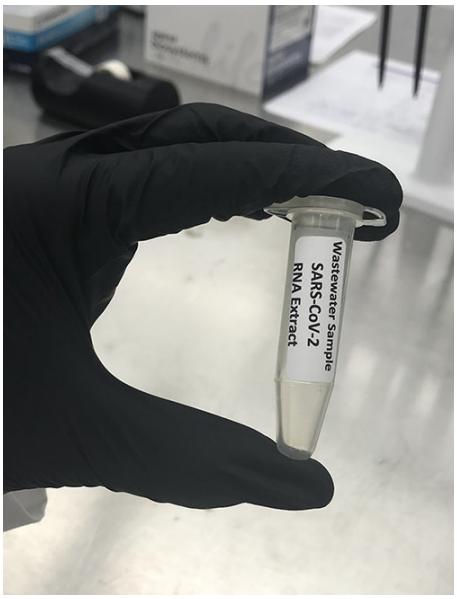
Science and Technology Evaluation for Practice (STEP)
Workgroup of the National Sewage Surveillance
Inter-Agency Leadership Council (NSSILc)



FEDERAL RESEARCH PRIORITIES TO SUPPORT WASTEWATER SURVEILLANCE FOR SARS-COV-2

March 30, 2022

Disclaimers

Views in this document are attributable to the NSSILc STEP Workgroup and do not necessarily reflect the official policy of the 9 federal agencies who participate in NSSILc.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Table of Contents

Executive Summary

Almost immediately after the start of the COVID-19 pandemic in 2020, researchers and wastewater utilities around the world began analyzing untreated wastewater for the SARS-CoV-2 RNA. These efforts were largely based on prior experience analyzing wastewater for poliovirus, presence of opioids, and other contaminants. Early reports from the Netherlands[1] indicated that detection of SARS-CoV-2 RNA in wastewater signaled the presence of the respiratory virus that causes COVID-19, in the community served by the wastewater utility before reported clinical cases. As word of these efforts spread, the public health community began to take interest in the approach and its value in informing public health responses such as resource allocation, community mitigation strategies (e.g., mask wearing), and resolution to conflicting individual based surveillance data. In the United States, environmental microbiology and engineering researchers and others began to rapidly pivot their focus to this emerging field of science in support of the COVID-19 response. Federal agencies supported the rapid development of this growing field by providing funding for wastewater surveillance research and implementation projects[2].

The urgency of the pandemic prevented the preparation of a coordinated national level strategy to articulate overall science and technology needs associated with wastewater surveillance. The establishment of the National Sewage Surveillance Inter-Agency Leadership Council (NSSILc) in the summer of 2020, convened by the United States Department of Health and Human Services (HHS) Assistant Secretary of Health, provided a venue for discussing and coordinating implementation and science and technology needs related to wastewater surveillance across the federal government. NSSILc quickly established two working groups, Implementation and Planning (IP) and Science and Technology Evaluation for Practice (STEP). Shortly thereafter, in the fall of 2020, the United States established a new public health program, the National Wastewater Surveillance System or NWSS[3] under the leadership of the United States Centers for Disease Control and Prevention (CDC).

STEP began by organizing federal agency representatives interested in wastewater surveillance and reviewing available information on the research projects that had been funded by agency extramural programs or were being conducted through agency intramural research programs. Each agency agreed to participate in a process to identify remaining science and technology needs for wastewater-based surveillance of SARS-CoV-2 and consider needs that would assist in further use of wastewater surveillance and preparation for future outbreaks and pandemics.

This document is the result of that process and presents a consensus set of science and technology needs for the advancement of wastewater surveillance as proposed by the federal agencies represented

[1] Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., and Brouwer, A. 2020. 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. *Environmental Science & Technology Letters*. (May 20, 2020). Available online at: https://pubs.acs.org/doi/10.1021/acs.estlett.0c00357.

[2] A Compendium of U.S. Wastewater Surveillance to Support COVID-19 Public Health Response. https://www.epa.gov/system/files/documents/2021-09/wastewater-surveillance-compendium.pdf

[3] Centers for Disease Control and Prevention. National Wastewater Surveillance System (NWSS). A new public health tool to understand COVID-19 spread in a community [cited May 30, 2021]. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html

by STEP. Given the broad range of mission areas, the list of priorities is not organized in order of importance nor does this document provide a linear sequence of research activities. Rather, the priorities are intended to articulate where additional research is needed and are grouped by subject area. This coordinated information can be useful to federal agencies to guide and inform federal extramural and intramural research efforts, evaluate research funding proposals, and potentially inform future funding opportunities. This document does not compel or impose any requirements on the agencies.

The five research priorities identified are:

1. Improve clinical understanding of gastrointestinal aspects of SARS-CoV-2 infections to associate infections with wastewater surveillance measurements.
2. Characterize SARS-CoV-2 decay, fate and transport, and variability within the sewer system and treatment train.
3. Support the harmonization of methods for quantifying and genetically characterizing SARS-CoV-2 shed into a sewer by a defined population under varying infection prevalence levels.
4. Develop biostatistical analytics for wastewater-based epidemiology to inform public health decision making.
5. Support wastewater surveillance approaches that exemplify best practices in environmental and social justice[4], protect privacy, follow the law, and prevent improper use of data and adverse community and individual outcomes.

[4] Environmental justice is the fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income, with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies (https://www.epa.gov/environmentaljustice). Social justice is the objective of creating a fair and equal society in which each individual matters, their rights are recognized and protected, and decisions are made in ways that are fair and honest. (https://www.oxfordreference.com/view/10.1093/oi/authority.20110803100515279)

Introduction and Background

This document was prepared by the NSSILc STEP Workgroup (Workgroup). The Workgroup is comprised of representatives from nine federal agencies and departments that have a role or interest in wastewater surveillance research and technology. The agencies and departments include CDC, National Science Foundation (NSF), Department of Homeland Security (DHS), Department of Defense (DOD), Environmental Protection Agency (EPA), National Institute of Standards and Technology (NIST), United States Geological Survey (USGS), Food and Drug Administration (FDA), and the National Institutes of Health (NIH). The Workgroup came together to develop a comprehensive set of research and technology priorities. This document does not require agencies to take specific actions. It is intended to bring about a consistent level of understanding of the research gaps and support coordination of agency activities. This information can be useful to agencies as they consider future investments in wastewater surveillance research and development for intramural and extramural programs and may assist other research groups domestically and internationally.

The COVID-19 pandemic prompted the rapid development of wastewater surveillance practices. This immediate scientific need required the reorientation of existing research and initiation of new research to assist in the response. Programs such as NSF's Rapid Response Research (RAPID) enabled the federal government to quickly support researchers in evaluating the usefulness of wastewater surveillance to support the COVID-19 response in close coordination with wastewater utilities, public health officials, elected officials, and others.

The experience gained from these early research projects and implementation of wastewater surveillance at utilities, universities and congregate settings also identified substantial challenges and research gaps that need to be addressed to enable expansion of wastewater surveillance in the United States, through the NWSS program, in preparation for future outbreaks and pandemics.

The research and technology priorities for wastewater surveillance are organized in a logical form, from sample collection to testing methods, to ethics and social science issues. The priorities are organized by topic and are not intended to follow a linear research trajectory. Although much consideration has gone into identifying the research gaps, the Workgroup acknowledges that it is likely not complete as the field is evolving and knowledge about the virus is continuously changing. Moreover, certain priorities may be of more interest to some agencies than others. There are critical topics for the implementation of wastewater surveillance programs not included in this document, such as attainment of health equity in communities, and training needs of agencies implementing wastewater surveillance programs. These issues were beyond the scope of this document, which is focused on science and technology gaps. The intention of this document is to continue coordination and cooperation among federal agencies on wastewater surveillance science and technology priorities to avoid the potential for duplication of effort and bring greater yields from complementary research investments. The overall goal of this document is to support development of a scientifically sound, widely adopted, wastewater surveillance system for the country.

Research Priorities

Priority 1: Improve clinical understanding of gastrointestinal aspects of SARS-CoV-2 infections to associate infections with wastewater surveillance measurements.

The relationships between SARS-CoV-2 RNA measurements in wastewater and human gastrointestinal viral shedding resulting from SARS-CoV-2 infections are critical for interpreting SARS-CoV-2 wastewater data. However, significant gaps exist in our understanding of human shedding rates throughout the infection period and of the relationship between fecal shedding and respiratory shedding used for case surveillance. Sound approaches are needed to close these gaps, as well as how SARS-CoV-2 variant infections and vaccination status impact human shedding. With this understanding, methods and models can be developed to determine if vaccination efficacy can be monitored through wastewater.

Objective 1.1: Characterize fecal shedding titers of SARS-CoV-2, and SARS-CoV-2 variants of concern, throughout the duration of human infection (pre-, peri-, post-, and asymptomatic).

- Evaluate models to correlate virus concentrations in wastewater with reported and unreported case numbers in sewersheds[5].
- Assess limitations of population infection estimation for public health use, including addressing
- community size limitations (e.g., is the community too small? would clinical testing be more appropriate?).
- Develop standardized approaches and validated methods to better characterize SARS-CoV-2 variants that may be present in the wastewater and their relationship to human infection.

Objective 1.2: Determine the relationship between human positive respiratory tests (saliva, nose swab, nasopharyngeal samples) and fecal shedding, both temporally and quantitatively, with respect to virus titer and across the ranges of disease severity and vaccination status.

- Improve and validate methods and models to better relate the respiratory infection tests with fecal shedding.

Objective 1.3: Evaluate the association between vaccination status and SARS-CoV-2 fecal shedding titers in humans and known animal carriers (e.g., mink).

- Improve the utility of wastewater-based surveillance to assess the impacts of vaccination rate and efficacy in a defined population over time.

[5] Sewershed is the area drained by a particular network of sewers in a community, with the drainage ultimately reaching a wastewater treatment facility.

Priority 2: Characterize SARS-CoV-2 decay, fate and transport, and variability within the sewer system and treatment train.

Centralized wastewater systems are complex. Many system operational factors, such as flow and input of industrial pollutants, can influence wastewater surveillance sampling results. For wastewater surveillance to be successfully implemented, robust tools and models (standardized and validated to the extent possible) are needed to better characterize the sewer system and relate sewer characteristics to SARS-CoV-2 levels in wastewater. Significant gaps exist in methods to evaluate the efficacy of treatment processes necessary for wastewater surveillance. Development of appropriate occupational safety and laboratory biosafety procedures throughout the wastewater sample collection, transport, and laboratory analysis needs additional research to ascertain the appropriate measures that should be taken.

Objective 2.1: Improve sewer system data and information to better characterize the fate of the virus within the system and efficacy of treatment processes to support wastewater surveillance.

- Measure and improve current models to determine sewage residence time, flow rates, temperature, interferants and other collection system characteristics, such as combined sewer overflows, that influence the measurement and fate of the virus in sewage.
- Develop and standardize mapping (including service connections and population served), and other tools to determine sewershed boundaries, building on existing requirements.
- Research the infectivity of the virus within the sewer system in treated as well as untreated effluents to support the most robust and efficient sewage surveillance programs.
- Develop Laboratory Biosafety and Occupational Safety Guidelines for testing and sampling by wastewater operators and other personnel (including on-site staff at laboratories and wastewater treatment plants).

Priority 3: Support the harmonization of methods for quantifying and genetically characterizing SARS-CoV-2 shed into a sewer by a defined population under varying infection prevalence levels.

The entire wastewater sampling and analysis workflow is subject to differing levels of natural variability as well as measurement uncertainty. Research gaps need to be addressed to decrease the level of uncertainty throughout this process from sampling, concentration, extraction, and detection. Sampling methods for wastewater need additional development to ensure that the samples are representative; at the same time, sampling and analysis timelines also need to be optimized to ensure data are useful for public health response actions. Understanding the representativeness of sampling methods upstream of the treatment plant is also needed to use wastewater surveillance as an infection screening tool at the individual building level. Other wastewater sample types, such as biosolids (also referred to as sludge) in conjunction with or as an alternative to liquid wastewater, need further geographical evaluation to determine when they are more appropriate. As infections decrease in communities, testing methods will likely require higher recovery and reduced detection limits to successfully detect low levels of infections in

a community. Finally, genetic sequencing methods for wastewater are emerging as a tool to indicate variant circulation within a community, but these tools need further evaluation and validation to determine if these techniques could be used to track mutations and relate them to variant detection in clinical cases.

Objective 3.1: Improve the quality and consistency of sewer system sampling methods to promote comparability of the results.

- Continue to develop and evaluate sampling methods to determine the most consistent and representative methods including grab, flow-weighted composite, time-weighted composite, and continuous composite, which should include both inter- and intra-validation studies.
- Evaluate the representativeness of wastewater solids as an alternative or in conjunction with sampling of the raw sewage.
- Evaluate representative sampling approaches for upstream sites (within the sewer system and at the building level).
- Develop guidance for testing data quality assurance and quality control, including reference materials and standards as well as inter- and intra-laboratory accreditation and proficiency. Also applicable to Objective 3.3.
- Work toward methods guidance and standardization that will allow for inter- and intra-laboratory reproduceable results and comparability. Also applicable to Objective 3.3.

Objective 3.2: Improve approaches to concentrate and extract the virus from wastewater samples.

- Continue to develop, evaluate, and validate wastewater concentration methods, such as Nanotrap® and others, with improved recovery efficiencies and reduced detection limits, which should include both inter- and intra-validation studies.
- Reduce the cost and time of testing and reduce the time between analysis and reporting, including defining optimal timeframes.
- Assess methods for characterizing process recovery and study the impact of concentrating and extracting nucleic acid methods on different detection technologies, to determine optimal regimes.

Objective 3.3: Evaluate and improve the performance, sensitivity, and specificity of molecular technologies for wastewater samples.

- Evaluate the performance, sensitivity, and specificity of wastewater molecular technologies for detection of SARS-COV-2 and quantification of known variants, and emergence discovery by sequencing.
- Develop reliable, standardized, and validated methods for detecting the abundance and genetic diversity of SARS-CoV-2 in wastewater, including viral variants.

- Develop guidance for testing data quality assurance and quality control, including reference materials and standards, and for testing inter- and intra-laboratory accreditation and proficiency.
- Work toward methods guidance and standardization that will allow for inter- and intra-laboratory reproduceable results and comparability.

Priority 4: Develop biostatistical analytics for wastewater-based epidemiology to inform public health decision making.

One of the primary objectives of wastewater surveillance is to inform-public health decision making to protect communities from outbreaks and pandemics. Substantial gaps exist in tools for connecting the wastewater surveillance data to other surveillance systems to inform mitigation and prevention strategies at the community level. Improvements are needed for better biostatistical analysis, epidemiological modeling, and prediction of disease.

Objective 4.1: Develop wastewater-based epidemiology approaches and methods that can incorporate wastewater surveillance data for public health decision making.

- Improve epidemiological modeling and predictive disease analytics using wastewater data.
- Determine the sensitivity and specificity of detection methods needed in wastewater samples for surveillance applications, especially for early detection of SARS-COV-2 and known variants.
- Establish appropriate biostatistical analytics that include trends and lead and lag analyses of wastewater surveillance trends based on various sampling approaches to inform public health response.

Priority 5: Support wastewater surveillance approaches that exemplify best practices in environmental and social justice, protect privacy, follow the law, and prevent improper use of data and adverse community and individual outcomes.

While there are substantial technical challenges to wastewater surveillance on a national level, there are also challenging aspects of establishing the surveillance system related to social sciences, legal considerations, ethics, and privacy issues. Without conducting research to inform properly created frameworks that will ensure environmental and social justice, protect privacy, and establish legal constructs and ethical practices, wastewater surveillance systems will not fully serve their intended purpose as a pillar of our national public health surveillance system.

Objective 5.1: Develop effective approaches to address environmental and social justice and privacy issues related to wastewater surveillance.

- Improve understanding of environmental and social justice, privacy and confidentiality, and ethics dimensions of wastewater surveillance.
- Engage the public and community in the development of effective models, based on data and analyses, to support the uptake of wastewater surveillance.
- Develop models of communication and community engagement tools.

Objective 5.2: Conduct policy research to develop legal frameworks for wastewater surveillance that outline authorities, liabilities, and other legal obligations and constraints.

- Develop analyses of wastewater surveillance legal authorities at the federal (Clean Water Act), state and local levels.
- Develop analyses of authorities and potential liability issues related to wastewater utilities.

Objective 5.3: Conduct research and data collection to ensure that wastewater surveillance programs are ethical and equitable and do not target or adversely affect individuals or communities while supporting public health response.

- Determine acceptable population size thresholds for ethical monitoring or reporting that distinguish populations from individuals and remove bias in data interpretation for specific communities (e.g., rural and septic systems).
- Consult and engage with communities to develop data reporting, data sharing, and other approaches to improve a community and individual's ability to understand and use data to inform public health action. These efforts should preserve anonymity and community and individual characteristics and prevent misuse of non-target sensitive data collected in wastewater (e.g., human DNA, pharmaceutical use, etc.).

Conclusion

Wastewater surveillance is a rapidly evolving area of science. The approach can provide significant benefits through early warning of disease in communities and can assist in informing and guiding public health response. CDC has established a permanent nationwide wastewater surveillance system, NWSS. To support this program for the COVID-19 response and other public health applications, additional science is needed to address existing research and technology gaps.

Although this set of research priorities is aimed at addressing needs related to wastewater surveillance of SARS-CoV-2, they could also be applicable to diverse surveillance targets in the future. Strengthening wastewater surveillance now could also assist in future development and deployment of tools to track other targets related to outbreaks and pandemics. These could include influenza, other coronaviruses, measles, Ebola, other emerging diseases, food, and waterborne illnesses, and antibiotic resistant genes. The NSSILc STEP Workgroup has developed a set of research priorities to foster a whole-of-government approach to address wastewater surveillance science and technology gaps, with the vision that this process of collaborative priority setting will continue and be refined as more is learned.