

Subject: DEP Guidance for Wastewater Utilities Engaging in Wastewater Collection and Analysis for SARS-CoV-2

Wastewater based epidemiology (WBE) is a rapidly evolving field that uses data collected from a wastewater stream as a non-invasive, broad scale screen for several biological or nonbiological endpoints. On small scale projects, this type of technology has been used to track the presence and abundance of illicit drugs and the presence of various human pathogens. Recently, using increasingly more robust and powerful tools, the concept was modified to track and report the detection of levels of the genetic material of SARS-CoV-2 (the virus that causes COVID-19).

Since there is currently no standard operating procedure for collecting and analyzing for the genetic signal of SARS-CoV-2 in wastewater, careful considerations must be undertaken when performing this type of analysis. To that end, the Department has prepared this *Guidance for Wastewater Utilities Engaging in Wastewater Collection and Analysis for SARS-CoV-2* for those that are considering or are already engaging in this type of work.

The Department is providing this guidance to ensure facilities are collecting and recording baseline minimum data so that scientists and decision-makers have confidence in the data to guide future action. The guidance provides background on WBE and informs decision-makers on pursuing a course of action for WBE at a facility. The guidance offers questions that may be helpful for facilities when interacting with other WBE-related entities. Please keep in mind, these questions and checklists in the guidance are not intended for a facility to provide directly to a potential consultant or contractor. If you have any comments or questions, please use the contact information provided in the guidance.

Thank you,

Division of Water Quality
NJ Department of Environmental Protection

Guidance for Wastewater Utilities Engaging in
Wastewater Collection and Analysis for SARS-CoV-2

July 20, 2020

Background

Wastewater based epidemiology (WBE) has been a rapidly evolving field that seeks to use the information in the wastewater stream as a non-invasive, broad scale screen for several biological or nonbiological endpoints. On small scale projects, this type of technology has been used to track the presence and abundance of illicit drugs, and the presence of various human pathogens. Recently, using increasingly more robust and powerful tools, the concept was modified to track and report the detection of levels of the genetic material of SARS-CoV-2, the virus that causes COVID-19.

Since March 2020, multiple countries, and several states have begun conducting pilot projects deploying WBE. However, because there is no single validated method for conducting this type of survey, detecting the virus genetic material in the wastewater, or doing a higher-level data interpretation and extrapolation, many questions and issues have arisen. To address these issues, organizations like the Water Research Foundation (WRF) enlisted national and international experts to survey literature, conduct surveys, and develop minimally acceptable criteria for undertaking this work. WRF's work emphasizes the importance of establishing sound methodologies for collection and analysis, including quality assurance and quality control measures. These types of controls are typical in field work and sampling conditions and their role is fundamental to the scientific integrity of the data that is collected.

DEP understands that some wastewater utilities are considering or are already engaging in this type of work. As there is currently no universally standard operating procedure for collecting and analyzing for the genetic signal of SARS-CoV-2 in wastewater, careful considerations must be undertaken when undertaking this type of analysis. To that end and to

To help increase data consistency as multiple groups conduct these studies, workgroups compiled by organizations such as WRF and vetted techniques from federal agencies such as EPA will ensure that there is confidence in both the data and any analyses conducted going forward. The checklist below summarizes existing resources, recommendations and considerations from these trusted sources.

Sample Collection

Sample collection influences the quality and therefore utility of the data generated. The following best practices will ensure that opportunities for sample contamination are minimized and controlled for. Proper PPE, training, and "safe handling" does not replace proper controls to ensure against contamination.

Metadata will vary based on sampling location. In many cases samples are being collected once weekly at the influent as a composite over 24 hours. Additionally, some utilities are also collecting non-composite "grab" samples within the sewershed.

Minimally Acceptable Criteria

- ☐ Collect and report appropriate metadata* for the type of study being conducted
- ☐ Collect samples in lot certified DNA/RNA free collection bottles, preferably polycarbonate
- ☐ Samples should be stored and shipped in temperatures that do not to exceed 4°C/39.2°F
- ☐ Field Blanks/Trip Blanks, and other appropriate Blanks (temperature) should be included to identify potential contamination
- ☐ A minimum of 500 mL should be collected for analysis

Additional Considerations

- ☐ If combining samples for shipping to lab, ensure that a sterilization protocol is developed and employed between sample handling

Sample Storage

Also, for sample storage, use of proper PPE, training, and “safe handling” does not replace controls to ensure against contamination. It is considered ideal to collect a 1-liter sample, which is split four ways:

- 250 mL for the purpose of measuring for quality control (recovery spiking);
- 500 mL for analysis; and
- 250 mL as an archival sample.

Samples should be collected and preserved in lot certified DNA/RNA free polycarbonate bottles, or otherwise using a suitable sterilization protocol. Upon collection, samples should be immediately stored at a cool temperature to prevent the loss of genetic signal. Separation into sub-containers should occur before freezing to prevent the sample from undergoing freeze-thaw cycling. Archival of unprocessed samples should occur at a minimum temperature of -20°C/-4°F; and samples being used for analysis/quality control should be processed as expeditiously as possible, with temperatures before processing not exceeding 4°C/39.2°F. Samples held at 4°C/39.2°F are expected to be stable for a few days, and samples held at -20°C/-4°F are expected to be stable for several months.

Molecular Analysis:

Enveloped RNA viruses, like SARS-CoV-2, may behave differently from those viruses typically surveyed in wastewater, such as Polio or Norovirus. The following represent the current best practices for isolating and amplifying the genetic material for SARS-CoV-2. As wastewater is an extremely complex medium, proper controls must be in place to ensure that signal measured is a “true” signal, and not a byproduct of the wastewater matrix. Multiple groups are working on protocols to address this issue, as well as other potential targets in the wastewater stream. At a minimum, all molecular work should include the following:

Minimally Acceptable

- ☐ A positive control of SARS-CoV-2 that is run in the reaction setup
- ☐ A negative control which has all the material of the reaction minus any genetic material to ensure no contamination from operator handling
- ☐ A known limit of detection
- ☐ The equivalent volume of the sample analyzed

- ☐ A list of the molecular targets being analyzed, and their sequences should be available upon your request
- ☐ Assurance that appropriate standards are being used for rt-qPCR for standard curve generation
- ☐ Written and available RNA extraction, and cDNA generating protocols
- ☐ A protocol that indicates which phase of the wastewater stream is being isolated

Optimally

- ☐ A standard curve should be run with each plate or whenever new reagent is used; this standard curve should be checked against previous standard curves generated for validity
- ☐ An internal inhibition control should be used with each wastewater stream sample to ensure no amplification inhibition is occurring
- ☐ Matrix spiking and recovery reporting should be ongoing to ensure method used is appropriate and no modifications are needed
- ☐ Archived samples (frozen) of unextracted sample should occur in the event methods change to ensure samples can be analyzed uniformly
- ☐ Archived samples (frozen) of extracted samples occur for data validation

Trend Analysis

There is great interest in the use of data collected from wastewater to estimate the burden of disease in a service area. However, experts agree that the uncertainties surrounding these types of estimation greatly affect the confidence in the results. The role of industrial discharges, stormwater inputs, and the amount and duration of virus shedding are just some of the factors that require further research before data can be reliably used for prediction. It is widely thought that confidence in the ability to predict prevalence of infection will increase as researchers address these issues, including the correlation of confirmed cases in the utility service area. As additional this data is collected and modeler confidence grows, or as refinements in the sampling process is conducted, this modeling may build higher confidence. Until that time, leading experts in the field agree that the best use of this data is to observe trends to serve as a possible signal of changes in disease occurrence in the population.

***Typical Critical Metadata that should be included on log sheets or reported with data submission**

- ☐ Sample Type (Grab/Composite)
- ☐ If Composite, Composite Type (Flow over Time, etc.)
- ☐ Time Sampled
- ☐ Sampler
- ☐ Location
- ☐ Weather Observations (if external)
- ☐ Flow
- ☐ Population Served (important for catchment studies)
- ☐ Sample Characteristics (TSS, BOD, Ammonia, pH, Temp, Chlorine Residual, etc.)
- ☐ Storage Temp
- ☐ Additional information as discussed with partner agencies for model development

Additional Information and Sources of this Information can be viewed:

Additional Resources:

The Water Research Foundation's Virtual International Water Research Summit on Environmental Surveillance of COVID-19 Indicators in Sewersheds - Opening Session

<https://www.waterrf.org/event/water-research-foundations-virtual-international-water-research-summit-environmental>

04-27-20

Virtual International Water Research Summit on Environmental Surveillance of COVID-19 Indicators in Sewersheds – Closing Session

<https://www.waterrf.org/event/virtual-international-water-research-summit-environmental-surveillance-covid-19-indicators>

04-30-20

Utah Department of Environmental Quality – SARS-CoV-2 Sewage Monitoring Pilot Project Dashboard

<https://udwq.shinyapps.io/pilot-ww-virus-db/#dashboard-1>

Water Research Foundation – RFQ for Interlaboratory and Methods Assessment of the SARS-CoV-2 Genetic Signal in Wastewater (WRF 5089)

https://www.waterrf.org/sites/default/files/file/2020-06/RFQ_5089.pdf