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Detection of SARS-CoV-2 in wastewater is an efficient and scalable approach for community infection monitoring

Introduction

During the COVID-19 pandemic, testing for SARS-CoV-2 has played an instrumental role in identifying infected individuals, directing them to appropriate medical care, guiding them in practices to prevent transmission to other individuals, and shaping public policy to protect the broader community. Individual clinical testing has played a key role in our pandemic response; however, as vaccination rates increase and positivity rates decline, there is a need for more unbiased, inexpensive, and scalable methods to assess transmission in a community. Because SARS-CoV-2 is shed in feces, it can be quantified in wastewater, providing an opportunity to obtain a pooled biological sample that can provide information about the health of a community.

The levels of SARS-CoV-2 RNA in wastewater have been shown to have a strong relationship to COVID-19 incidence[1–6]. Wastewater monitoring can serve as a reliable indicator of disease that is not impacted by challenges with clinical testing such as inequitable access and test seeking behavior - a particular concern for COVID-19 because transmission by asymptomatic and pre-symptomatic individuals is common, which can result in underestimation of community prevalence. Not only has wastewater monitoring proven useful for COVID-19, it can also be extended to other pathogens that are shed in feces and urine and present in wastewater.

In order for a wastewater monitoring platform to be useful for regular, prospective monitoring of COVID-19 incidence rates, it is essential that the methods used are also 1) simple and inexpensive to deploy, 2) sensitive enough to detect low concentrations of SARS-CoV-2, 3) scalable to allow for rapid processing and communication of data to public health officials, and 4) comparable for consistent interpretation across plants and laboratories providing data.

Wastewater sampling can focus on either liquids or solids

Wastewater contains both liquids and solids, with the solids primarily consisting of fecal material. Most methods for analyzing wastewater use liquid samples. These samples may be collected from wastewater flowing into a wastewater treatment plant, from within the sewer network, or from the outflow of a building. Because SARS-CoV-2 RNA is relatively dilute in liquid wastewater, these methods require the use of techniques to concentrate viral RNA in the sample prior to quantification.

Methods that focus on the solids in wastewater are currently less commonly used, but are gaining recognition as a superior source for pathogen detection. Samples that are rich in solids can be collected from the treatment plant directly, or settled out of all of the types of

liquid samples described above. Because

SARS-CoV-2 is naturally concentrated in the solids in wastewater, this approach circumvents the need for a concentration step.

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Verily's wastewater monitoring platform uses solids to produce data indicative of COVID-19 transmission using sensitive, scalable methods. Results from solids demonstrate higher concentrations of SARS-CoV-2 on a per mass basis, higher rates of detection when COVID-19 incidence is low, and less preprocessing labor before analysis. These characteristics make the analysis of solids an attractive solution for large scale analysis.

Solids are simple to collect

Solids can be collected from the primary clarifier, a sedimentation tank commonly used in wastewater treatment plants to settle solids out of wastewater as one of the first steps in the treatment process (**Figure 1**). This sample is much higher in solids content than wastewater entering the plant and aggregates solids over a period usually lasting at least several hours. If there is no primary clarifier at a plant, collection from that location is undesirable or difficult, or samples from sewers or buildings are targeted, standard methods can be used to settle solids out of wastewater influent after collection[7].

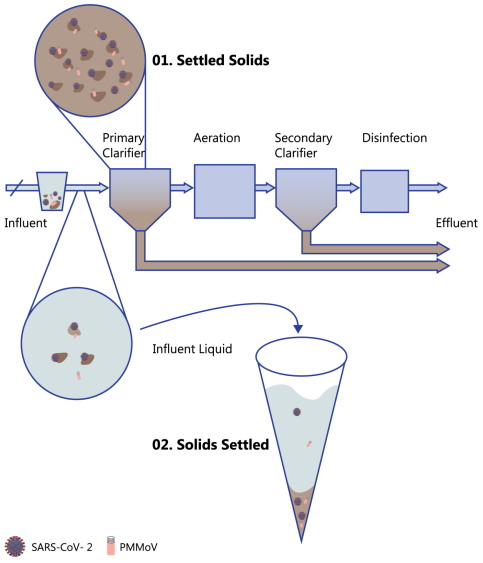

Figure 1. Schematic of a typical wastewater treatment plant showing possible sampling locations. Influent wastewater coming into the plant is mostly liquid and contains lower concentrations of SARS-CoV-2. It must be concentrated prior to analysis. Alternatively, at collection point (1), settled solids can be sampled from the primary clarifier, where they have already thickened. Another option at collection point (2) is to mimic this process by settling the solids out from a liquid sample in an Imhoff Cone. This is a standard method and can also be used for liquid samples from within the sewer network.

Enveloped viruses have a high affinity for wastewater solids

Coronaviruses such as SARS-CoV-2 have a high affinity for the solids in wastewater[8]. Solids in wastewater are largely fecal in origin, and SARS-CoV-2 in wastewater primarily originates from the feces of infected individuals. Because the virus adheres to the solids, the concentrations remain high in wastewater solids even after mixing with water in the sewage system.

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The settling of solids naturally serves as a concentration step that can be leveraged to allow for analysis with minimal pre-analytical processing. Direct comparisons of solids and influent show that SARS-CoV-2 concentrations in wastewater are 3-4 orders of magnitude higher in the solid fraction than in the liquid fraction on a per mass basis[2,9], making solids results a more sensitive sampling method. Higher concentrations on a per-mass basis result in a lower limit of detection (LOD) in solids, so that SARS-CoV-2 RNA can be detected and quantified to observe trends during periods of low COVID-19 incidence when it may not be detectable using methods for liquid samples.

Importantly, these measurements from solids are also shown to be highly associated with COVID-19 incidence rates in communities. Multiple studies have shown strong associations between measurements in wastewater solids and COVID-19 incidence from a diverse set of plants across North America [1,2,4,5,9].

Verily's solids method results in higher concentrations on a per-mass basis

Head to head comparisons of the Verily solids methods against two methods of concentrating and quantifying liquid wastewater build on this evidence. For six sites across the United States, Verily's solids methods result in several orders of magnitude higher quantification than two alternative methods utilizing liquid samples from the same plants and dates.

There are a range of technologies available for concentrating liquid wastewater (e.g. filtration, flocculation, magnetic beads). Both the PEG precipitation (Liquid 1) and magnetic beads (Liquid 2) methods for concentration were compared to sampling solids. The Liquid 2 methods show an advantage over Liquid 1 by producing consistent detection with quantifiable results (**Figure 2**). Despite consistent detections, the Liquid 2 method results in concentration estimates several orders of magnitude lower than that in solids on a per-mass basis.

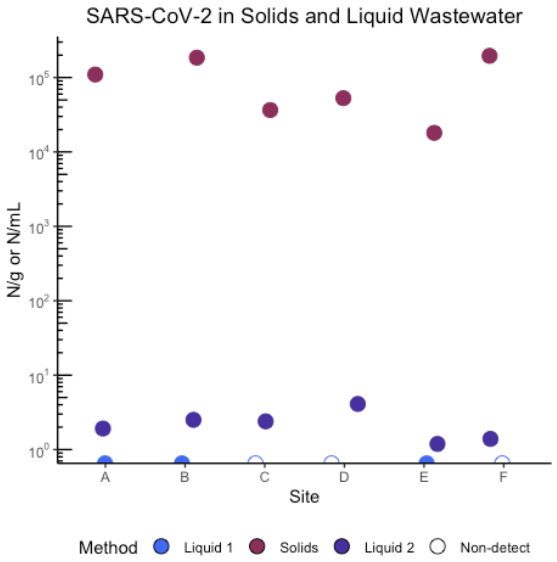

Figure 2. Comparison of results quantifying the N gene of SARS-CoV-2 in terms of cp per either mL or g in solids and liquids. Verily's methods were used for direct extraction from solids and quantification with ddPCR. Liquid 1 utilizes PEG precipitation methods followed by q-RT-PCR and and Liquid 2 used magnetic beads to directly capture viral RNA in the influent followed by ddPCR.

Solids methods are scalable

The methods that Verily uses for analysis of solids take advantage of the higher concentrations in solids to circumvent the need for lengthy and laborious concentration steps.

The methods were developed in partnership with researchers at Stanford University and the University of Michigan with the goal of taking successful methods for solids analysis and optimizing for scalability[10–12]. The resulting approach has been implemented in a program of daily sampling at plants across Northern California since November 2020,[13] with plans to scale to a national monitoring network enabling passive, anonymized epidemiological monitoring from more than half of the US population.

After a solids sample is collected (either from a primary clarifier or settled from a liquid sample) the sample is centrifuged to further dewater the solids. These solids are then resuspended in a buffer that liberates and then stabilizes viral RNA. RNA extraction is performed using an automated extraction system followed by removal inhibitors that are present in wastewater. Robotic liquid handling systems are used to streamline this process and ensure consistent application of methods.

Finally, the purified RNA is used as a template for two droplet digital PCR (ddPCR) reactions - one for SARS-CoV-2 targets (the N, S and, ORF1a genes) and one for analytical controls. ddPCR can also be successfully used for probe-based assays quantifying the proportion of SARS-CoV-2 viral RNA originating from variants of concern (e.g. alpha, delta, etc).

Pepper Mild Mottle Virus (PMMoV) is a virus found in many solanaceous plants that is also excreted in human feces and present in high concentrations in wastewater globally. It serves as an endogenous control allowing for normalization of pathogen detection by controlling for variation in RNA recovery and the fecal strength of a sample.

Because there is no concentration step, this process is simpler and more scalable than other methods. Verily has successfully used these methods to provide daily results within 24 hrs of sample collection for 8 Northern California plants participating in the first phase of this work over a period of more than six months (Figure 3).

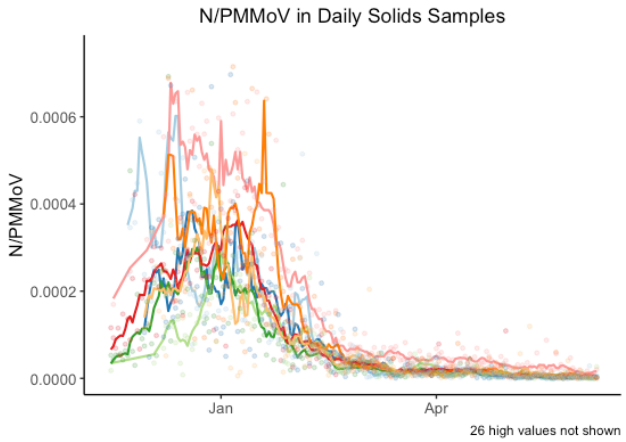

Figure 3. Time series illustrating wastewater data (N/PMMoV) from daily samples for 8 plants in Northern California over a period of 6 months from late 2020 - June 2021. Wastewater trends are reflective of the “winter surge” in COVID-19 incidence in the area and were utilized by public health officials via a public website (wbe.stanford.edu) to aid in decision making.

Solids results are comparable across methods

Finally, when measurements from wastewater solids are expressed in copies per gram (cp/g)/ of solids dry weight and normalized by the concentration of PMMoV in a sample, the values can be compared across results from different sampling locations and laboratories. This is because PMMoV adjusts for the recovery of viral RNA in the sample and the amount of the

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sample that comes from fecal material. This provides consistent results from a network of samples despite variations that exist in wastewater and processing.

Summary

Wastewater monitoring is an effective approach to estimate levels of SARS-CoV-2 transmission in a community. Wastewater solids are the best option to efficiently produce the most sensitive data to detect outbreaks. Verily's methods for the detection of SARS-CoV-2 in solids with ddPCR are scalable, effective, and available for widespread use.

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