Development and Validation of a Simplified Method for Analysis of SARS-CoV-2 RNA in University Dormitories

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Table S1: SARS-CoV-2 signal obtained from ESP-extracted RNA decays slower than signal obtained from raw wastewater stored at 4°C or room temperature over the course of 11 days.

Figure S1: Spiked eGFP RNA was detected using a multi-bottle composite sampler. A plot of detected spike RNA levels vs. time suggests that RNA persists in the effluent system at least on the order of a few hours, which validates our sampling cadence of 20 minutes.

Supplementary Information

Degradation Characterization:

To measure the effect of temperature on SARS-CoV-2 degradation we spiked free RNA or heat-inactivated virus (both obtained from BEI Resources) into SARS-CoV-2 negative wastewater at nominal concentrations of 1,000 and 10,000 copies/mL, respectively. Sample aliquots were aged at room temperature for zero to up to 21 days. Then, ESP extraction and RT-qPCR was performed (as described in the main text). Data was fit to a best-fit exponential decay function using MS Excel and the time constant was extracted from this equation. RNA half-life was calculated using this time constant. Free RNA exhibited a half-life of 3.5 hours while heat-inactivated whole virus exhibited a half-life of 3 days. Therefore, we assume that a large portion of free RNA is lost to degradation during composite sample collection and that the 4-24 hour sampling window limits degradation of intact virus. Further, we observed that ESP-stabilized RNA stored at room temperature exhibited improved stability over 11 days compared storage of raw wastewater at either 4°C or room temperature (Table S1).

	Raw Sample at 4°C	Raw Sample at Room Temp.	ESP Extracted at Room Temp.
Day 0 Conc.	2057 cp/mL	2057 cp/mL	2314 cp/mL
Day 3. Conc.	2006 cp/mL	1139 cp/mL	2376 cp/mL
Day 11 Conc.	983 cp/mL	181 cp/mL	2083 cp/mL
% Signal Remaining	35%	23%	88%
Half-Life	9.62 days	3.11 days	63.01 days

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Transit Time Evaluation

There is an expected transit delay between an individual shedding SARS-CoV-2 into the sewer system and its uptake by an autosampler. Variable flow rates and sewer architecture affect how long a biomarker takes to reach an autosampler and how long it may persist in the sewershed. To measure transit time from toilet to sampler, we flushed food-grade dye into the waste stream of a residential building. We visualized the dye at 15 minutes post-flush pass by the manhole approximately 200 feet from the toilet. In order to assess how long a nucleic acid signal is detectable in a wastestream, we flushed RNA and sampled effluent wastewater from the facility.

Spike Selection

We used an RNA sequence that encodes enhanced green fluorescent protein (eGFP) expressed in the jellyfish *Aequorea Victoria.* This sequence was chosen because it is highly unlikely to occur in residential wastewater streams and it can be harvested in large quantities from specialized cells lines already available in our lab.

Composite Sampling

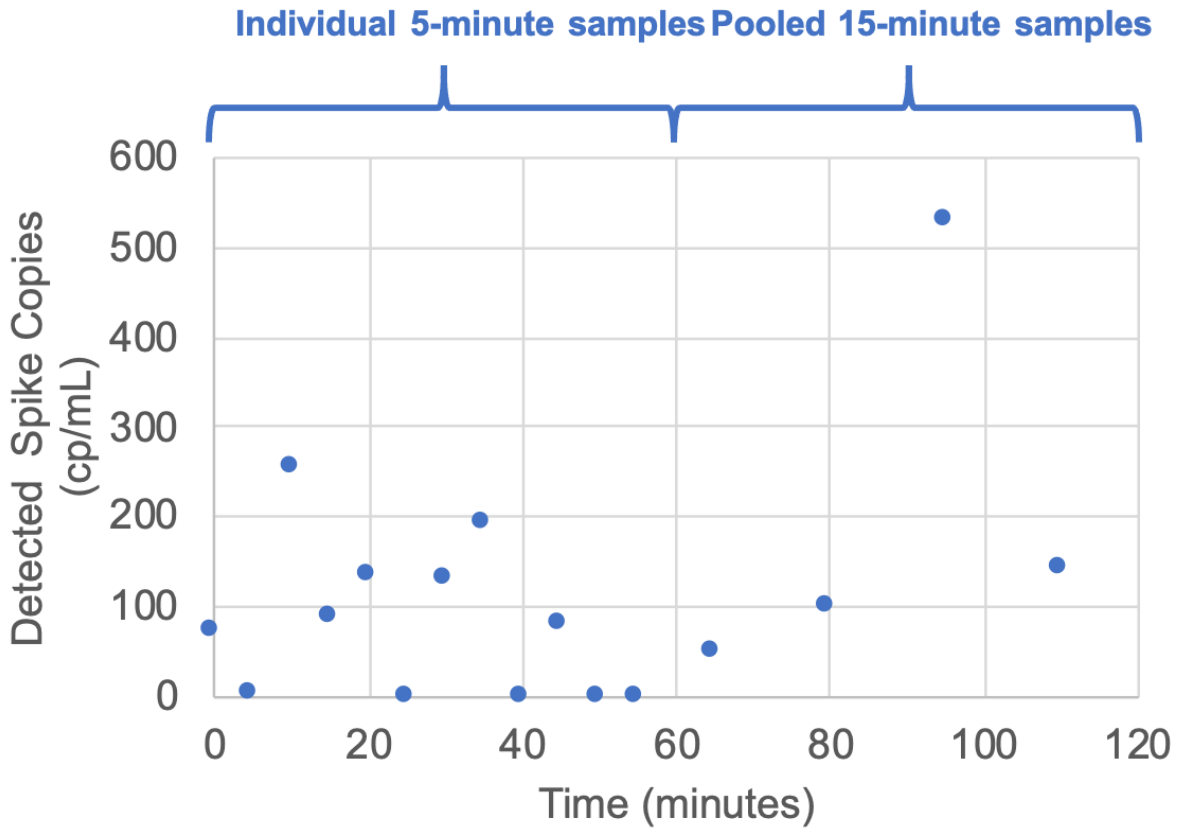
Sampling was performed using a Teledyne ISCO 3700c composite autosampler. This device is capable of collecting effluent samples and distributing them into a 24-bottle configuration. To maximize temporal resolution, we collected 24 100-mL samples in 5-minute intervals for the first hour of sampling, and then 15-minute pooled samples for the second hour.

Each fraction of wastewater was then extracted in triplicate and analyzed using RT-qPCR as described in the main text

Results

800 million copies of eGFP were diluted in DI water and spiked into the facility's waste stream. While the food dye was observed to peak between 5- and 15-minutes post-flush (albeit with a low sensitivity visual readout), we were able to detect RNA over much of the two-hour sampling window (Figure 2). Interestingly, this included early and late peaks, which are at least partially explained via the expected stochasticity associated with detection of dilute molecules. The composite autosampler takes roughly 1 minute to prime, purge, and pull a sample, which may explain the signal present at the first data point. The high signals during second hour may be explained by secondary and tertiary flushing events, which may act to propel RNA lodged in hydrodynamic stagnation points downstream.

Interpretation: From these experiments, we learned that flushed RNA would maintain detectable levels within the two hours of testing. Further, the high signal observed near the end of the two-hour window combined with calculations indicating that only 20% of the spike was sampled (adjusted to account for the fractions of wastewater that were not collected or collected and not tested), suggest that the RNA signal likely persisted beyond our test window of two hours. Therefore, assuming that our spike model is a valid representation of SARS-CoV-2 RNA, an infectious individual contributing to the wastewater at a residential level should be detected with a sampling cadence of 20 minutes.



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