Standardizing sample preparation and clarification methods for wastewater surveillance monitoring of SARS-CoV-2



(L)CSTE

CONTRIBUTOR: Courtney Marx, Microbiology III, Supervisor, Pennsylvania Department of Health

CATEGORY: Epidemiology and Laboratory Capacity (ELC)

The Pennsylvania Department of Health developed a protocol to standardize the sample preparation and clarification methods for wastewater surveillance monitoring of SARS-CoV-2. This study led to the validation of a test method, which is now used to test wastewater samples weekly from several wastewater treatment plants to provide early detection of COVID-19 in communities across Pennsylvania.



The "What"

In response to the COVID-19 pandemic, the Centers for Disease Control and Prevention (CDC) launched the National Wastewater Surveillance System (NWSS). NWSS was developed to track the presence of the SARS-CoV-2 virus shed through feces in wastewater. Wastewater surveillance provides an early detection method to observe the presence of COVID-19 in a community. Early detection allows communities to act quickly and prevent the spread of the virus.

No standard method exists for the sample preparation and clarification activities for the quantification of SARS-CoV-2 N1 and N2 gene targets from an effluent wastewater sample. The Pennsylvania Department of Health (PA DOH) Bureau of Laboratories (BOL), which received funding through the Epidemiology and Laboratory Capacity for the Prevention and Control of Emerging Infectious Diseases (ELC) cooperative agreement, developed a protocol to standardize the sample preparation and clarification methods for wastewater surveillance monitoring of SARS-CoV-2. The data showed that heattreated samples indicated viral RNA degradation when compared to the non-heattreated controls and that the preferred sample clarification method is centrifugation.

The "So What"

Wastewater samples were collected from various wastewater treatment plants to detect SARS-CoV-2 ribonucleic acid (RNA) under different pasteurization and clarification processes. The steps included pasteurization, clarification, viral concentration, RNA extraction, and quantification by digital polymerase chain reaction (dPCR). Three different conditions of pasteurization and clarification processes were investigated to determine their effects on the detection of SARS-CoV-2 RNA. The data showed that heattreated samples indicated viral RNA degradation when compared to the non-heat-treated controls and that the preferred sample clarification method is centrifugation.

PA DOH BOL presented a poster summarizing the process and results at the Association of Public Health Laboratories (APHL) Infectious Disease Laboratory Conference in March 2023. The method studies also strengthened relationships of different bureaus within PA DOH and forged partnerships with wastewater treatment plant personnel through interactions and education of processes to maintain safety of the community.

The "Now What"

The results determined a new reliable, accurate, sensitive, and reproducible method to determine the presence of the SARS-CoV-2 virus in wastewater samples. This study led to the validation of this test method, which is now used to test wastewater samples weekly from several wastewater treatment plants across the Commonwealth of Pennsylvania.

The results are reported to the PA DOH Bureau of

Epidemiology and will be entered into NWSS for the CDC. PA DOH BOL plans to onboard additional wastewater treatment plants for weekly testing as well as develop test methods to detect other pathogens of interest. Further, the connections that have been made with community partners and the framework that has been established will serve to quicken response time for surveillance efforts of the next pandemic.

Funding source: This work was supported in part by the Centers for Disease Control and Prevention (CDC) cooperative agreement #NU38OT000297. Its contents are solely the responsibility of the authors and do not necessarily reflect the views of the CDC.

Key contributors to this project include Danielle Glatts, Pennsylvania Department of Health; Lisa Dettinger, Pennsylvania Department of Health; Jalpa Shah, Pennsylvania Department of Health; Dongxiang Xia, Pennsylvania Department of Health.