Predictive Values of Whole Campus Wastewater Surveillance for SARS-CoV-2

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Abstract
Screening university wastewater for SARS-CoV-2 emerged as an attractive method to provide ancillary data to bolster detection of viral circulation, as it had the potential to detect the presence of asymptomatic cases or cases that are part of the campus community but not the student population. Our objective was to evaluate the positive and negative predictive values of wastewater screening for COVID-19 cases. We developed and undertook a pilot wastewater screening program for the Fall semester of 2020. Homogenized wastewater influent was collected once per 24 hours, and extracted nucleic acids were interrogated for the presence of SARS-CoV-2 by quantitative reverse-transcriptase PCR. Dates of positive detection were overlayed with dates of confirmed cases in the student population to determine assay sensitivity. SARS-CoV-2 was detected numerous times and was often, but not always, followed by cases of COVID-19. Positive student cases were preceded by detection of SARS-CoV-2 in wastewater by up to 48 hours at each detection. The positive predictive value of wastewater detection for student COVID-19 cases was 0.8105, and the negative predictive value was 0.991. Wastewater screening has the potential to serve as a tool for community-level SARS-CoV-2 surveillance, and is particularly powerful as a negative predictor of disease activity.

Keywords
COVID-19, SARS-CoV-2, Wastewater, Surveillance

Introduction
The novel Betacoronavirus SARS-CoV-2 emerged in late 2019, resulting in a global pandemic of CoRonaVIrus Disease 2019 (COVID-19) [1]. College campuses were among the many communities tasked with developing methods for surveillance to detect the presence of both positive cases and the virus itself due to its high capacity for horizontal transmission in dense community settings [2-4]. While SARS-CoV-2 is transmitted person-to-person via inhalational spread, the virus can be detected in wastewater. Wastewater screening can therefore be a useful tool to monitor viral presence in communities, as is done for multiple pathogens [5-7]. The University of New England’s main campus features a stand-alone wastewater treatment facility serving 1,000 students that is distinct from the surrounding municipal treatment plant, indicating that any detection of SARS-CoV-2 can be definitively linked to the campus community. We utilized this setup to evaluate the positive and negative predictive values (PPV, NPV) of wastewater screening for COVID-19 activity.

Wastewater collection
Influent was collected in sterile tubes containing DNA/RNA Shield™ (Zymo Research) at a 4:1 v/v ratio. Organic material was concentrated onto MF-Millipore® hydrophilic mixed cellulose ester 0.45 μm membranes (Sigma-Aldrich) by vacuum filtration [8], removed from the filter surface with sterile cell scrapers, and homogenized in 1 mL fresh DNA/RNA Shield™.

Nucleic acid extraction and molecular testing
Total RNA was extracted using Quick-RNA™ reagents (Zymo Research), and cDNA was generated using qScript™ cDNA Supermix (QuantaBio) per the manufacturer’s instructions. Samples were interrogated for the presence of SARS-CoV-2 by quantitative reverse-transcriptase PCR.

Materials and Methods
We developed a wastewater screening program for the Fall semester of 2020. Homogenized influent was collected from the primary settling tank at the wastewater treatment facility once per 24 hours (N = 84 samples).

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has excellent potential for establishing and maintaining a community as free of SARS-CoV-2 as the pandemic wanes and cases become less frequent.

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