Executive Summary

Interlaboratory and Methods Assessment of the SARS-CoV-2 Genetic Signal in Wastewater (project 5089)

With the spread of the COVID-19 pandemic across the nation and the globe, there is growing interest in additional approaches for assessing community health. Traditionally, such assessments have been based on clinical testing of individuals within the population. The shortcomings of this approach have been evident during the COVID-19 pandemic as the number of available tests frequently could not match the demand. Because infected individuals shed the virus in their feces and other body secretions, measuring the concentration of SARS-CoV-2 in wastewater has the potential to provide complementary insight on the level of infection within a population. Unlike clinical data—which may be biased toward the evaluation of symptomatic individuals—wastewater contains regular inputs from the entire population representing all stages of infection from symptomatic to pre-symptomatic to asymptomatic individuals. The international water community responded rapidly by developing methods to measure SARS-CoV-2 genome concentrations in wastewater. The simultaneous efforts of these groups, however, resulted in a wide diversity of methods, each pursuing a common goal: the reliable and reproducible quantification of SARS-CoV-2 concentrations in wastewater.

In April 2020, The Water Research Foundation (WRF) hosted an international summit to evaluate the use of wastewater surveillance as an indicator of COVID-19 in communities (WRF 2020). The participants identified two priority applications for the use of wastewater surveillance data: tracking trends in occurrence and assessing the degree of community prevalence. One of the prerequisites for these applications, however, is the identification of reliable, reproducible, and sensitive methods. To help address this issue, the project 5089 research team performed an interlaboratory evaluation of 36 different methods used to assess the genetic signal of SARS-CoV-2 in untreated wastewater. The nationwide study included 32 U.S. laboratories from 19 different states, each processing split samples of two different raw wastewaters emanating from populations known to have high levels of infection. The project sought to identify if and how the SARS-CoV-2 findings were impacted by multiple methodological differences such as sample concentration method, pasteurization pre-treatment, primer/probe selection, and solids removal steps. The effort did not intend to standardize a single method, but rather evaluate whether the existing methods provide sufficient reliability and reproducibility to track trends in occurrence and assess the prevalence of community infection.

A nationwide call for laboratory participants led to the identification of 32 participating labs. Because some labs evaluated multiple approaches, 36 different methods were included in the study. The project team divided the methods into eight major groups based on two major methodological differences: the presence or absence of both a solids removal step and a sample concentration step. While each laboratory followed its own standard operating procedure (SOP), each lab was required to adhere to the project’s quality assurance project plan (QAPP; Trussell Technologies 2020), which described the quality assurance/quality control (QA/QC) requirements. The QAPP was constructed to ensure uniformity in sample collection, shipping and handling, quality control for the analytical methods, data management, and validation. Key elements of the QAPP included requirements for blind matrix spikes, positive and negative controls, inhibition controls, and
method blanks. By specifying these QA/QC requirements, a handful of data that failed these checks were identified and justifiably eliminated from the dataset, allowing the team to focus on methodological sources of variability. The QAPP is available on the 5089 project page of the WRF website.

A comprehensive presentation and discussion of the results can be found in the manuscript entitled, "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 U.S." [Pecson et al., submitted]. Key findings from the study include the following:

- The interlaboratory methods comparison showed a high degree of reproducibility in the quantification of the SARS-CoV-2 genetic signal in wastewater. Of the eight method groups representing 36 different methods, 80% of the results fell within a band of approximately +/- 1-log genome copies per liter (GC/L).
- When corrected for recovery efficiency, the results did not show a systematic impact from solids removal or concentration method used. Other differences between the methods (e.g., pasteurization, primer set selection, and PCR platform) generally resulted in small differences compared to other sources of variability.
- Factors promoting interlaboratory reproducibility include (a) the relative insensitivity of the findings to methodological differences, (b) the implementation of strict QA/QC requirements, (c) the use of a quality assurance project plan to normalize the findings and account for important sources of variability, and (d) implementing a shared SOP among different laboratories.
- The findings support the use of wastewater surveillance for tracking trends in the concentrations of SARS-CoV-2 within communities. They also highlight methodological challenges related to modeling incidence and prevalence.
- Additional metrics should be used to select the best methods for future efforts including method sensitivity, cost, equipment requirements, and simplicity.

References


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