for this type of testing because, unlike traditional environmental testing for regulatory-based prevention monitoring, the wastewater data are used for public health action. As such, these wastewater data are more similar to clinical test results for public health surveillance and response, which require rapid coordination with public health partners. Public health laboratories will also play an important role in expanding wastewater surveillance targets beyond COVID-19, informing future target development with community needs.

BENEFITS FOR ESTABLISHING WASTEWATER TESTING IN THE PUBLIC HEALTH LABORATORY

- Ability to rapidly adjust testing scale and surveillance targets based on public health need
- Facilitate reporting of testing data to epidemiologist and public health decision makers
- Robust interpretation of data resulting from this unique sample type that may be unfamiliar to many epidemiologists and medical doctors
- Potential for future harmonization of methodologies/data interpretation
- Network of connected labs where new needs could be rapidly implemented across the nation

Wastewater surveillance provides a flexible platform to tailor targets as needed, from antibiotic resistance to emerging pathogens. Figure 1 describes the data flow for NWSS and highlights the role of laboratories in coordinating between key stakeholders at wastewater utilities and public health departments. See NWSS for more information on wastewater data reporting and use of wastewater surveillance data.

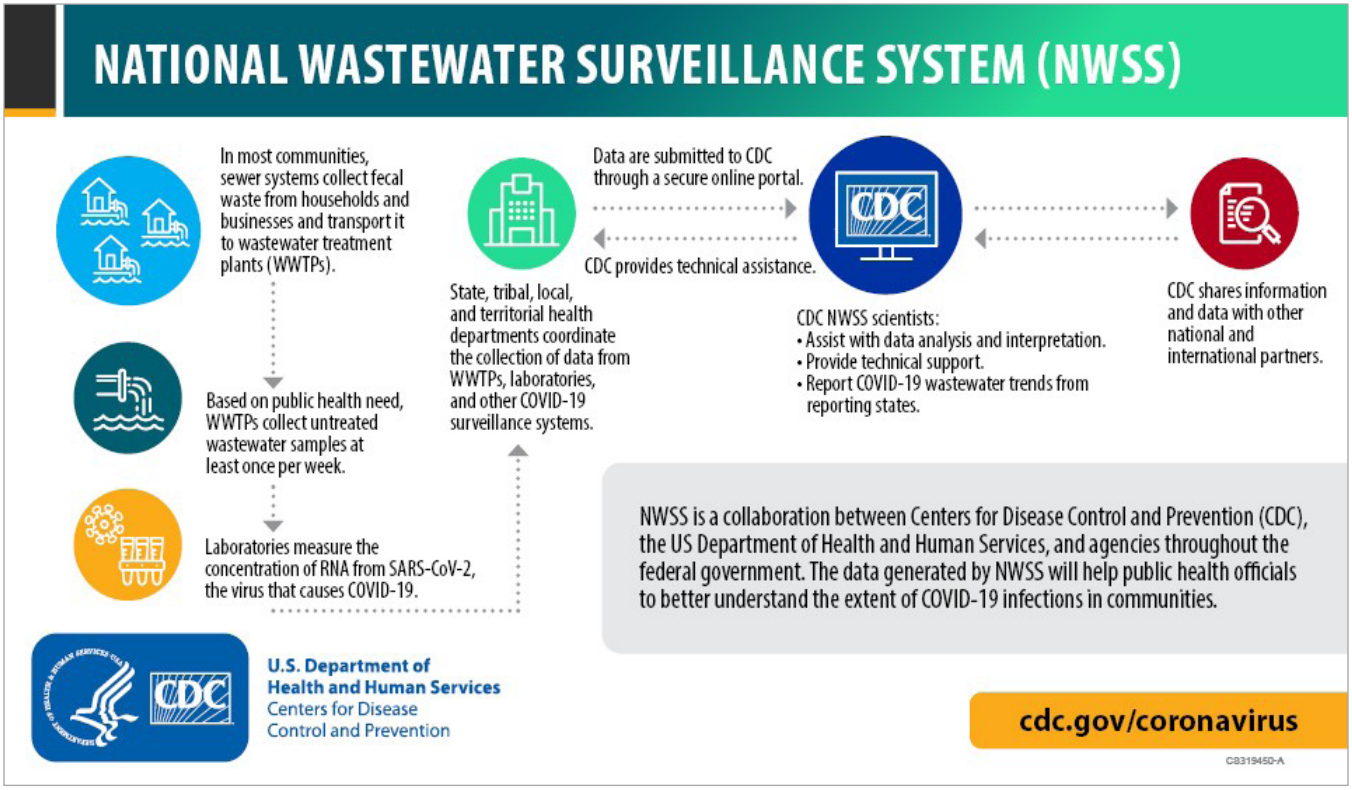

Figure 1. Schematic of the data flow for the United States CDC National Wastewater Surveillance System (NWSS). Public health laboratories conducting wastewater testing serve as an efficient link between utilities collecting and submitting samples and public health epidemiologists and decision makers analyzing and reviewing the data. Source: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/data-reporting-analytics.html

WASTEWATER SURVEILLANCE TESTING METHODS

There is no single method for SARS-CoV-2 testing for wastewater surveillance. The amount and type of processing required to concentrate a wastewater sample for viral quantification will be dependent on a variety of factors, including the number of infections in the community and the sampling location. For example, sludge samples, also called biosolids, can only be collected at certain wastewater treatment plants. In addition, COVID-19 infection rates have been sufficiently high in certain locations where concentration of a wastewater sample was not needed for quantification of the virus. Instead of a single standard method, there are standard performance criteria and quality controls required for wastewater testing mandated by public health reporting systems, such as NWSS. A public health laboratory will choose a wastewater testing method from a suite of high performing options based on laboratory capacity and both local and national public health data needs.

Figure 2 presents an overview of the wastewater testing process. Following sample collection, the first step in SARS-CoV-2 wastewater testing is sample preparation, consisting primarily of homogenization of the sample through processes like blending or sonication. During this step, a matrix recovery control should be spiked into the sample. The second step is sample concentration of the virus, through procedures such as filtration or pelleting by centrifugation. The third step is RNA extraction from the concentrated wastewater sample using methods that are specifically designed to remove high concentration of environmental inhibitors. The final step is RNA measurement by reverse transcription quantitative

polymerase chain reaction (RT-qPCR), RT digital PCR (RT-dPCR), or droplet digital PCR (ddPCR). These steps in the testing methodology are further described on the NWSS Testing Methods website and in the resources provided for this section.

Example wastewater testing protocols that have demonstrated robust method performance are provided by NWSS on Protocols.io.

Wastewater Samples

Sample type is an important consideration for collecting representative samples and will depend on the sample collection location and factors specific to the wastewater treatment system. There are two wastewater surveillance sample types: untreated wastewater and primary sludge. Untreated wastewater includes waste from household or building use (e.g., toilets, showers or sinks), which contains human fecal waste, as well as waste from non-household sources (e.g., rainwater or industrial use). Untreated wastewater may be sampled from wastewater treatment plant influent prior to primary treatment, or upstream in the wastewater collection network. Primary sludge comprises suspended solids that settle out of wastewater during the first solids removal ("sedimentation") process at a wastewater treatment plant. Primary sludge is distinct from secondary sludge following primary treatment. Primary sludge may not be available at all wastewater treatment plants.

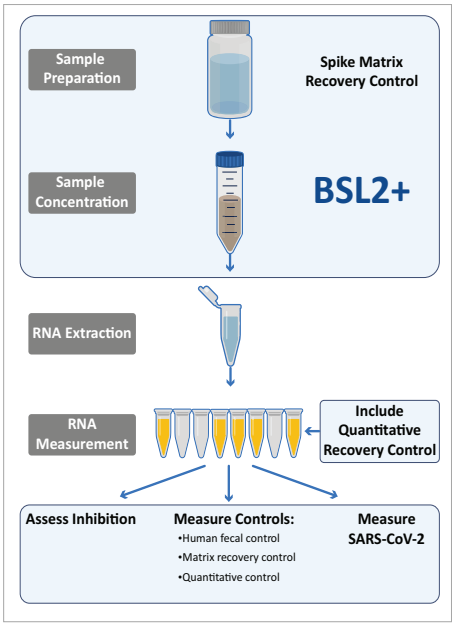

Figure 2. Overview of wastewater testing process. While untreated wastewater is visually represented, the major steps are consistent for sludge and swab sample types. Source: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/developing-a-wastewater-surveillance-sampling-strategy.html

There are two predominant sample collection methods for wastewater-based disease surveillance: grab sampling and composite sampling. Grab samples represent a single point in time, can be collected rapidly, and do not require automated equipment. Composite samples are collected by pooling multiple grab samples at a specified frequency over a set period—typically 24 hours—or are collected using a swab placed within the wastewater for a specified time.

KEY CONSIDERATIONS WHEN COLLECTING WASTEWATER FOR DISEASE SURVEILLANCE

- All samples should be kept cold during collection and transport at 4°C (2-8°C) until processing.
- Collecting and sending at minimum two processing volumes worth of sample to the processing lab from a given site allows for immediate processing and back-up, archiving, or replication.
- Sample collection metadata should be recorded at the time of sampling on the chain of custody form. Metadata include details such as total and average flow over the sampled time period, sample type (grab or composite), wastewater type, collection date and time and collection location. Total suspended solids, water temperature, time, pH and biological oxygen demand (BOD) may also be included.
  Note: If a laboratory wishes to report data to NWSS, they must be able to collect the required data needed for public health interpretation which includes wastewater plant, sample collection, and analysis information. Examples of sample collection fields include sample type (grab or composite), wastewater type, collection date and time, collection location, and collector name.
- For untreated wastewater and sludge, the volume of sample to collect will depend on the sample type (wastewater or sludge). The volume of sample that is concentrated and quantified will determine the lowest amount of SARS-CoV-2 RNA that can be detected. The sensitivity of the testing needed will depend on the phase of the epidemic monitored, with higher sensitivity needed when cases are emerging or disappearing from a community, and lower sensitivity being adequate when cases are relatively high. For example, SARS-CoV-2 could be quantified in a one milliliter sample of wastewater without concentration during certain periods of high case incidence during the COVID-19 pandemic.

Therefore, composite or swab samples can be more representative of the inputting population because these methods capture diurnal variation in wastewater flows and inputs. The US Environmental Protection Agency (US EPA) protocol for wastewater sampling may be a helpful resource:

- National Pollutant Discharge Elimination System (NPDES) Compliance Inspection Manual - Chapter 5 (epa.gov)
- Procedures for Collecting Wastewater Samples | US EPA

If the laboratory does not have the capacity to sample, it is common practice for a public health system partner to assist. These partners can include wastewater treatment plants, utilities and health department or environmental agency staff. The Water Research Foundation has compiled a useful best practices document.

Wastewater Testing Protocols

The major wastewater testing steps include 1) sample preparation (including homogenization through blending, sonification, etc.) and inactivation for biosafety; 2) sample concentration through filtration or pelleting by centrifugation; 3) extraction of RNA through methods that are specifically designed to remove high concentration of environmental inhibitors; and 4) viral quantification by RT-qPCR or digital (or droplet digital) RT-qPCR (Table 1).

The wastewater testing steps unique from clinical testing include preparation, concentration of the virus depending on the infection rate within the community, and quantification of the virus and associated controls (Figure 2). The quantification controls include using standard curves (i.e., dilution series of known amounts of the genomic material of the target organism), quantitatively assessing the impact of any inhibition, spiking in a matrix control to account for loss of the virus during processing, and/or measuring the human fecal load to account for changes in the population contributing to the sewer over time and dilution of the virus from non-human waste inputs. For each of these steps, a specific method and control material will be chosen based on several interconnected factors including cost, equipment available, level of automation desired, personnel expertise and public health data needs.

KEY CONSIDERATIONS FOR SELECTING A WASTEWATER TESTING METHOD

- Wastewater samples should be processed as soon as possible - preferably on the day of receipt. Otherwise, samples should be stored at 4°C until processing (no longer than four days) and never frozen until archiving at -80°C either in raw wastewater or extracted nucleic acid form.
- Sampling programs can be structured to minimize hold time prior to processing, such as coordinating most samples to arrive on specific days of the week more conducive to the initial time-consuming concentration steps.
- Method selection for wastewater testing will be dependent on staffing capacity, making certain workflows work better than others (e.g., membrane filtration or sludge testing versus often more time-consuming PEG precipitation and centrifugation of larger volumes). The *Testing Scale and Reporting Time* on page 7 has more details on this topic.

Given that quantitative estimates of SARS-CoV-2 levels may vary across laboratory methods, it is advisable for a laboratory to select a single method and use it consistently over time to enable reliable comparisons of concentrations. For this reason, methods less susceptible to supply chain limitations are preferred. The exception to this rule is methods may be altered in response to categorical changes in community COVID-19 case levels during different phases of the pandemic. Changes may be made to improve method sensitivity when case levels are low or to reduce laboratory resource requirements when levels are high. For a testing laboratory to decide the most cost-effective and successful testing and data reporting methodologies, programmatic questions need to be addressed by the wastewater surveillance program team that includes epidemiology, wastewater plant personnel and the laboratory. See Appendix C for a list of programmatic questions to be considered when selecting a testing method.

Table 1. Common sample types and methodologies for wastewater testing of SARS-CoV-2 surveillance. Names of concentration and extraction kits are provided as examples commonly used and are not an exhaustive list or endorsement of a product. See required equipment in in the *Laboratory Space and Equipment Needs* section on page 8.

Inactivation	Concentration	Extraction	Quantification	Targets

Heat	• PEG-centrifuge
	• Centrifuge
	• Membrane filtration
	• Nanobeads:
	  ○ CeresNano
	  ○ Invitrogen Dynabeads
	• Ultrafiltration
	• InnovaPrep filter pipette
		Kit-based:
		• QIAGEN PowerViral
		• QIAGEN PowerSoil (sludge)
		• ThermoFisher MagMax Wastewater
		• IDEXX Magnetic Bead
		• Promega Wizard Enviro
		Kit-free:
		• Trizole
			• RT-qPCR
			• Digital-RT-PCR
			• Droplet-RT-dPCR
				• N-gene
				• N1
				• N2
				• E-gene
				• ORF-gene

Variant Tracking Through Wastewater

As a pathogen mutates and spreads over time, tracking emerging variants through wastewater allows public health officials to better understand the dynamics and transmission of infections in the broader community, monitor the spread of variants of concern (VOC), and potentially identify emerging mutations in the community before clinical detection. While the science behind variant tracking in wastewater is still rapidly developing, a multifaceted approach can be used to leverage genomic data for variant tracking in wastewater for public health decision making. This could include combining data learned from more than one approach, such as:

- Quantifying of one or more known mutations found in a variant of interest or concern to evaluate presence and trends over time.
- Applying bait capture and/or whole genome amplification laboratory technologies for identifying possible novel mutations or variants.
- Using clinical genomic data to confirm presence of a variant in a community upon indication in wastewater.
- Evaluate variant data to assess clinical testing algorithms.

Laboratory equipment, space and expertise needed for targeted mutation-specific tracking and sequence-based variant tracking are similar to the requirements for qPCR and clinical variant sequencing, respectively. However, it is important to note that clinical bioinformatic pipelines for variant detection cannot be directly applied to wastewater/environmental samples because:

- Nucleic acid can be highly fragmented in wastewater.
- There is likely a diversity in variants present.
- Potential for cross reaction with non-human strains (e.g., rat coronaviruses).

Development and evaluation of bioinformatic pipelines for variant detection in wastewater samples is ongoing.

The National Center for Biotechology Information (NCBI) and NWSS provide guidance for submitting wastewater surveillance sequence data to GenBank, an NCBI database that facilitates global coordination of genomic disease surveillance data.

Testing Scale and Reporting Time

To utilize the data for real-time public health response, wastewater testing data must be reported within two to three days of sample receipt. Therefore, laboratory capacity must be structured to achieve minimum turnaround time. Sample throughput will depend on many factors including the virus concentration method, extraction automation, PCR format (96 or 384 well), and number of markers (i.e., viral targets and controls) quantified. Extraction automation is recommended for sample loads of greater than 50 per week, and, if possible, PCR and extraction equipment should be reserved solely for wastewater testing to maximize capacity and limit cross-contamination with clinical samples.

Throughput can be greatly increased if infection burden within a community is sufficiently high for viral quantification from direct extraction of wastewater, in-lieu of a concentration step. Similarly, testing of primary settled sludge (also referred to as biosolids) can increase throughput due to smaller sample volume required for equivalent or lower sensitivity to untreated wastewater (liquid and solids combined).

GUIDELINES FOR WASTEWATER TESTING WEEKLY THROUGHPUT

- **Small scale:** < 50 samples per week
- **Medium scale:** 50-150 per week
- **Large scale (high throughput):** >150 samples per week, often commercial laboratories

LABORATORY INFRASTRUCTURE FOR WASTEWATER TESTING

Laboratory Personnel

Previous experience with environmental samples is especially valuable in understanding the complexities of wastewater SARS-CoV-2 analysis. Laboratory staff who would have these types of skills include those with experience conducting recreation water monitoring for molecular targets (e.g., *Escherichia coli* or enterococci qPCR monitoring), microbial source tracking (MST) for species-specific molecular fecal markers (e.g., HF183), or rare species tracking with environmental DNA surveillance. Masters and PhD level individuals may have this experience through research or experience in a regulatory or utility laboratory. Bachelors level individuals can have this experience through working within a regulatory or utility environmental laboratory.

The number of laboratory full-time employees (FTEs), personnel and bench space needed for testing wastewater from sample collection to result per sample or batch of samples will depend upon the scale of the operation, staff expertise and the use of automated equipment (e.g., automated extraction or molecular extraction preparation). Scale and use of automation will be driven by the necessary turnaround (e.g., within 2-3 days of receipt) and types of data reported (e.g., minimum NWSS reporting, quantitative mutation detection, sequencing). Additional field staff will be needed if the laboratory is also collecting the wastewater samples (additional time needed will include travel, cleaning and disinfection of personnel and equipment, etc.).

GUIDELINES FOR STAFFING NEEDS BASED ON TESTING THROUGHPUT

- **Small scale:** 1.5 FTE trained laboratory scientist and 1 FTE PhD level (or equivalent) lead
- **Medium scale:** 3 FTE trained laboratory scientist and 1 FTE PhD level lead
- **Large scale (high throughput):** at minimum, staff for medium scale but dependent on level of automation

Laboratory Space and Equipment Needs

Designated bench and floor space are required for specialized and/or dedicated wastewater processing and testing equipment. While wastewater laboratory equipment may be applicable to other testing needs, equipment may need to be dedicated to wastewater surveillance activities due to biosafety requirements (see *Biosafety* section on page 10). If cross contamination with clinical samples is a concern, necessary bench space should be solely dedicated to wastewater testing. Laboratory space needs will be in part dependent on the testing method, number of samples processed per week, and extent of automation. Below are lists of general laboratory equipment required for conducting wastewater testing and of optional equipment based on method selected.

General Required Equipment

- Refrigerator (4 °C) with sufficient space to temporarily store sample volumes and nucleic acid extracts until processing is complete—depending on number of samples and sample volume received weekly, more than one

fridge may be required
- Freezer (-80 °C) with sufficient space to store RNA/DNA extracts for archive and/or future testing (e.g., sequencing, other health targets, controls, and reagents) if remaining unprocessed wastewater is archived for future testing, more freezer space may be needed
- Freezer (-20 °C) is used as interim storage for frequently accessed supplies such as reagents, RT-PCR Master Mix, cold block and extracted material prior to its storage at -80 °C
- Biosafety safety cabinet and bench space dedicated for wastewater testing - the amount of bench space required will depend on concentration method used and automation equipment
- Large autoclave for biological waste disposal (can be shared with non-wastewater laboratories)
- Homogenizer equipment for pre-concentration sample mixing (i.e., shaker, blender, stir plate)
- Vortex mixer for small volume sample mixer
- Microcentrifuge for spinning down mixed samples
- Heating block for warming reagents (if needed)
- Nucleic acid quantification equipment (i.e., microvolume spectrophotometer or fluorometer)
- UV crosslinker for molecular sterilization of PCR equipment and consumables
- PCR hood for dedicated clean space to prepare Master Mix
- Pipettors and micropipetters (10, 200, 1000 µL) and corresponding tips
- Tube racks (appropriate sizes)
- 2 mL cryovials
- Cooling block for cryotubes, enzymes
- Chemical fume hood or exhaust hood when instruments don't fit in fume hoods due to size restrictions.

Method Specific Equipment

Note: This list is not exhaustive. Refer to detailed method descriptions for full equipment and consumable lists and approximate footprints.

- **Sludge**: laboratory oven with exhaust hood for dry weight solids measurement
- **Pasteurization**: water bath or heat block depending on volume
- **Concentration**
  - PEG/Ultrafiltration/Centrifugation methods: large volume (15 mL to 500 mL) high speed (up to 10,000xg) centrifuge (preferably refrigerated to at least 4 °C to minimize decay of genomic material during processing); trip scale for balance centrifuge rotors
  - Membrane filtration: vacuum manifold(s), funnels, autoclave or alternate sterilization systems for reusable filtration apparatus
- **Extraction**
  - Manual: bead beater (or vortex adaptor for bead beating)
  - Automated extraction system: example equipment includes QIAcube (QIAGEN), Maxwell® (Promega), or KingFisher™(ThermoFisher)

Spatial Layout for Contamination Control

The key to a wastewater testing laboratory spatial layout design is to dedicate separate spaces to handling steps that have different biosafety risk and cross-contamination profiles. Commonly, separation is achieved by separating profiles by rooms, but in laboratories with larger rooms, separate areas can be designated for each profile if there is low probability of cross-contamination. For the most controlled levels (Level 1 or 2), separate, contained equipment or working space is preferred. This includes laboratory tools and reagents that are used solely for these purposes. The personnel working in these levels may require personal protective equipment (PPE) specifically for these areas or designate specific personnel to work in certain areas on a given day. For levels 3 or 4, ensuring that the workspace is properly cleaned and separated from other ongoing laboratory work is typically sufficient, but should be decided by the user.

EXAMPLE PROFILE SCHEME MOVING FROM THE MOST CONTROLLED SPACE (LEVEL 1) TO THE LEAST CONTROLLED (LEVEL 4)

Level 1: Master Mix preparation only and no RNA or PCR product storage/use allowed

Level 2: PCR plate preparation with no PCR product storage - a separate L2 area can be used for standard dilution

Level 3: PCR reactions and product storage

Level 4: Wastewater processing and extraction

Biosafety

Biosafety should be determined by the testing laboratory through site- and activity-specific biosafety risk assessments and will vary based on the sample matrix and test method(s) being performed. Procedures that concentrate viruses, such as precipitation or membrane filtration, can be performed in a BSL-2 laboratory with unidirectional airflow. Procedures with a high likelihood of generating aerosols or droplets should be done using a certified Class II Biological Safety Cabinet (BSC). Additional precautions to provide a barrier between specimen and personnel should be considered to reduce the risk of exposure to personnel including PPE, physical barriers, facility controls and equipment controls.

At a minimum, laboratories will need appropriate BSC space (depending upon sample numbers and type of viral concentration method). Initial intake of raw wastewater is recommended in a dedicated BSC located in a different room than used for PCR. Pasteurized samples may be approved for handling outside of a BSC.

When working with a potentially infectious agent, it is important to consider the National Institutes of Occupational Safety and Health (NIOSH) Hierarchy of Controls (Figure 3). This inverted triangle depicts the most effective and protective forms of control are found at the top (elimination) with the least effective and protective at the bottom (PPE). Following this hierarchy will lead to less transmission of disease in the laboratory.

More information on wastewater testing biosafety and CDC's guidance can be found on CDC's website. For more general information on biosafety, please see the *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition*.

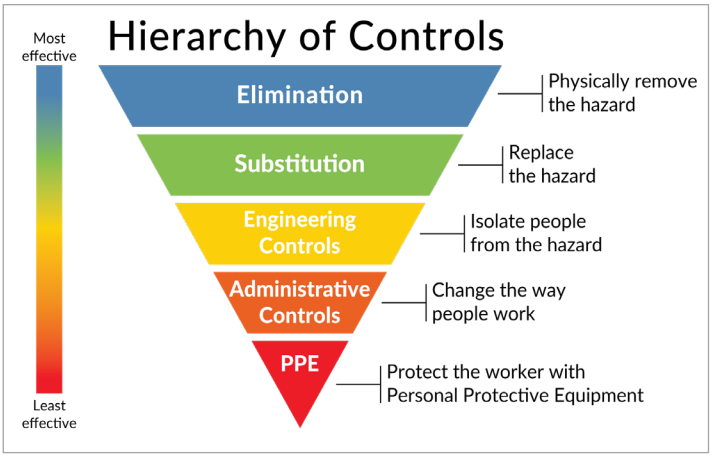

Figure 3. National Institutes of Occupational Safety and Health (NIOSH) Hierarchy of Controls. Source: https://www.cdc.gov/niosh/topics/hierarchy/default.html

Risk Assessment

All laboratories should perform a site-specific and activity-specific risk assessment. APHL has developed example risk assessment for Ebola testing that can aid in developing a wastewater testing specific risk assessment.

Risks and mitigation steps should be prioritized based on likelihood of occurrence and potential negative outcomes. As with any risk assessment, it is important to consider the whole picture as well as each step. For example, some methods include a water bath pasteurization step prior to processing. For maximum biosafety risk reduction, the water bath could be placed in the BSC during that process. However, this could also create more risk of injury to staff, a greater water spill/splash risk, and risk of dropping/breaking the equipment to move the bulky water bath in and out of the BSC for the inactivation step. During pasteurization when the capped samples will be in a sealed bagged and sitting in a water bath,

the risk of generating aerosols is very low. Therefore, the risk created by moving the water bath in and out of the BSC was more likely and greater than the risk of processing wastewater samples outside the BSC.

KEY CONSIDERATIONS WHEN DETERMINING RISK

- Testing volume
- Sample matrix
- Procedure(s) performed
- Likelihood to generate infectious aerosols or droplets.

KEY CONSIDERATIONS FOR DETERMINING MITIGATION MEASURES

- Laboratory biosafety level and facility controls
- Competency of personnel
- Laboratory equipment.

Waste Disposal

Based on the designated biosafety level designation, decontaminate and/or dispose of all samples, reagents and other potentially biohazardous or contaminated materials in accordance with local, state and federal regulations.

WASTE CONSIDERATIONS FOR WASTEWATER TESTING

- The unused portion of non-pasteurized samples should be autoclaved. The unused portion of pasteurized samples may be poured down the drain. If using commercial products, follow the disposal instructions provided by the supplier.
- Do not autoclave the PCR plates. Autoclaving the sealed plates will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.

Information Technology, Security, and Data Coordination

If data will be sent to a public health agency, information technology (IT) infrastructure requirements should be considered. For laboratories with experience submitting other types of health surveillance data, the IT infrastructure and processes are likely similar and already in place. For laboratories new to submitting surveillance data, it can be a substantial time and resource investment to set up procedures for automating data entry where possible, and validating manually-entered data where manual entry is required. Additionally, secure data storage and transfer software will likely be necessary if sensitive or personally identifiable information are recorded. For both new and experienced laboratories,

KEY CONSIDERATIONS FOR WASTEWATER DATA SYSTEMS

- **Simultaneous entry and revision of data by multiple parties**. Need for the laboratory, the sample collector, and the wastewater utility (if different from the sample collector) to collect and collate linked data pertaining to wastewater sample metadata and laboratory results. A key challenge for many teams has been that some important metadata, such as wastewater flow rate or endogenous wastewater control test results, may not be available for days following the SARS-CoV-2 test result. Additionally, quality control measures may result in the need to retrospectively alter data.
- **Relatively large number of fields to report**. Given the variety of protocols and amount of metadata needed to interpret wastewater surveillance test results, over 40 fields are required (as of September 2021) for submission to the NWSS DCIPHER platform.
- **Reporting quality control data**. Many laboratory information management systems (LIMS) do not have this feature built into the system so laboratories will need a mechanism for retrieval and reporting.
- **Sample tracking through LIMS from receipt to storage and finally discarding**. As part of sample tracking, laboratories and surveillance programs should determine a plan for sample archive and disposal, as well as policies for potential additional testing.

it is advisable to begin conversations about reporting formats and requirements and data security concerns early, as these requirements may impact laboratory workflows and take time to set up.

Example: Robust Data Management System

- Electronic test ordering procedure that produces test request form and barcoded sample labels.
- LIMS that allows data entry and revision by sample collection partners and/or wastewater utilities in a way that automatically links to electronic test order.
  Note: Providing LIMS access to utility, academic, or commercial partners may be a hurdle, and the implications for data entry, transfer, and revision should be considered. Some laboratories receive a paper chain of custody form with wastewater metadata from the utility, and then digitize those data along with their test results.
- LIMS that allows all data submission partners to view results in real time.
- Automatic data transfer using modern data messaging protocols from the LIMS to epidemiologists/program managers. If this is not possible, then a secure way to transfer data files from the LIMS to epidemiologists/program managers should be used.
- Automated data validation schemes should be implemented in the LIMS to the extent possible. Additional validation may be implemented outside the LIMS as necessary.
- Contact NWSS about data transfer at NWSS@cdc.gov.

PROGRAM PERFORMANCE EVALUATION

Evaluating a laboratory wastewater testing program's performance includes both training of laboratory staff and initial validation of protocols, as well as ongoing verification of the protocols. Continued verification of the protocols can inform any needed modifications to the program and can include periodic internal blind testing both within and between laboratories. Verification processes can also provide opportunities to benchmark performance of new team members, where substantive differences in both the nature and scale of the checks can be identified. Performance evaluation includes several factors related to methodology and data quality, as described below, and should be outlined in the laboratory's quality assurance program plan.

Training and Proficiency Testing

The cornerstone skill preferred for management of a wastewater testing laboratory is molecular environmental microbiology (PhD level). This person(s) will lead method selection and program development—both of which will require adjustments throughout the phases of outbreak, depend on public health data needs, and need to account for the unique aspects of the target pathogen, in this case SARS-CoV-2. Troubleshooting oversight will be the major activity for this person, particularly during the method selection and staff training phases. If possible, leadership by junior molecular-trained personnel is ideal for training other staff and managing day-to-day laboratory operations, including data review, supply ordering, equipment maintenance and quality management.

Laboratory staff conducting wastewater testing can be Bachelor's (minimum) or Master's level microbiologists with environmental microbiology and/or molecular microbiology experience. Specific methodology training can be gained from method-experienced staff. As all steps in wastewater testing require attention to detail, the following skills are required:

- Precise micro-pipetting ability
- Knowledge and attention to contamination controls
- Understanding and experience with laboratory biosafety

All entry-level/support staff should be required to demonstrate method proficiency by performing an initial demonstration of capability (IDC) and annual continuing demonstration of performance for each specific aspect of the method. If possible, at least one support staff person should be skilled in raw molecular data-processing and validation.

Internal Quality Assessment and Method Validation

When initially gaining experience and validating a method, internal quality assessments are important, which primarily

involves duplicate sample processing, particularly at the concentration stage (i.e., two separate filters or two separate PEG precipitations). During routine sample processing and testing, archiving a duplicate of the concentrated sample is a stable and long-term "archiving" approach compared to freezing raw wastewater due to freezer space constraints (e.g., small membrane filter, PEG precipitate or extracted nucleic acid).

The major components of a robust quality assessment program are within (intra-) laboratory quality assessments that evaluate precision, accuracy and contamination—both overall and within major steps (e.g., concentration, extraction, quantification). In addition, evaluation of inhibition, recovery efficiency, method limit of detection, and limit of quantification are critical validation/verification components for the quantification step.

1. Precision

Laboratories should first evaluate method precision when assessing method performance. Precision characterization (i.e., method uncertainty and variability) is fundamental to the interpretation of the data in the context of epidemiology and public health decision making. Precision should be evaluated at each step in the wastewater testing method during method development to assess method robustness and/or validation of a new method. Precision characterization should also remain a component of the method's routine performance evaluation but at reduced effort.

Precision is assessed from "replicate" samples—the nature of the replicates will vary depending upon which step of the process you are evaluating (i.e., concentration, extraction, quantification). Regardless of the step being evaluated, it is critical to have homogenous samples with which to make these step-specific precision assessments. A recommended approach for new method validation is to conduct at least seven replicates for each step listed below. An overall method precision represented by the relative standard deviation (RSD) target at the 50,000 genomic copies (gc) per liter level of +/- 30% is reasonably acceptable.

Strategies for ongoing precision assessment may vary between laboratories with the greatest emphasis usually on molecular replicates. The extent of a laboratory's ability to incorporate replication will in part depend on the level of automation available and PCR platform (e.g., 384-well qPCR plates allow more replication). Spiking and assaying for exogenous and morphologically similar viral targets into sample, as well as assaying for surveillance target (e.g., N1 and N2 SARS-CoV-2 targets), adds substantial values to these precision assessments by distinguishing between replicate variability from insufficient mixing versus methodological and technician inconsistency.

Precision Evaluation by Wastewater Testing Steps

- **Concentration**: Processing replicates of homogenized raw influent, processed identically through all wastewater testing steps. One approach is to routinely triplicate every sample at the concentration step (e.g., filter three separate aliquots from a homogenized wastewater sample), but NOT routinely further process more than one filter (e.g., archive the other two filters at -80°C). Archived filters can then be pulled for trouble-shooting purposes or proficiency testing of analytical staff.
- **Extraction**: Generating a large quantity of pooled homogenized concentrate aliquoted into replicate extraction vials for replicate extraction (e.g., at least duplicate).
- **Quantification**: Environmental molecular microbiology best practice recommends triplicate PCR (dPCR, qPCT or ddPCR) for each assay on every sample (see EMMI guidelines in Appendix D), and if the relative standard deviation (RSD) across these triplicates is >20%, re-running the PCR should be considered, particularly at higher Cq- (also referred to as Ct-) values at which there is higher measurement uncertainty.

2. Accuracy

While a more challenging performance metric to measure and interpret, accuracy should be assessed at multiple levels, including sample, plate, and batch. Accuracy also includes accounting for variability in target recovery and contributes to data quality and utility.

Quantitative Positive Control

Quantitative positive control (target RNA or cDNA), also referred to as a template control, of a known SARS-CoV-2 target concentration should be run at least in duplicate with each PCR instrument run. Multiple small aliquots should be maintained to avoid extensive reuse/freeze-thawing (≥ 30 µl per aliquot). Quantitative values should be within +/-

15%; if not, positive control and aliquots should be re-made.

Calibration (real time qPCR only)

To ensure optimal performance of the RT-qPCR protocol, standard curves of genomic material should be run in duplicate (at minimum) with each instrument run. Metrics, such as correlation coefficient and efficiency should be documented, with the following criteria:

- R^2: >0.95
- Efficiency: 90-110%
- Exponential Amplification Value: 1.95-2.05

Multiplex Efficiency Confirmation

If you plan to test for multiple targets per run (multiplexing), confirm the accuracy of the multiplex by comparing with each marker single-plexed. This should be performed during method development and re-evaluated if standard curves do not meet the criteria above.

Matrix Recovery Controls

Spiking in a matrix recovery control (e.g., Bovine coronavirus) into every sample immediately upon receipt in the laboratory prior to pasteurization (if possible) is recommended. Consistent recovery of spiked matrix controls should be a goal of the initial method development and validation. While in analytical/biochemical assays a recovery of 1% would be considered unacceptable, wastewater recoveries of 1% and even as low as 0.1% are acceptable. Substantial variations in recovery can be observed between wastewaters, and the drivers of this variability are not well understood. Despite this limitation, matrix recovery controls are essential for flagging samples for re-testing or adding an outliner comment to the report due to low recovery (<1%). As the field develops, these controls will improve the mechanistic understanding of low and variable recoveries. A spiked external certified reference material (CRM) or standard reference materials (SRM) is ideal to evaluate accuracy for every sample given the instability of wastewater matrices. However, those are currently not available for wastewater testing.

Within Testing Recovery Control

Spiking of heat inactivated or genomic material of target(s) at a known concentration into wastewater testing steps post-concentration can evaluate recovery at each step, such as extraction. This should only be performed during method development and evaluation or if a run returns aberrant results, not on every instrument run.

Direct Extraction

Extracting wastewater without concentration circumvents the highly variable steps of the wastewater testing method (i.e., pre-concentration and lysing of microbial materials), enables more replication and, most importantly, enhances method accuracy. Direct extraction (DE) can be done by inputting multiple replicates of the homogenized wastewater sample (100-400 µL) directly into the extraction protocol. DE can provide an estimate of the pre-concentration step effectiveness (recovery) by comparing it to the matrix control recovery of the sample put through the entire testing method. The DE approach is also useful for troubleshooting samples that are exhibiting poor pre-concentration (low virus recovery) and/or (unexpectedly) low virus levels.

The two major limitations of the DE approach are:

- It is restricted to samples with relatively high virus concentrations (to achieve Ct values of <35, endogenous virus concentrations must exceed several hundred thousand gc/L).
- It is only representative of the small aliquots of wastewater.

Higher volume capacity extraction protocols and more sensitive PCR (e.g., dd or dPCR) will help mitigate these limitations.

Environmental Data Reasonableness

While caution is advised in the amount of weight put into the metric, evaluation of environmental reasonableness by experienced laboratory personnel can be useful in identifying suspect samples that may need additional examination. Approaches to evaluating reasonableness include asking:

1. Do the data fit with the current trend at a given site?
2. Is the magnitude reasonable?

3. Are the data consistent with other markers measured?

3. Contamination

Contamination should be consistently monitored, with checks at each step in the wastewater testing process to determine whether measured virus levels are real. Contamination can occur at any step, including sample cross-contamination or reagent contamination, and blanks can help determine which step was compromised. If contamination is observed in blanks, discarding all reagents and thoroughly cleaning any tools used from the contaminated step is the most efficient mitigation strategy. Any data associated with the contaminated reagents should also be marked and samples re-run or re-processed. For this reason, using small-volume working stocks of reagents helps prevent reagent waste when amplification of blanks or contamination controls occurs. Maintaining working stocks can save time, money and reagents if contamination does occur.

Method Blank

A method blank (also referred to as a full process blank), including the concentration step, should be run consistently based on program sampling scheme and laboratory resources (e.g., weekly or every 20 samples processed) and when training new laboratory staff to evaluate cross-contamination of samples. A method blank (e.g., molecular grade water) is processed in the exact same process as the wastewater sample. If the method blank amplifies, the plate must be re-run and a root-cause analysis carried out.

Extraction Blank

During the nucleic acid extraction process, a blank sample (e.g., molecular grade water) should be processed in parallel to wastewater samples. This control ensures there is no contamination of extraction reagents or cross contamination of samples during the extraction process. If any amplification is observed in the extraction blank, all reagents in the extraction kit/protocol should be discarded due to contamination risk and cross-contamination potential during the extraction process should be evaluated. Cross-contamination potential steps to consider include but are not limited to, bead beating or vortexing to lyse samples and centrifugation of spin columns with tubes open. The nucleic acid should be re-extracted from an aliquot of the same wastewater sample(s), and the plate should be re-run. At least triplicate PCR of each extraction blank should be run.

No Template Control (NTC)

PCR without template should be run on every qPCR plate/batch in triplicate (at minimum) to evaluate the presence of contamination in PCR reagents. If any amplification is observed in NTCs, the plate must be re-run and it is recommended to throw away all reagents used in contaminated plate (determination of contaminated reagents is possible, but time consuming).

4. Inhibition

Wastewater matrix composition (biological oxygen demand, total suspended solids, metals, industrial contributions, disinfectants, etc.) can contribute to poor virus recoveries, which may manifest in PCR inhibition. There are several approaches for mitigating or evaluating the extent of inhibition present in a molecular reaction. With the use of RT-qPCR commercial Master Mix and polymerase enzymes designed for environmental samples, PCR inhibition has been infrequently observed in wastewater surveillance testing. This suggests inhibition evaluation may not be required in routine evaluation but is useful during initial method validation or when testing wastewater from a new location.

Spike Control Virus

Spiking a control of known quantity (e.g., Bovine Respiratory Syncytial Virus) into each sample nucleic acid extract can be used to evaluate and quantify the extent of molecular amplification inhibition. If any (>5%) inhibition is observed, the sample extract is diluted and retested (see Extract Dilution).

Extract Dilution

Samples with documented PCR inhibition are diluted and re-run. It may be necessary to reprocess (concentrate) the sample with a smaller volume. Conversely, sample dilutions can be concurrently run with undiluted extracts to save time, though this approach will increase costs. While dilution can resolve inhibition, it can also dilute out the target

signal if the undiluted sample target is near the detection limit. Processing a smaller sample volume can help reduce inhibition, though would also increase the method detection limit (e.g., reduce sensitivity).

ddPCR/dPCR Platform

Droplet and digital-based PCR platforms have been demonstrated to be less subject to inhibitors than traditional RT-qPCR. In particular, the reverse transcription reaction occurring in small volumes (e.g., after droplet formation) has been shown to significantly reduce inhibition when testing sludge samples.

5. Endogenous Wastewater Controls

Endogenous wastewater controls are used to account for wastewater matrix variability. Human fecal normalization controls are assays targeting organisms or compounds specific to human feces that can be measured in wastewater to estimate its human fecal content. These human fecal controls (e.g., pepper mild mottle virus, *Bacteroides* HF183, crAssphage) are used as endogenous wastewater controls as these targets accompany virus targets through the sewage system and sample processing and thus can be considered subject similar decay and recovery. Therefore, normalization of quantitative virus data with these controls can help improve accuracy by accounting for variability in human fecal content and viral loss from source to test.

6. Limits of Detection (LOD)

LOD is commonly defined as an assay's ability to detect the analyte at low levels. A recommended approach for LOD assessment for environmental samples is outlined and published by Klymus et al., (2019). The Klymus protocol utilizes a published R script to generate three curves, which identify the 95% confidence interval of an LOD given a standard curve specific to a lab's protocol. The LOD will be unique to a testing method and lab performance, and it should be established using standards before processing unique samples. The LOD measures the sensitivity of the overall testing method, establishing a threshold of target copies/ml wastewater below which signal cannot be accurately detected but could be reported qualitatively. NWSS requires reporting of LOD by testing method.

7. Limit of Quantification (LOQ)

LOQ is commonly defined as the lowest amount of analyte in a sample that can be quantitatively determined with a stated precision under stated experimental conditions. The determination of this threshold will help a laboratory optimize the quantitative precision of their assay. Values below the LOQ should be evaluated qualitatively, as detections or non-detections. The assay's LOQ should not be lower than the assay's LOD (Klymus et al. 2019).

External Quality Assessment and Accreditation

Interlaboratory performance comparisons with established or national laboratories using similar protocols is also a critical step for evaluating method performance, with greater importance during initial testing method validation or when onboarding new wastewater sites. This comparison does not provide for absolute accuracy, but instead provides information on data comparability.

Establishing a formal mentoring program to pair a new laboratory with a reference laboratory for method performance comparison using shared samples and for troubleshooting support during development and validation can also be beneficial and efficient for rapid startup of a new laboratory. A mentor laboratory can also provide guidance and decision-making processes for laboratory set up, method selection and equipment and reagent purchasing. Finally, participating in multi-laboratory validation studies can be critical in evaluating the robustness of method performance when the same samples are split and tested by various laboratories or in evaluating the impact of wastewater matrices on method performance when laboratories exchange samples collected from distinct systems.

Participating in a formal proficiency testing (PT) program is one of the best approaches to document a laboratory's initial and ongoing method performance, as well as to compare performance with other laboratories. While there are several PT programs for SARS-CoV-2 in clinical matrices, PT programs for wastewater-based disease surveillance are still under development.

Participating in a wastewater testing PT program can involve:

- Regularly (e.g., 2-4 times per year) receiving an unknown wastewater sample, with or without a negative control/blank.
- Returning testing results to the PT vendor within a defined time (e.g., 2-3 days).
- Receiving testing performance evaluation from the PT vendor by referencing an absolute known value or a most probable number based on the median data value of all participating laboratories.

As with standard public health laboratory best practices, wastewater testing laboratories should be continuously evaluating ways to improve performance and data quality. This process can include activities such as:

1. Participation in workshops and surveys.
2. Engagement in NWSS community of practice discussions.
3. Evaluation and implementation of new methodologies and equipment that improve performance and efficiency or reduce cost at a comparable quality.
4. Consistently plotting and reviewing quality control data for performance metric trends.

Laboratory Data Reporting Metrics

For disease surveillance systems, it is important to evaluate program performance from a data reporting standpoint. While the preceding sections outline considerations for evaluating method and technician performance, this section outlines considerations for evaluating laboratory data reporting timeliness, completeness and other factors that are key for interpretation and use of disease surveillance data.

Reporting Timeliness

Minimizing the time from sample collection to data receipt at the health department (or other end users) helps public health decision makers take advantage of wastewater's potential as an early surveillance indicator. This turnaround time comprises three general components:

1. Time from sample collection to receipt at the laboratory.
2. Time from receipt at the laboratory to generation of results.
3. Time from generation of results to receipt of results at the health department (or other end users).

Systematically evaluating the duration of each component can help identify strategies for minimizing the total turn-around time, which may differ by wastewater utility, courier or shipping service or day of the week. To perform this evaluation, it is important for laboratories to track the date and time of each of these steps for each sample received. Note that data received by NWSS is sufficient to monitor the time from sample collection to test result and test result to data submission to NWSS.

Reporting Completeness

Completeness may be measured as the proportion of required fields that are completed across all submitted observations. High completeness is critical for analyzing and using wastewater surveillance data. An additional measure may also be the proportion of optional fields that are completed, which can be helpful for understanding the richness of the metadata available for different sampling sites or produced by different laboratories.

Reporting Quality

Data reporting quality is a measure of how well laboratory-generated data conform to data reporting specifications, including conforming to allowed values, correct data types, correct data schema (variable names and order) and the reasonableness of data fields (whether the values make sense). Quality markers can include metrics such as analytics flags, which is a marker used in the NWSS data platform.

Reporting Method Changes

Variation or shifts in laboratory methods can impact SARS-CoV-2 RNA quantification in wastewater; therefore, documenting and reporting changes to laboratory methods is critical for interpreting wastewater surveillance data. When

possible, side-by-side comparison of lab methods is critical during troubleshooting to understand the degree of impact from method changes. Differences in lab methods may limit the comparability of SARS-CoV-2 concentrations. If there is a major method change, validation experiments should again be performed.

NWSS enables laboratories to submit information on laboratory method changes to indicate that they are sufficiently different and that caution should be used when comparing SARS-CoV-2 concentrations across them. Differences in laboratory methods may limit the comparability of PCR target concentrations. When major changes occur in laboratory testing methods, NWSS submitters are able to flag and document the changes on the data submission using the *major_lab_method* field. These differences may result from any laboratory processing or quantification steps, including detection of different PCR targets.

APPENDIX A: LIST OF ACRONYMS

APHL: Association of Public Health Laboratories

BSC: Biological safety cabinet

BSL2: Biosafety Level 2

CDC: Centers for Disease Control and Prevention

COVID-19: Coronavirus Disease 2019

Cq: Cycle quantification

CRM: Certified reference material

Ct: Cycle threshold

DCIPHER: Data Collation and Integration for Public Health Event Responses

DE: Direct extraction

DNA: Deoxyribonucleic acid

- **cDNA:** Complementary deoxyribonucleic acid

FTE: Full-time employees

gc: Genomic copies

IDC: Initial demonstration of capability

NCBI: National Center for Biotechnology Information

NTC: No template sontrol

NWSS: National Wastewater Surveillance System

PCR: Polymerase chain reaction

- **dPCR:** Digital polymerase chain reaction
- **ddPCR:** Droplet digital polymerase chain reaction
- **droplet RT-dPCR:** Droplet-reverse transcription-digital polymerase chain reaction
- **qPCR:** Quantitative polymerase chain reaction
- **RT-qPCR:** Reverse transcription quantitative polymerase chain reaction

PEG: Polyethylene glycol

PPE: Personal protective equipment

PT: Proficiency testing

RNA: Ribonucleic acid (the genomic material of SARS-CoV-2)

RSD: Relative standard deviation

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

SRM: Standard reference material

USGS: United States Geological Survey

VOC: Variant of concern

WWTP: Wastewater treatment plant

APPENDIX B: GLOSSARY OF TERMS

Bait capture: Used to enhance sequencing power by hybridizing a target, typically with biotin, to allow for that region to be selected, amplified by PCR, and then sequenced.

Bioinformatics pipeline: Software algorithms executed in a predefined sequence to process and analyze raw sequencing data.

Biosolids: solid organic matter found in certain wastewater treatment processes.

cDNA: Complementary DNA synthesized from a single-stranded RNA template produced by reverse transcriptase.

Cq value: The number of cycles taken to detect a signal from the sample when using RT-PCR. Also referred to as the Ct value.

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.

GenBank: An open access, annotated collection of all publicly available nucleotide sequences and their protein translations.

Human fecal normalization controls: Organisms or compounds specific to human feces that can be measured in wastewater to estimate its human fecal content.

Inhibition (PCR): inhibition of PCR amplification caused by interfering substances that may be present in samples.

Lineage (viruses): Group of closely-related viruses with a common ancestor. SARS-CoV-2 has many lineages; all cause COVID-19.

Master mix: Mixture of dNTPs, MgCl2, Taq polymerase, and pH buffer in nuclease-free water used to amplify DNA templates in PCR reactions.

Matrix recovery control: Process control used to assess virus concentration lost during sample processing.

Metadata: Data that are used to describe or provide more information about data.

Method validation: Process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

Multiplex PCR: Simultaneous amplification of multiple targets in a single reaction with different pairs of primers for each target.

Mutation: A mutation refers to a change in a virus's genome (genetic code).

Nucleic acid extraction: Process of extracting nucleic acids from cells, breaking open cells, removing proteins, lipids and other contaminants, and concentrating nucleic acids.

Pasteurization: Heating wastewater sample at 60 °C to reduce potential pathogen exposure risk from bioaerosol-generating procedures during sample processing.

Proficiency testing (PT): Testing of unknown samples sent to a laboratory by an approved PT program.

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.

Sensitivity: The ability of a method to accurately detect a true signal (i.e., presence of genetic material). This can also be thought of as how effectively a target is amplified and quantified if it is present.

Singleplex PCR: Assay to detect one target sequence of DNA or RNA.

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

Spiked control virus: Known quantity of virus added to each sample nucleic acid extract to evaluate and quantify the extent of molecular amplification inhibition.

Standard curve: A tool used to estimate virus concentration in an unknown sample by comparing concentrations to standards with known RNA concentrations; also used to determine the efficiency, linear range and reproducibility of a qPCR assay.

Target: An RNA or DNA sequence or gene of interest.

Template DNA: Sample DNA that contains the target sequence.

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.

Wastewater: Also referred to as "sewage," includes water from household/building use (i.e., toilets, showers, sinks) that can contain human fecal waste, as well as water from non-household sources (e.g., rainwater and industrial use).

Wastewater surveillance: Strategic sampling and testing of wastewater to detect pathogens or other health targets to better understand disease burden and spread within a community.

APPENDIX C: PROGRAMMATIC DECISIONS INVOLVING TESTING LABORATORIES

Wastewater laboratory testing is one aspect of the overall wastewater surveillance program. For a testing laboratory to decide the most cost-effective and successful testing and data reporting methodologies, these programmatic questions need to be addressed by the wastewater surveillance program team that includes epidemiologists, wastewater plant personnel, as well as the laboratory. These questions should also be reflected upon throughout the wastewater surveillance period to ensure that systems are efficient and adapting as needed to those using the data.

Data Use and Communication

- How do you envision the data being used? And by whom?
- What is the public health data need addressed by the testing? Include case load data information – presence/absence, trend analysis, data limitations, and gaps the data fill.
- What public health response actions do you envision taking based on the testing results?
- Can our epidemiologists use this data to inform interventions to prevent further spread in areas where detection is just starting or re-emerging? If so, what kind of data do they need?
- Who has access to this data? Can we post this data online for our community to be more informed about their environment?
- How will the data be communicated?

Sampling and Data Analytics

- What frequency and volume of sampling is needed to support analytic needs?
- Are community interventions effective for preventing spread/further spread of disease?
- Can community activity guidance be adapted based on presence/absence of disease in a defined community?
- Are public health intervention efforts working? And how will you measure this impact?
- Are facilities being monitored? If so, what public health interventions could the wastewater data inform? Are wastewater data providing confirmation of other public health data?
- What variants are emerging or are present in our population?

Programmatic

- Who will I need to collaborate with and how?
    - Department of Health program staff (they would coordinate with water utilities, develop sampling plans, keep testing focused on public health, provide direction to reach vulnerable and underserved populations)
    - Wastewater treatment plants and epidemiologists
    - If working with a specific facility, or with a vulnerable population, how can you ensure that data privacy and ethics are communicated effectively?
    - Universities
        - Expertise in environmental microbiology and up-to-date on the latest research
        - Local university graduate students for extra staffing
- How will the program identify funding sources and/or secure equipment, reagents, consumables and staff to implement testing?

APPENDIX D: ADDITIONAL RESOURCES

- Using Wastewater Surveillance Data to Support the COVID-19 Response
- Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands
- Early-pandemic wastewater surveillance of SARS-CoV-2 in Southern Nevada: Methodology, occurrence, and incidence/prevalence considerations
- Wastewater Surveillance of the COVID-19 Genetic Signal in Sewersheds: Recommendations from Global Experts
- High-frequency, High-throughput Quantification of SARS-CoV-2 RNA in Wastewater Settled Solids at Eight Publicly Owned Treatment Works in Northern California Shows Strong Association with COVID-19 Incidence
- Evaluating recovery, cost, and throughput of different concentration methods for SARS-CoV-2 wastewater-based epidemiology
- Example protocols for each step of the wastewater testing process
- List of environmental microbiology and engineering laboratories across the United States and skills applicable
- Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition
- Laboratory biosafety guidance related to novel coronavirus
- APHL Risk Assessment Best Practices
- Reporting the limits of detection and quantification for environmental DNA assays
- Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the US
- The environmental microbiology minimum information (EMMI) guidelines: qPCR and dPCR quality and reporting for environmental microbiology
- Comparative analysis of rapid concentration methods for the recovery of SARS-CoV-2 and quantification of human enteric viruses and a sewage-associated marker gene in untreated wastewater
- Minimizing errors in RT-PCR detection and quantification of SARS-CoV-2 RNA for Wastewater Surveillance
- Example Laboratory Risk Assessment Process: A Laboratory Risk Assessment during the Coronavirus (COVID-19) Pandemic

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Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

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