Passively sensing SARS-CoV-2 RNA in public transit buses

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HIGHLIGHTS

- Passive, non-destructive sensing of viral presence on existing urban transit infrastructure is possible.
- Analysis of varying methods to overcome environmental factors
- Existing metro transit infrastructure can be used for viral surveillance.
- Amenable to pooled testing as a supplement to wastewater monitoring

GRAPHICAL ABSTRACT
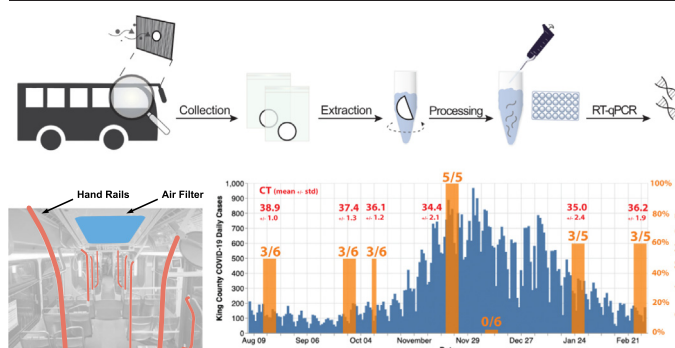
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ABSTRACT

Affordably tracking the transmission of respiratory infectious diseases in urban transport infrastructures can inform individuals about potential exposure to diseases and guide public policymakers to prepare timely responses based on geographical transmission in different areas in the city. Towards that end, we designed and tested a method to detect SARS-CoV-2 RNA in the air filters of public buses, revealing that air filters could be used as passive fabric sensors for the detection of viral presence. We placed and retrieved filters in the existing HVAC systems of public buses to test for the presence of trapped SARS-CoV-2 RNA using phenol-chloroform extraction and RT-qPCR. SARS-CoV-2 RNA was detected in 14% (5/37) of public bus filters tested in Seattle, Washington, from August 2020 to March 2021. These results indicate that this sensing system is feasible and that, if scaled, this method could provide a unique lens into the geographically relevant transmission of SARS-CoV-2 through public transit rider vectors, pooling samples of riders over time in a passive manner without installing any additional systems on transit vehicles.

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1. Introduction

The global pandemic of COVID-19 has exceeded 50 million reported cases in the US and 270 million confirmed cases worldwide as of December 20th, 2021 (Centers for Disease Control and Prevention, 2020; World Health Organization and others, 2020). The virus causing COVID-19, SARS-CoV-2, is primarily transmitted through airborne respiratory droplets via face-to-face contact (Buonanno et al., 2020; Zhang et al., 2020; Wiersinga et al., 2020) with asymptomatic or pre-symptomatic infected individuals. (Bai et al., 2020; Rothe et al., 2020; Yu et al., 2020; Hu et al., 2020a; Yu & Yang, 2020; Huff & Singh, 2020). Therefore, disease monitoring via viral presence testing is essential for managing potential outbreaks. Current disease monitoring is focused primarily on testing individual members of the population. However, frequent widespread testing across the entire population can be cost-prohibitive in many communities, even with pooled testing (Augenblick et al., 2020). While this resource intensive sampling strategy is useful for capturing the overall presence of a disease, alternative environmental sampling can serve as a warning sign of early-stage disease presence in a community prior to symptomatic patients testing positive (Daughton, 2020; World Health Organization, 2020).

One example of passive viral sensing is testing for SARS-CoV-2 in community wastewater plants (Daughton, 2020; Wurtzer et al., 2020; Wu et al., 2020; Peccia et al., 2020; Medema et al., 2020). However, wastewater surveillance methods suffer from a fixed, coarse granularity since sampling happens far downstream from the individual source. Leveraging multiple environmental sampling techniques through additional infrastructural media, such as public transit, can make viral monitoring more robust. Wastewater sampling has been explored for commercial aircraft and cruise ships (Ahmed et al., 2020), but these approaches cannot be extended to public transport vehicles without wastewater management facilities. Public transit such as buses, light rails, and trains may be valuable targets for surveillance sampling, since they are linked to the population's geospatial mobility. The United Nations estimated >50% global population lives in urban centers (Neiderud, 2015), such as Seattle, where nearly 50% of urban commuters use public transit (S. D. of Transportation, 2019).

Viral particles expelled from the respiratory system of an infected individual can circulate through the air into Heating, Ventilation, and Air Conditioning (HVAC) systems, and have been detected in air filters in hospitals treating infected individuals (Kim et al., 2016; Ong et al., 2020; Guo et al., 2020; Chirico et al., 2020; Horve et al., 2021), suggesting a similar approach for public transit. The HVAC system in King County Metro buses, which involve an air intake, MERV-rated filter, and recirculation, pull air from the front or rear passenger-seating areas on buses and are designed to be running all-day long to provide fresh air for passengers. Therefore, they represent an opportunity for passive sensing if viral presence can be detected in the system. Prior work has examined risk of transmission for passengers on buses, trains, and airplanes at local, national, and international scale (Hu et al., 2020b; Zheng et al., 2020; Zhao et al., 2020; Browne et al., 2016; Hoehl et al., 2020; Shen et al., 2020; Luo et al., 2020; Bae et al., 2020; Yang et al., 2020; Kong et al., 2020; Kasper et al., n.d.; Harvey et al., n.d.; Silcott et al., 2020); however, prior work has not explored community monitoring on public transit. One potential reason for this is the cost and time associated with known sampling methods with adequate Limits of Detection (LOD) to sense the low number of copies of virus expected in filters without employing active systems of collection, such as environmental swabbing or vacuum-like Personal Environmental Monitor (PEM) equipment (Moreno et al., n.d.). Rapid and inexpensive RNA extraction methods have detected 10–20 copies/reaction, which may be above the viral copies recovered from passive HVAC systems in non-concentrated settings outside of hospitals (Panpradist et al., 2021a). Additionally, virus particles can remain viable for 7 days on porous surfaces, like air filters, and 3 days on non-porous surfaces, like metal handgrips (Aboubakr et al., 2021; Matson et al., 2020). Therefore, air filters may accumulate and maintain virus over a longer time than swabbed surfaces, capturing data from more individuals with a single sample, enabling

pooled testing. Notably, viral presence does not imply infectivity, as a disinfected surface may still hold a dead virus with RNA that can be detected while the virus is no longer capable of infecting someone.

Here, we explore the feasibility of passive surveillance sampling in public buses by installing fabric sensors in vehicle air filtration systems. We demonstrate that sensitive methods of detection can be used to detect small virus copy numbers from samples collected from bus filters, using viral lysis, RNA extraction, and RNA detection via reverse transcription quantitative polymerase chain reaction (RT-qPCR) in a combination not proven in prior literature. In contrast to prior work studying building air filters (Horve et al., 2021; Rosario et al., 2018), we show that a novel combination of methods enables passive, scalable sampling while maintaining the potential for finer-grained community spread monitoring in localized areas via known bus routes. A requirement for scalability of this method is leveraging the already-operating HVAC systems in the bus for sensing while maintaining high analytical sensitivity and specificity for low concentration environments. Thus, we evaluated our in-house method in samples collected from actively circulating buses from August 2020 to March 2021 to demonstrate the detection of SARS-CoV-2 RNA in real-world environments, and we present herein an analysis of how this method may be related to citywide cases for future disease monitoring use cases.

2. Methods

2.1. Sample collection from public buses

Between August 2020 and March 2021, environmental samples were collected from 15 actively deployed buses in the Seattle King County Metro fleet (Fig. 1A). Bus selection was narrowed down to the main bus depot that serviced the Downtown Seattle area, which has the highest ridership. Individual buses were selected to be sampled via a convenience sampling approach based on which buses could be made available at the depot on a regular basis between 7:00–9:00 AM for sample retrieval.

Air filters and environmental swabs were used to capture samples on buses. For air filtration testing, four different materials were tested as supplementary air filters: foam biopsy pads (22-038-221, Fisher Scientific), PolyPro fabric (25PPMB, CanvasETC), mixed cellulose ester filters (A020A025A, Thomas Scientific), and paper filters (Fig. 1). Supplementary 5 cm^2 filters were placed in front of the existing air filter in the bus HVAC system in both the front and rear of the bus. Mixed cellulose ester filters were preferably used when they were available due to prior literature confirming their effectiveness at capturing and retaining biological material (Bartlett et al., 1997; Junter & Lebrun, 2017). On filter collection day, four different environmental swab materials were also tested: PolyPro fabric, microporous paper separators, mixed cellulose ester filters, and EnviroMax Swabs. Swabbing was performed by running one swab across common hand-hold areas (Fig. 1B in red) in the front and rear of the bus. When EnviroMax swabs were available, a second swab of the front face of the main bus filter (Fig. 1B in blue) was performed.

All bagged samples were placed in a plastic secondary container, which was wiped with bleach-based disinfectant, and transported to an approved lab facility. All procedures involving the untreated filter material were performed in a BSL2-certified Class II A2 biosafety cabinet. All types of filters that were used are shown in Fig. 1C. Due to safety-related lab space and chemical SOP limits for phenol-chloroform isoamyl extraction, a maximum of $n = 6$ buses (2 replicates for each of the two methods - filter and swab) could be tested in a single experiment. Notably, passenger safety measures were in effect for public transit throughout the sampling period in response to the COVID-19 pandemic. This included a mask mandate, which required all passengers to wear masks while riding the bus, as well as nightly spraying and wiping of all commonly-touched surfaces, such as seats, handrails, payment readers, stop-request pull cords, and door handles, with the Virex quaternary ammonium disinfectant (04332, Diversey).
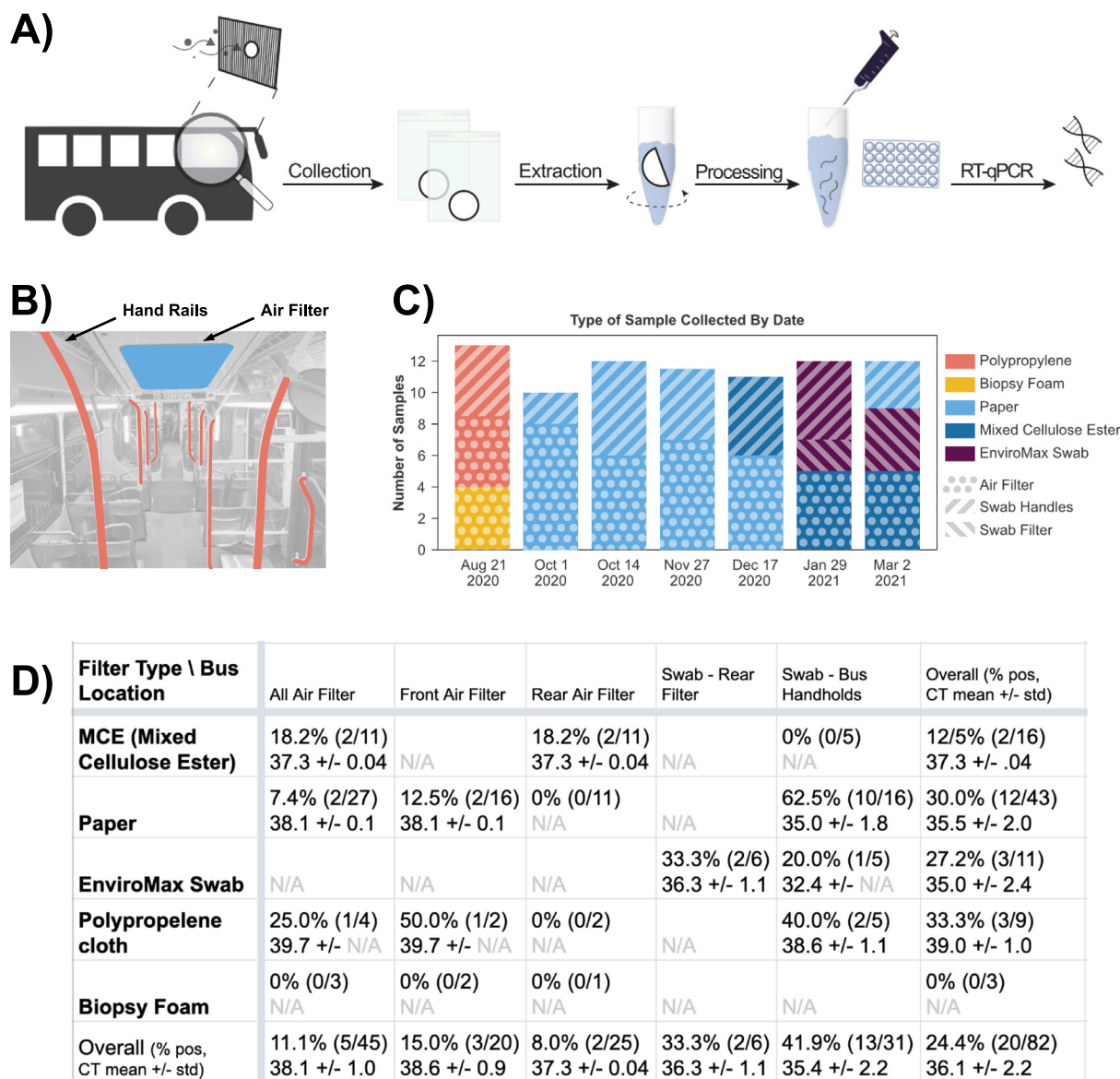
Fig. 1. Detection of the samples collected from the metro bus using our in-house extraction protocol. (**A**) Workflow for passive sensing SARS-CoV-2 RNA including sample collection, sample transfer from papers or swabs, RNA extraction, and RT-qPCR for detection. (**B**) Sampling occurred via two methods in different areas of the bus. We collected supplementary pre-filters after more than 7 days of being installed inside the HVAC systems of actively-used metro buses (blue). We also swabbed commonly-touched surfaces on the bus (red). (**C**) Sample types and collection methods used during the course of the study. (**D**) Positivity rate and average CT value breakdown by collection material and location. Sampling from both air filters as well as surfaces returned traces of SARS-CoV-2. Swabs from bus handholds made up the majority of SARS-CoV-2 detections with 42% positivity rate (13/31), while materials placed in air filters had the lowest positivity rate at 11% (5/45).

2.2. Detection of SARS-CoV-2 RNA in filters

Sample extraction for testing was performed within the same day of the sample collection from metro buses. Detection of SARS-CoV-2 RNA consisted of the following steps: viral extraction and lysis, RNA isolation via phenol-chloroform isoamyl extraction, and RNA detection via RT-qPCR (Fig. 1A).

2.2.1. RNA extraction and isolation

Filters collected from buses were cut into 2-cm^2 pieces. Two pieces, considered sample replicates, were placed into microcentrifuge tubes containing 200 μL lysis buffer (50 mM EDTA pH 8.0, 250 mM Tris-HCl pH 8.0, 50 mM NaCl, 1% (w/v) SDS) (Miura et al., 2011). The tubes were placed on a foam tube rack attached to a vortexer and agitated for 15 min, at

high speed, at room temperature. After vortexing, 600 μL TRIzol was added to each tube, pipette-mixed 10 times, and then the resultant 800 μL solution was transferred into a new tube. The solutions were incubated at room temperature for 5 min to allow complete dissociation of viral particles into the upper media and inactivation of any potentially remaining active virus in the solution. The RNA was then isolated from protein and DNA following the standard TRIzol phase separation procedure (Rio et al., 2010). Precipitation of RNA was carried out by adding 1 mL 200-proof ethanol and 1 μL RNA-grade glycogen (R0551, ThermoFisher) to each tube, followed by 1-min vortexing. Each tube was then incubated overnight at −20 °C. Following overnight precipitation, the supernatant was discarded and residual ethanol was allowed to evaporate. The RNA pellet was washed following the standard TRIzol RNA wash procedure and subsequently re-suspended in 8 μL nuclease-free water.

2.2.2. Detection of SARS-CoV-2 RNA using RT-qPCR

Each 8 TRIzol isolation product was assayed with TaqPath 1-step RT-qPCR (A15299, ThermoFisher Scientific) in 20 μL reactions. We used probes from the CDC SARS-CoV-2 qPCR probe assay targeting two regions in the N gene, designated N1 and N2 (10,006,713, Integrated DNA Technologies), one for each sample replicate. To avoid cross-contamination, the reactions were loaded into non-adjacent wells in a 96-well plate on ice at a separate bench from where the RNA isolation step was performed. Wells also were covered with Parafilm between loading samples. RT-qPCR was carried out on a Quantstudio 3 (ThermoFisher) using the CDC-recommended protocol (C. for Disease Control, 2020).

2.3. Positivity determination

Positive results were determined by amplification before a specified PCR cycle threshold (CT). Raw RT-qPCR amplification data were loaded via a custom Python script (Supplementary Information) that determined CT based on a common threshold across all samples (50,000 RFU) which delineated between amplified samples and non-amplified samples in control experiments. For environmental samples from buses, a positive result for a bus was declared if one replicate from one of the sample methods from that bus had a CT <40. Positive and negative controls experiments were conducted by dripping AccuPlex enveloped RNA reference material (0505–0126, Seracare, Milford, MA) onto unused filter, and the full extraction and detection method was performed to confirm that positive results were a result of viral presence in the air filter.

3. Results

3.1. Results on environmental samples from buses

Out of 82 samples (164 total with replicates) tested, 24% (20/82, 95% CI: 16–35) of samples tested positive for SARS-CoV-2 RNA (Fig. 1D), indicating viral presence but not necessarily infectivity. Samples collected by the swabbing method showed the highest positivity rate at 42% (13/31, 95% CI: 25–61) while filters had the lowest positivity rate at 11% (5/45, 95% CI: 4–24). 1 replicate amplified in all positive bus samples (Table A.3). This indicates that the viral particles may not be distributed evenly across the sampling material or that the sample methods may be sensing viral presence from different signal sources (i.e. riders who breathe may not touch the railing). Most positive samples had SARS-CoV-2 RNA near or below the LOD of the RT-qPCR assay (Fig. A.4) and thus were confirmed correct product sizes by fragment analysis (Fig. A.12).

3.2. Method comparison

We compared our TRIzol-based RNA extraction method with a more commonly used column-based RNA extraction from Qiagen. After dividing five filters in half and processing in parallel, we found a bus positivity rate of 60% (3/5) and 80% (4/5) (Table A.4) in TRIzol-based and column-based methods, respectively. Interestingly, our TRIzol-based method did not yield positive results from any samples collected by EnviroMax swabs, which were positive with the column-based method. We observed black particle residues in samples using EnviroMax swabs (Fig. A.13), which were filtered out by the column-based extraction. These residues ended up in the RT-qPCR reactions when EnviroMax samples were extracted by the TRIzol-based method, which may have inhibited the RT-qPCR reaction. On the other hand, the column-based method displayed 0% positivity rate on all air filter samples, while the Trizol-based method detected 40% (2/5) positivity on the same air filters. We hypothesize that the debris broken from filters could interfere with the binding of SARS-CoV-2 RNA to the silica membrane in the columns or the SARS-CoV-2 RNA might remain trapped in the columns. These filters are made from mixed cellulose ester which have different surface properties and porous structures from those of polyurethane foam structures (EnviroMax swabs) which reportedly had a high release efficiency even with minimal agitation (Panpradist et al., 2014).

All air filters were installed on buses for more than 7 days, and thus can represent pooled samples of all riders for the prior 7 days (Fig. 2). One exception is that, for one sampling date on October 14, 2021, filters were installed and collected in one day. In one-day testing, 0 filters returned positive, indicating that one day may not be enough filter exposure time to build up a detectable viral load. However, a relatively high rate (60%) of swab samples returned positive, which may be attributable to lack of surface decontamination mid-day. Metro cleans buses nightly, and the morning sampling period for all 2-week samples occurred the morning following the decontamination, in between which no riders would have ridden the bus.

### 3.3. Results compared to population testing

Fig. 2 shows the bus testing results juxtaposed with the SARS-CoV-2 case counts and bus ridership counts for the 7 sampling periods. While the sample size is too low to demonstrate positive correlation, we do see that a higher proportion of buses sampled return positive results when SARS-CoV-2 cases in King County were high, with the exception of December 14th, which showed no positive results. This was likely due to the fact that only 2/6 buses sampled had any ridership during that week, reducing exposure and decreasing likelihood of detection. October 1st and November 24th also returned positive results for buses which did not leave the station (zero riders), denoted by the empty circles in Fig. 2. This finding indicates that some results in this study may be a signal of continued viral RNA presence after more than 7 days or infected maintenance workers who entered a bus during the sample period.

Metro's ridership data was compared to bus positivity results to determine whether ridership was correlated with the positivity rate for buses, and a small Pearson's Correlation of 0.255 was observed. King County population testing data was also compared to average CT value of positive results, and a Pearson's Correlation of −0.687 was observed (Fig. A.7). The negative trend indicates that the strength of the detected signal on buses may increase as more people test positive in the city. The correlation observed may be reduced due to the small sample size and infection control measures taken by King County Metro. This included required rider masking and nightly surface disinfection (see Methods) throughout the entire sampling period, likely resulting in an increased number of false negative results due to a decreased number of viral particles escaping the mask of infected riders and reduced viral presence on hand rails. Ventilation and virus collection on filters may have been affected by driver and rider behavior, as well, as it is the driver's choice whether to activate the ventilation system (though most drivers do) and passengers may open the windows if they choose, which would modulate ventilation and likelihood of viral capture on filters. Additionally, the population of metro riders from which the testing was sampled is not fully representative of the overall population from which individual testing was performed.

3.4. Control validation

In control experiments, extracting spike control from filters yielded 6/9 (66.7%) replicates and 7/9 (77.8%) replicates of spike controls directly in solution amplifying and displaying positive detection using our method, while only 1/18 (5.6%) of the replicates of negative controls returned positive with a high CT value of 37.3 (Fig. A.5). Based on a PCR standard curve from the same experiment (Fig. A.6), the extraction efficiency is approximately 33% (average of 134 out of 400 copies) for filter extraction controls and 115% (average of 459 out of 400 copies, which is within the error range for PCR extraction) for direct in solution extraction controls (Table A.2). This suggests that about one-third of viral material may be lost during the filter extraction step, but not much is lost during RNA extraction. This may have resulted in some false negatives in bus testing but demonstrates that positive detection results were likely a result of viral material collected from bus sampling.
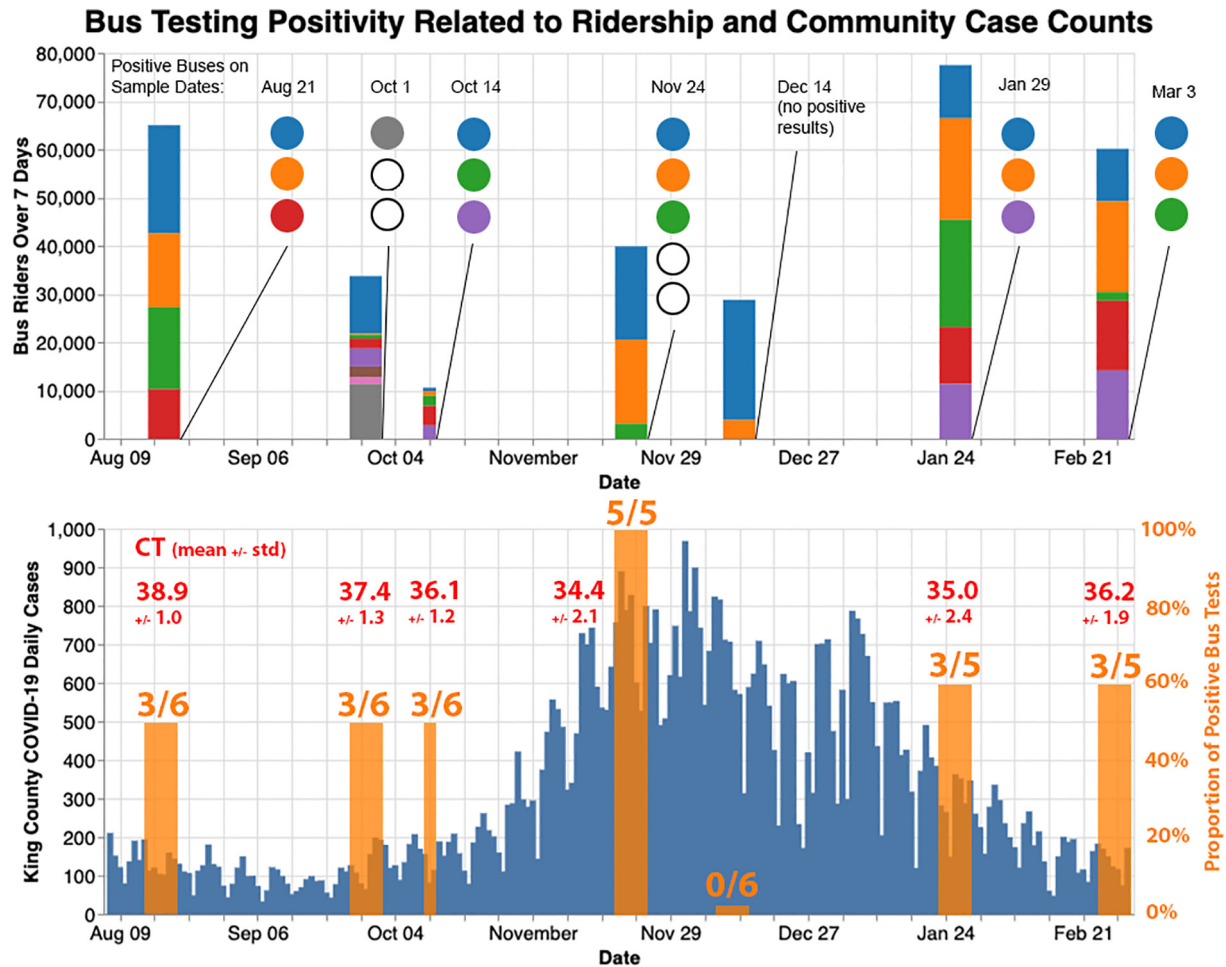
Fig. 2. **Top** chart shows total riders per bus per 7-day period of filter installation before sampling. Each color denotes a unique bus that week. The color of associated circles denotes a positive result from that bus. An empty circle denotes a positive sample for a bus with 0 riders during that week. **Bottom** chart shows new cases of SARS-CoV-2 in King County (blue) superimposed with the proportion of buses sampled that week returning positive results (orange). CT values for positive results are listed by date (red), and showed a -0.687 Pearson's correlation when compared to King County individual testing positivity (Fig. A.7).

4. Discussion

Here we show that passive infrastructure-mediated sensing of viral presence may be feasible. By leveraging a passive viral sensing method such as ours in parallel with other environmental and individual testing methods, epidemiologists could inexpensively monitor a community to identify locales of transmission and estimate case numbers within local regions. Our method may be more valuable when cases within the general community are low, leveraging the fabric sensors to passively pool respiratory droplet samples from riders temporally over the filter installation period and spatially over bus routes. This monitoring would be enabled by sufficient resourcing to enable daily sampling and testing of a subset of bus routes to ensure adequate coverage, which was not possible with the small study team in this proof-of-concept study.

This study is limited by its sample size ($n = 39$ total buses). Sample filters were placed and recovered manually by the research team and metro collaborators, which could be scaled by larger research teams (Table A.5). False negatives in air filters may have been caused by the limited coverage of the sample media over the air vent and air currents diverting around the sample filter media, resulting in a lower positivity rate than swab samples. In addition, mask mandates were in effect for riders during the sample period, likely reducing the number of viral particles expelled into the air by breathing of infected riders landing on the filters. Finally, Metro's nightly cleaning process is designed to reduce the overall viral load in the bus, even if it did not completely remove the viral presence signal. Considering

these effects, the small viral loads (Fig. A.4) of some samples are unsurprising, but may fall below the typical LOD for many commercial PCR kits, including our chosen Taqpath 1-step kit (C. for Disease Control, 2020). Alternative sensitive assays that amplify multiple regions in SARS-CoV-2 may be useful to detect these samples with low concentrations (Kline et al., 2021; U. Food, 2021). We also note that our method does not necessarily identify the risk to bus riders, but rather the presence of inactive SARS-CoV-2 RNA. Viral viability tests and more frequent sampling are needed to understand the risk to riders.

4.1. Conclusion

Future research into scalable, sensitive viral detection for environmental samples would enhance this approach. Studies evaluating filter placement, size, and material, incorporating further control experiments in simulated environments, could further validate the sensitivity of the method and optimal materals for sampling (Buonanno et al., 2020; Holmgren et al., 2010). City-wide deployments enabled by scalable detection methods, such as rapid diagnostic lateral flow detection for on-site detection enabled by miniaturized amplification devices (Panpradist et al., 2021a; Panpradist et al., 2021b) or sequencers (Cardozo et al., 2021), could gather more data, enabling network analysis techniques to study probability of SARS-CoV-2 transmission on a neighborhood level. This method could be adapted and deployed to provide an early signal of community outbreak of SARS-CoV-2, or other viruses transmitted by

respiratory droplets, when case counts are low in the population. This could provide a relatively inexpensive early warning system and ongoing monitoring insight into the local routes of viral transmission for current and future respiratory pathogens.

CRediT authorship contribution statement

Jason Hoffman: Conceptualization, Methodology, Validation, Software, Investigation, Resources, Data Curation, Writing - Original Draft, Visualization, Project Administration, Funding Acquisition. **Matthew Hirano:** Methodology, Validation, Investigation, Resources, Writing - Original Draft, Visualization. **Nuttada Panpradist:** Methodology, Formal Analysis, Investigation, Writing - Original Draft, Supervision. **Joseph Breda:** Software, Writing - Original Draft, Investigation, Data Curation, Visualization. **Parker Ruth**: Funding Acquisition, Software, Writing - Original Draft, Visualization. **Yuanyi Xu:** Validation, Investigation. **Jonathan Lester:** Writing - Reviewing and Editing, Supervision, Funding Acquisition. **Bichlien Nguyen:** Writing - Reviewing and Editing, Visualization, Supervision, Funding Acquisition. **Luis Ceze:** Conceptualization, Supervision, Resources, Funding Acquisition. **Shwetak Patel:** Conceptualization, Supervision, Funding Acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152790.

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