Wastewater Monitoring of SARS-CoV-2 with Early Warning™ COVID-19 Test Provides Insights on Community Prevalence

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Abstract

Wastewater monitoring to track community health status of the SARS-CoV-2 virus enables understanding and mitigation of community transmission for municipalities, academic and industrial campuses. As a strategic community response for wide-scale and regular COVID-19 testing of the American population, CDC-guided grab sampling methodology is highly cost effective and scalable. Specifically, composite effluent is sampled from either wastewater treatment plants (WWTP) or recently accumulated solids through remote sub-sampling at municipal locations identified by manhole covers. Collection is followed by immediate submersion in an inactivation transport and storage buffer for preserving sample quality at ambient temperatures.

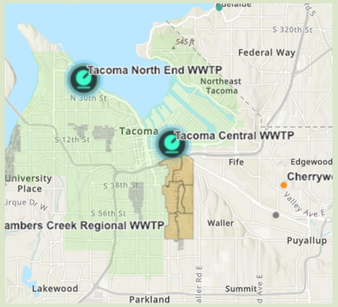
Preliminary results show the correlation of viral copy numbers detected with publicly available data of COVID-19 case counts from March 2020 to March 2021 in Pierce County, WA.

Introduction

Testing human wastewater has been successfully used for decades to monitor the presence of pathogens and guide public health officials' decision-making processes. As part of the effort to respond to the pandemic, testing wastewater for the presence of SARS-CoV-2 has come into very intense focus as tool for tracking the health status of communities, from small school campuses to large municipalities. Considering sampling and collection strategies, viral concentration protocols, RNA preservation, RNA extraction methods and SARS-CoV-2 RNA detection, we developed a workflow for a sensitive, affordable, timely wastewater-based early warning system for the presence of SARS-CoV-2.

Testing Area

Working directly with the municipality in the City of Tacoma, samples were collected from WWTPs at City of Tacoma's North End (T3) and Central (T1), representing the catchment area shown in green. In addition, we tested manhole locations to characterize viral signals at smaller catchment areas and neighborhoods (data not included here). Results were reported for this analysis as number of estimated viral copies per sample.



Screen shot of interactive COVID-19 sampling map illustrating sampling locations and results of testing over time.
www.rainincubator.org/services/covid-dashboard

Sampling Strategy

It has been shown that, during SARS-CoV-2 infection, the virus replicates in the human intestinal tract and thereby is shed in the feces in potentially substantial numbers, estimated at 5,000 to 4,000,000 copies/ml. However, once in the sewage system, the concentration of viral RNA is inevitably diluted, down to an estimated 2-1000 copies/ml (reviewed in [5]). Total viral RNA concentrations depend on a large variety of factors, like individually highly variable fecal shedding dynamics, rain prevalence and overall influent rates, solids concentrations, chemical disinfection efforts, etc.

For the purposes of early warning, reporting estimated copies per mL is unnecessary and tradeoffs in cold storage requirements and RNA degradation diminish its benefits. Since the subsample from WWTP primary sludge is done by swab, the sample can be immediately sub-merged in an RNA/DNA shield (Zymo Research) transport and storage buffer, thereby alleviating the need for immediate refrigeration. Therefore, we are reporting as copies per sample. We also avoid exposure of the potential RNA to flow turbulence after sampling that is known to contribute to RNA degradation[6] and optimize our ability to detect RNA when in low abundance[7,8].

Wastewater Treatment Facility

1. Collect in cup, move swab about
2. Prep tube with RNA/DNA Shield
3. Immerse swab head in buffer and mix
4. Break off shaft and seal

Manhole

1. Long pole, paper towel secured at end
2. Sampling of the collected sludge
3. Immersion of swab into RNA/DNA shield, cut off shaft and seal

It has been shown that sampling from primary sludge might yield better results when overall RNA concentrations are low[7,8], favoring a one-time grab sample over a 24-hour automated composite sample, provided attention is given to collecting primarily sludge over flow water.

Trends in Case Numbers, RNA Copies, Influent Rate

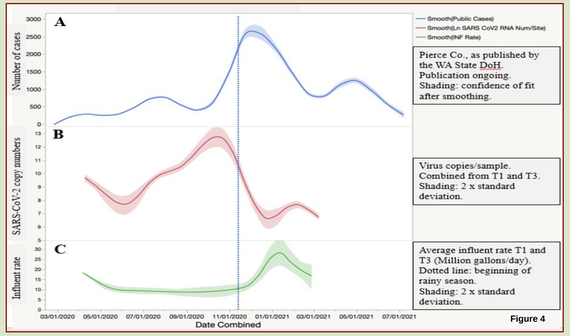


Figure 4

To assess if and how the wastewater results correlated with the number of publicly reported COVID-19 cases in Tacoma, WA, Pierce County[9], the time series for confirmed (positive molecular test for SARS-CoV-2) and probable (antibody positive but no positive molecular test) cases (Fig. 4A, blue line) was compared with the wastewater testing results over the same time period period (Fig. 4B: red line). Test data include results from all samples collected and tested from both the Central (T1) and North End (T3) WWTPs to represent their combined catchment areas. (Since all manhole sampling sites are contained in the combined catchment areas of T1 and T3, the data presented here focus on the results obtained for the WWTPs only.)

Fig. 4C (green line) depicts the average influent rate combined at both WWTP locations. Additional influent is primarily due to water influx from stormwater entering the WWTPs.

Analysis

Our data show that over the course of a year of longitudinal observation, there were three waves of increased SARS-CoV-2 positivity, roughly in April 2020, September-November 2020 and February-March 2021. These trend waves were observed both in the data for WWTPs and data for the manholes (data not shown). Importantly, there were also three waves observed in the number of individual COVID-19 cases in Pierce County (confirmed and probable), as captured by the Washington State DoH (Figure 4). The waves and peaks of positivity in wastewater occurred slightly earlier than the corresponding spikes in cases. Data also show that a strongly increased influent rate during the rainy season (November 2020 - January 2021) corresponds to a significant decrease in copy numbers. Despite the impact of additional influent rates, likely due to rainwater in the combined catchment areas, SARS-CoV-2 was still detected at levels to accurately report presence of virus in the community.

For the data presented here, statistical analysis employed a linear least squares regression model with 2 degrees of freedom. This model shows a highly significant relationship between the number of cases and the SARS-CoV-2 copy number trend, as indicated by P<0.0001 for the viral copy number per site.

The combined influent rate into the WWTPs had an inverse relationship to case load. That relationship was still significant (P=0.02) but not as strong as the copy number as predictor of the number of cases.

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-295.0227	112.4933	-2.62	0.0095*
Ln SARS CoV2 RNA Num/Site	108.67766	10.82812	10.04	<.0001*
INF Rate	-9.275838	3.974807	-2.33	0.0207*

This correlation of wastewater positivity (copy number increasing) with the number of individual cases in the county overall supports that sampling and testing of wastewater in a particular catchment area can serve as an early indicator of a rise in individual cases. Hence, the sampling strategy and method, and the processing workflow were collectively named Early Warning Wastewater Surveillance and are offered as a commercially available testing service by Neogen Genomics.

Acknowledgements

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Rainwater Effect

While the trends of increasing and decreasing rates of infection in the community were evident in the wastewater, it is not currently possible to infer the actual number of individual infections from the magnitude of the collective RNA signal in the wastewater. Variables that prohibit such modelling include differential rates of fecal shedding over the course of an infection. Consequently, a given SARS-CoV-2 RNA signal from wastewater can be made up of a single strong shedder's active infection or many moderate shedders' infections. Additionally, quantification of copy numbers by qPCR does not distinguish whether the detected RNA comes from live virus particles, indicating active current infection, or dead and disrupted virus particles. For locations that receive heavy rain fall, the dilution factor presented by large additional influx of stormwater in Pierce County and similar regions can be expected to diminish the ability to detect the RNA signal.

Conclusion

Changes in viral load in the wastewater may act as an efficient indicator of community spread (increase or decrease), provided influent rate is taken into account. Determination of trends in wastewater positivity can thus be an affordable and effective community-wide ongoing surveillance strategy. It can detect trends of new emergence, resurgence, subsiding and elimination of SARS-CoV-2 signal in the population as part of the ongoing continuous response to the SARS-CoV-2 pandemic. Further work by this group will focus on characterizing the dynamics of the lag time between positivity in the wastewater and the case load in the community, as well as defining the effect of water flow on viral counts in the sewer. We expect that aggregation of cases for smaller catchment areas defined by manholes will demonstrate increases in resolution and the ability of such testing to enable targeted rapid responses to outbreaks of smaller proportions.

Additionally, as the focus might shift from SARS-CoV-2 to other human enteric pathogens, wastewater-based surveillance might be expanded to include other emerging biological threats.

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Influent rates downloaded from:
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- https://apps.ecology.wa.gov/paris/ComplianceAndViolations/ViewDMRData.aspx?PermitNumber=WA0037214&FacilityName=&City=&County=&Region=0&PermitType=0&StartDate=01/01/2020&EndDate=01/14/2021 (T3)