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A sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level

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HIGHLIGHTS
• A Moore swab method was developed for COVID-19 wastewater surveillance.
• 442 swab samples were collected from the wastewater of the student residence halls, hospital, and quarantine buildings. 148 (33.5%) were positive for SARS-CoV-2.
• Paired weekly wastewater samples from the university hospital with COVID-19 patients showed that Moore swab method was sensitive than grab sampling method.
• Weekly Moore swab wastewater samples at the quarantine building indicated that Moore swab was sensitive to detect few cases in the building.
• This method is suited for COVID-19 wastewater surveillance of buildings or communities where COVID-19 prevalence is relatively low.

GRAPHICAL ABSTRACT

ABSTRACT

SARS-CoV-2 is a respiratory virus, but it is also detected in a significant proportion of fecal samples from COVID-19 cases. Recent studies have shown that wastewater surveillance can be a low-cost tool compared to massive diagnostic testing for tracking COVID-19 outbreaks in communities, but most studies have focused on sampling from wastewater treatment plants. Institutional level wastewater surveillance may serve well for early warning purposes because specific geographic areas/populations with emerging cases can be tracked and immediate action can be executed in the event of a positive wastewater signal. In this study, a novel Moore swab method was developed and used for wastewater surveillance of COVID-19 at an institutional level. Of the 442 swab samples tested, 148 (33.5%) swabs collected from the three campuses and two buildings were positive for SARS-CoV-2 RNA. Further study of the quarantine building with a known number of cases indicated that this method was sensitive enough to detect few cases in the building. In addition, comparison between grab samples and Moore swab samples from the hospital sewage line indicated that Moore swabs were more sensitive than grab samples and offer a simple, inexpensive method for obtaining a composite sample of virus in wastewater over a 24–48 h period.

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1. Introduction

Since the novel SARS-CoV-2 coronavirus was identified in Wuhan, China in December 2019 (Wang et al., 2020a), it has quickly spread to many countries and led to a global pandemic of COVID-19. Although COVID-19 is primarily transmitted person to person via respiratory droplets, studies have shown that SARS-CoV-2 is also shed in feces. A number of studies have reported that SARS-CoV-2 RNA was detected in a considerable proportion (25–80%) of fecal samples from COVID-19 cases in both adults and children (Zhang et al., 2020a; Xiao et al., 2020; Holshue et al., 2020; Xu et al., 2020; Wang et al., 2020b). In addition, a high proportion of cases had persistently positive viral tests from rectal swabs even after nasopharyngeal swab results became negative (Zhang et al., 2020a; Xiao et al., 2020; Xu et al., 2020), suggesting that the duration of virus shedding in the gastrointestinal tract could be longer than the respiratory tract. SARS-CoV-2 shedding in the gastrointestinal tract enabled the detection of SARS-CoV-2 RNA from wastewater collected at an urban wastewater treatment facility in Massachusetts in mid- to late-March 2020 and the SARS-CoV-2 RNA was confirmed by sequencing from the S gene (Wu et al., 2020).

Wastewater surveillance has been traditionally performed using two methods: grab sampling and composite sampling. Grab sampling is a simple and convenient method that involves wastewater collection at one point in time; however, interpretation is limited because the samples only represent a snapshot at one moment. Composite sampling has been considered as a more representative method due to its ability to collect numerous individual samples at regular time intervals, and the individual samples are subsequently combined in proportion to the wastewater flow rate (Johannessen et al., 2012). Composite samples can be collected manually or by using automated samplers, such as flow-weighted samplers or continuous composite samplers. Automated composite samplers must have built-in refrigeration capacity and require electricity, whereas manual composite samples require samplers to repeatedly return to the same location multiple times over a period of time. Composite sampling is, therefore, more costly and time-consuming, and it may not be feasible under certain environmental conditions (e.g., no electrical hook-ups) or when resources are limited. However, composite samples can provide a more representative wastewater sample – especially for smaller catchment populations with intermittent flows and virus shedding.

Wastewater surveillance for SARS-CoV-2 can be performed by collecting sewage samples at “downstream sites,” such as a wastewater treatment facility, or at “upstream sites,” such as manholes adjacent to residential buildings such as campus dormitories or apartment complexes (Wang et al., 2020c). Sampling influent entering a wastewater treatment plant is a useful method to evaluate community-level trends in COVID-19 infection, but the catchment area contributing sewage to this point will be large because wastewater treatment plants in urban areas may serve tens of thousands of people. Alternatively, wastewater surveillance using samples upstream from the treatment plant can be used to detect SARS-CoV-2 RNA in smaller catchment populations. For example, collecting wastewater samples from manholes at an institution level provides researchers and decision-makers with a better understanding about SARS-CoV-2 RNA presence in wastewater and probable COVID-19 cases on the premises.

The “Moore swab” method is an environmental surveillance method that has been used for decades by public health practitioners around the world to detect and isolate enteric pathogens from water. Consisting of a strip of cotton gauze tied with string or fishing line that is suspended in flowing water or wastewater, this sampling method acts as a filter that allows collection of microorganisms over an extended period of time. Compared to automated composite sampler that costs about $2333 (WS700R, Pollardwater, New Hyde Park, NY, USA) to $6187 (AS950, Hach, Loveland, Colorado, USA) and requires electricity and skills to set up, Moore swab sampling is a low cost (about $1.2/swab) and an easy method to deploy. The Moore swab was first described by Brendan Moore in 1946 to trace Salmonella Paratyphi B from sewage in North Devon, England to determine the sources of infection responsible for sporadic outbreaks of paratyphoid fever. Since this first application, the Moore swab method has been utilized throughout the world to detect several excreted pathogens, such as coxsackieviruses, poliovirus (Kelly et al., 1957; Sattar and Westwood, 1977; Matrajt et al., 2018; Zdrazilik et al., 1977), human norovirus (Tian et al., 2017), Escherichia coli O157:H7 (Tian et al., 2017; Cooley et al., 2007), Vibrio cholerae O1 (Barrett et al., 1980; Merson et al., 1977; Simanjuntak et al., 1993), and S. Typhi (Sikorski and Levine, 2020; Liu et al., 2021). Over the years, the technique once used for tracing chronic carriers of Salmonella Paratyphi B has expanded to environmental surveillance, investigation of ongoing outbreaks, and pathogen detection in a range of water and wastewater (Sikorski and Levine, 2020).

The objectives of this study were to develop a practical, low-cost, and convenient Moore swab method for composite wastewater sample collection and analyses to provide early warning of COVID-19 outbreaks at an institutional level. A positive RT–PCR signal from a Moore swab placed in wastewater from specific buildings can be used to help inform health authorities about the spatial distribution of COVID-19 cases at an institution and the need to initiate diagnostic testing of residents in a specific building. For these objectives, we were interested in the presence or absence of SARS-CoV-2 RNA in sewage samples rather than quantitative measures of viral RNA in the samples. This manuscript describes: 1) the development of a standardized and efficient method for processing Moore swabs specifically for SARS-CoV-2 that includes an elution buffer to elute viruses off the swab, the use of a potato ricer (and more recently a stomacher) to process the swab samples, and subsequent virus concentration, RNA extraction, and detection of SARS-CoV-2 RNA by RT-qPCR, and 2) the application of this method to wastewater from a hospital, student residence halls on three campuses, and a quarantine building for known and suspected COVID-19 cases.

2. Materials & methods

2.1. Moore swab seeding experiment

The Moore swabs were made by cutting pieces of cotton gauze approximately 120 cm long by 15 cm wide and firmly tying the center with nylon fishing line. The cost of ten Moore swabs was approximately $12 for materials and 30 min of technician time. To examine the limit of detection for this method, a Moore swab was placed in a plastic container filled with 2 L distilled water, and the end of the fishing line was attached to a shelf located above the container. A stir bar was placed in the container to mimic wastewater flow observed in the field. The distilled water was spiked with 50 equivalent genome copies (EGC)/mL, 5 EGC/mL, and 0.5 EGC/mL of inactivated SARS-CoV-2 (ATCC, Manassas, VA) and Bovine Respiratory Syncytial Virus (BRSV, INFORCE 3, Zoetis, Parsippany, NJ). The swab was submerged in the flowing water for 24 h to trap the inactivated SARS-CoV-2 and BRSV as shown in...
The swabs were then processed and tested as described below. The same concentration of inactivated SARS-CoV-2 was also seeded into surface water samples collected from a small lake on the Emory Main Campus, and the experiment was repeated as described above.

2.2. Moore swab sample collection and processing

Manholes for sample collection were selected based on a detailed map of the sewerage lines and direction of flow in order to capture the wastewater directly downstream from only the target residence halls, the Emory University Hospital, and the quarantine building. For sample collection, a Moore swab was placed in the outflow stream of wastewater from selected buildings between 8 and 10 am on a weekly basis, secured by tying the fishing line to a hook at the top of the manhole. After leaving the swab in manhole for 24–48 h during weekdays (Main Campus, Clairmont Campus, Emory University Hospital, and quarantine building) and for 72 h (over the weekend on the Oxford Campus due to the schedule of the facilities staff), the swab was retrieved from the manhole, stored in a Ziploc bag, and transported to the lab on ice. In the beginning of the project, the swabs were manually squeezed in a beaker to capture all the trapped liquid. Since January 2021, the swabs were squeezed by a stainless-steel potato ricer, which was more standardized and efficient. After squeezing, the liquid was poured into a beaker. The swab was then submerged in 100 mL of elution buffer consisting of 0.01% sodium polyphosphate, 0.01% Tween 80, and 0.001% antifoam Y-30 emulsion (Liu et al., 2012). After gentle kneading for 2 min, the swab was squeezed again, and the liquid was then combined with the initial liquid squeezed from the swab. This step was repeated one more time until the total eluate volume was approximately 250 mL. Finally, the eluate was centrifuged at 5,000 rpm for 15 min at 4 °C to remove additional solids, and the supernatant was saved for further processing (Fig. 2). Total time for Moore swab processing was approximately 1.5 h for ten samples.

2.3. Grab sample collection and membrane filtration

Grab samples were only collected from the sewage outflow stream from the Emory University Hospital from a single manhole around the lab on ice. In the beginning of the project, the swabs were manually squeezed in a beaker to capture all the trapped liquid. Since January 2021, the swabs were squeezed by a stainless-steel potato ricer, which was more standardized and efficient. After squeezing, the liquid was poured into a beaker. The swab was then submerged in 100 mL of elution buffer consisting of 0.01% sodium polyphosphate, 0.01% Tween 80, and 0.001% antifoam Y-30 emulsion (Liu et al., 2012). After gentle kneading for 2 min, the swab was squeezed again, and the liquid was then combined with the initial liquid squeezed from the swab. This step was repeated one more time until the total eluate volume was approximately 250 mL. Finally, the eluate was centrifuged at 5,000 rpm for 15 min at 4 °C to remove additional solids, and the supernatant was saved for further processing (Fig. 2). Total time for Moore swab processing was approximately 1.5 h for ten samples.

Fig. 1. Moore swab seeding experiments using distilled water and surface water seeded with inactivated SARS-CoV-2 and BRSV at 50 EGC/mL, 5 EGC/mL, and 0.5 EGC/mL. A). Flow diagram showing Moore swab processing and testing procedures in the seeding experiments; B). Moore swab photo; C). Seeding experiment setup.

Fig. 2. Flowchart of Moore swab processing of the field samples.
8 am on a weekly basis, and the wastewater was processed by membrane filtration. 1 L of wastewater was collected from the hospital manifold because it had an adequate sewage outflow and the sample was transported to the lab in a cooler with ice. The sample was first pasteurized at 65 °C for 1 h to inactivate pathogens before further processing. 500 mL of the sample was centrifuged at 5,000 rpm for 15 min at 4 °C to remove solids in the sample that could clog the membrane filter. Before filtration, 10^5 equivalent genome copies (EGC) of BRSV was added to the sample as a process control. The sample was then filtered through two 0.45-μm-pore-size, 47-mm-diameter nitrocellulose filters (Millipore Sigma, Burlington MA) in order to maximize filtration amount after pH adjustment to 3.5 and 25 mM of magnesium chloride was added. The sample volume that was filtered varied between 80 and 500 mL due to differences in turbidity. After filtration, the membrane filter was placed into a microcentrifuge tube and 800 μL of RLT buffer from the RNeasy Mini Kit (QIAGEN, Hilden, Germany) was added immediately. The sample was vortexed at maximum speed for 10 min and then subjected to RNA extraction as described below.

2.4. Skimmed milk flocculation concentration

Most of the Moore swab samples were processed by the skimmed milk method (Melgaco et al., 2016). Briefly, a 5% (w/v) skimmed milk solution was prepared by dissolving 5 g of skimmed milk powder (BD, #232100, Sparks, MD) in 100 mL of distilled water. Before the solution was prepared by dissolving 5 g of skimmed milk powder (BD, followed by addition of 10^5 EGC of BRSV and shaking for 2 h.

2.5. PEG precipitation

In addition to the skimmed milk method, a small proportion of Moore swab samples were processed by PEG precipitation method in the beginning of the project. Virus was concentrated from the eluate from the Moore swab samples by adding 12% PEG (Sigma, St. Louis, MO, USA), 0.9 Mole sodium chloride, and 1% bovine serum albumin (Sigma, St. Louis, MO, USA) (Liu et al., 2012). To monitor the RNA extraction procedure and possible PCR inhibition, 10^5 EGC of BRSV were added into each sample as an extraction processing control prior to PEG precipitation. A stir bar was added to each flask, and the samples were stirred overnight at 4 °C (Liu et al., 2012).

2.6. RNA extraction

After skimmed milk or PEG concentration, the samples were centrifuged at 10,000 rpm for 1 h at 4 °C. The supernatant was removed and the side of the centrifuge bottle was rinsed, and the remaining pellet was dissolved using 800 μL RLT buffer from the RNeasy Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer’s protocol. If the pellet was too big, a bulk of the pellet was removed to reduce clogging of column because of excess material. One negative extraction control was included in each bath of samples. The final volume of extracted RNA from each sample was 100 μL (Fig. 2).

2.7. Quantitative real-time RT-PCR method

SARS-CoV-2 RNA was detected via real-time quantitative reverse-transcription polymerase chain reaction (RT-qPCR) using the N1 primers developed by CDC and the TaqPath™ qPCR Master Mix (ThermoFisher Scientific, Waltham, MA) (Lu et al., 2020). Each reaction contained 5 μL of 4x Master Mix, 0.2 μM probes and 0.2 μM primers specific to either SARS-CoV-2 or BRSV, 8.5 μL of molecular water, and 5 μL of RNA sample loaded in duplicate wells in a 96-well plate and placed into the Bio-Rad CFX PCR thermocycler (Bio-Rad, Hercules CA). The RT-qPCR program consisted of 25 °C for 2 min, 55 °C for 15 min, 95 °C for 2 min, 95 °C for 3 s, and 55 °C for 30 s, with a total of 45 cycles. Synthetic SARS-CoV-2 RNA (ATCC® VR-3276SD, Manassas, VA) that contained the region of the SARS-CoV-2 nucleocapsid (N) gene served as a positive control and molecular water was used as a negative control. BRSV was detected using the primers/probe described by Boxus et al. (2005) RNA extracts from each sample were tested in duplicate wells. Potential PCR inhibition was examined in some samples through testing dilutions (1:2, 1:5 and 1:10) and comparing the results to those from the undiluted RNA.

3. Results

3.1. Moore swab method for recovering seeded SARS-CoV-2 and BRSV in distilled and surface water

When SARS-CoV-2 and BRSV were seeded at 50 EGC/mL in distilled water, all three replicate swabs showed positive RT-qPCR results. When the seeding level was reduced to 5 EGC/mL, SARS-CoV-2 could only be detected in one of the two experiments, but BRSV was detected in both experiments. At the 0.5 EGC/mL seeding level, both SARS-CoV-2 and BRSV RNA could not be detected, indicating the limit of detection for both SARS-CoV-2 and BRSV using the Moore swab method in 2 L of distilled water was approximately 5 EGC/mL. The same concentrations of SARS-CoV-2 and BRSV were seeded into surface water in one trial, and the limit of detection for both SARS-CoV-2 and BRSV was 50 EGC/mL (Table 1).

3.2. SARS-CoV-2 RNA detection using Moore swabs on Emory campuses

Wastewater from multiple residence halls at three Emory campuses (Main, Clairmont, and Oxford) was sampled on a weekly basis by placing Moore swabs at manholes directly downstream from the buildings. From mid-August 2020 through March 2021, a total of 442 Moore swab samples were collected and analyzed. The overall proportion of swabs that were positive for SARS-CoV-2 RNA by RT-qPCR ranged from 16.8% to 42.1% for the three campuses with the highest detection rate on the Main Campus (42.1%) and the lowest detection rate from the Clairmont Campus (16.8%). Moore swabs were also collected from the quarantine

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Table 1

| Seeding Level (EGC/ml) | Distilled water | Surface water |
|------------------------|-----------------|---------------|
|                        | SARS-CoV-2 Positive Swabs/no. Swabs | BRSV Positive Swabs/no. Swabs | SAS-CoV-2 Positive Swab/no. Swab | BRSV Positive Swabs/no. Swab |
| 50                     | 3/3             | 3/3           | 1/1           | 1/1               |
| 5                      | 1/2             | 2/2           | 0/1           | 0/1               |
| 0.5                    | 0/2             | 0/2           | 0/1           | 0/1               |

* Swab samples were processed by skimmed milk method.
* Equivalent genome copies.
* Ct values of RT-qPCR positive were between 30.0 and 39.8 with replicate wells showing <2 Ct difference.
3.3. SARS-CoV-2 RNA detection in grab and Moore swab samples of Emory University Hospital sewage

To validate our methods and compare Moore swabs to grab samples, we collected paired weekly wastewater samples (both grab samples and Moore swabs) from July 2020 to March 2021 from a manhole directly downstream from the Emory University Hospital that was a major healthcare provider for COVID-19 inpatients in the metro Atlanta area. A total of 18 (69.0%) of 26 grab samples were positive, and 24 (92%) of 26 Moore swab samples were positive for SARS-CoV-2 RNA. Two sets of samples were negative by both methods (Table 3). There were some discrepancies between the results of the grab samples and Moore swab samples. For 8 negative grab samples, 6 paired swab samples tested positive. The concordance between the two methods was 77% (20/26).

3.4. Detection of SARS-CoV-2 RNA in wastewater using Moore swabs at the quarantine building

Students living in residence halls who tested positive for COVID-19 and suspected cases who were waiting for diagnostic test results were moved to a quarantine building near the Main Campus. The building had one nearby manhole to place Moore swabs. Starting in October 2020, Moore swab samples were collected weekly from the manhole, and the number of confirmed COVID-19 cases in the building each week was recorded and provided to the lab at the end of the study period. When there were 1 to 4 cases in the building, the proportion of swab samples that were positive for SARS-CoV-2 by RT-qPCR was 71.4%. When there were 5–10 cases in the building, the swab positive rate was 100%. When there were more than 11 cases in the building, there were no confirmed COVID-cases in the building.

Table 3

| Grab sample results | Moore swab results |
|---------------------|--------------------|
| +                   | 18                 |
| -                   | 6                  |
| Total               | 24                 |

* Moore swabs were collected and were processed by the skimmed milk method.

Table 4

| Weekly total confirmed cases | Number of weekly swabs tested | Positive (%) |
|-----------------------------|------------------------------|--------------|
| 0                           | 2                            | 2 (100.0)    |
| 1–4                         | 7                            | 5 (71.4)     |
| 5–10                        | 7                            | 7 (100.0)    |
| 11–81                       | 7                            | 7 (100.0)    |

* Moore swab samples were processed by the skimmed milk method.

4. Discussion

These results indicate that Moore swab is a sensitive, simple, and low-cost method for SARS-CoV-2 RNA detection in wastewater at a building and institutional level with relatively small catchment populations. Compared to automated composite samplers that are expensive and require electricity and specialized installation, the Moore swab is an extremely affordable, easy to deploy and use method. Moore swabs can be set for 24 h in wastewater, deployed weekly or more frequently as needed, and the sample processing can be standardized as described here. The application of Moore swab sampling at “upstream” locations such as residence halls/buildings on university campuses gives timely alert information that can provide an early warning of the presence of COVID-19 cases in specific geographic areas and populations. In the present study, we developed the Moore swab method for sewage sampling at manholes near buildings of interest, and laboratory processing of the swab samples for SARS-CoV-2 RNA detection using a standardized procedure that was sensitive and had good replicability. This process included squeezing the wastewater from the swab with a simple potato ricer, the use of a virus elution buffer developed in our lab, RNA extraction, and finally RT-qPCR detection of SARS-CoV-2 RNA. The limit of detection of the Moore swab method, evaluated using inactivated SARS-CoV-2 and BRV, was 5 EGC/mL in distilled water and 50 EGC in surface water. Of the 442 swab samples collected and tested from residence halls on the three campuses, the quarantine building, and the hospital, a total of 148 (33.5%) were positive for SARS-CoV-2 RNA. As expected, a high proportion of the swab samples from the quarantine building were positive for SARS-CoV-2 RNA (91.7%), and these results confirmed that this method was sensitive to detect SARS-CoV-2 RNA in wastewater in a building with a small number of COVID-19 cases. The detection rate of SARS-CoV-2 RNA in wastewater from the student residence halls ranged from 16.8–42.1%, and positive samples led to subsequent diagnostic testing of building residents, and in some cases, to the identification of one or more COVID-19 cases in the building. In addition, comparison between grab and Moore swab methods for the hospital sewage samples indicated that Moore swab method was more sensitive than the grab sampling method. These results suggest that the Moore swab is a sensitive, practical, and easy to use method, and it can be applied for any building, such as schools, nursing homes, prisons, and public/private buildings. This method is particularly appropriate for rapid response to COVID-19 in low-resource settings and at an early stage of infection in communities because the positive signal from a sewage sample could alert specific catchment populations to conduct intensive diagnostic testing, isolation of infected individuals, and contact tracing to prevent further spread of the virus. We did detect SARS-CoV-2 RNA from two swab samples when there were no confirmed COVID-19 cases in the quarantine building which raises the question of false positive results. This building housed confirmed and suspected COVID-19 cases intermittently. It is possible that the SARS-CoV-2 excreted by cases in the previous week persisted in the sewer lines in the building and was captured in the Moore swab sample from the following week. It is also possible that some of the suspected cases in the building were shedding virus before they were identified as confirmed cases.
Pathogen detection in wastewater can be a valuable surveillance approach—especially for diseases with pre-symptomatic or asymptomatic transmission, such as COVID-19. From a surveillance perspective, wastewater surveillance is advantageous because it can monitor the infection status in specific populations and provide an early warning of potential outbreaks in a relatively large population without having to collect individual clinical specimens, and it captures both symptomatic and asymptomatic infections. In addition, collecting and analyzing wastewater samples is relatively simple, rapid, cost-efficient, non-invasive, and does not require informed consent. Wastewater surveillance provided an early warning of cholera in Peru in the early 1990’s and has been an integral part of the global polio eradication program for several decades. It has proven useful for early warning of wildtype poliovirus circulation in a range of countries and populations (Hovi et al., 2012; Deshpande et al., 2003; Nakamura et al., 2015; Njile et al., 2019). Studies from several countries (Deshpande et al., 2003; Nakamura et al., 2015) indicated that wastewater surveillance was able to detect wildtype poliovirus in sewage even in the absence of reported acute flaccid paralysis cases. This may also be true for COVID-19 surveillance since recent publications from several countries (Mizumoto et al., 2020) have reported that asymptomatic infections of SARS-CoV-2 were very common, and estimates of prevalence primarily based on diagnostic testing of symptomatic individuals might be significant underestimations.

Detection of SARS-CoV-2 RNA in untreated wastewater has been reported in a number of countries, including the Netherlands (Medema et al., 2020), USA (Kaplan et al., 2021), Australia (Ahmed et al., 2020), China (Zhang et al., 2020b), Spain (Randazzo et al., 2020), and Italy (Riva et al., 2020). All these studies focused on sample collection at wastewater treatment plants, and the positive PCR signal may represent thousands of SARS-CoV-2 infections in the catchment areas. When COVID-19 prevalence is high, samples collected at the wastewater treatment facility tend to be consistently positive by PCR. The results from these samples may be useful for surveillance at a municipal level and to monitor overall trends in disease prevalence in the catchment population. However, this surveillance does not provide spatial information on the locations of the cases which limits how it can be used for public health response from the catchment area for targeted population interventions. When the disease prevalence is low, the virus is highly diluted in the sewage system, samples collected from the wastewater treatment plant may not be sensitive enough to detect SARS-CoV-2 RNA from a small number of COVID-19 cases. Wastewater from upstream sites is less diluted and represents a smaller catchment area. Detection of SARS-CoV-2 from these sites can be useful to indicate where there are new infections, which may lead to early warning and early intervention to prevent further transmission.

Recently, Cynthia et al. reported an effective COVID-19 wastewater surveillance program in on-campus student dormitories at the University of North Carolina at Charlotte (Gibas et al., 2021). Similarly, Smruthi et al. employed a powerful high-throughput approach to monitor SARS-CoV-2 RNA in the wastewater from a large number of campus buildings (Karthikeyan et al., 2021). These studies indicated that institutional-level wastewater surveillance serves well for early warning purposes since the catchment population is known and an immediate public health response can be executed in the event of any positive signal to prevent further transmission within the catchment population as well as to the community beyond.

There are several fundamental challenges to collecting wastewater samples from a building when SARS-CoV-2 presence may be transient. The challenges include small wastewater flow at the sampling site (e.g., manhole), depth of the manhole, intermittent excretion of viruses, unknown viral peak time, and identifying optimum sample collection time and frequency. Among these challenges, collecting a composite sample over time using an automated composite sampler can be a good solution to overcome some of these challenges, but there are some drawbacks to this approach. The Moore swab can act as a composite sampler that can trap microorganisms in wastewater flow over time. In our experience, the cost and time of Moore swab assembly and processing was considerably less than that for a wastewater sample collected as a grab sample or from an automated composite sampler. The Moore swab cost was approximately $12 for the materials for 10 swabs and required 30 min to prepare and a total of approximately 1.5 h to process using the skimmed milk method described here. Recently, we have been able to reduce processing time to 2 min per swab by using a stomacher (BagMixer®400, Interscience for Microbiology, Woburn MA, USA). Grab samples of wastewater required 1–3.5 h to process by membrane filtration depending on the turbidity of the sample. The cost of commercially available refrigerated automated composite samplers ranges from $2333 (WS700R, Pollardwater) to $6187 (AS950, Hach), and have recently become difficult to purchase because of increased demand due to the number of communities that are attempting wastewater surveillance.

We recently applied Moore swabs successfully for wastewater-based surveillance for typhoid fever at the city and neighborhood level in Kolkata, India, and our data indicate that this method was more sensitive than grab samples (Liu et al., 2021). In that study, we successfully developed and demonstrated the value of the Moore swab method for wastewater surveillance of Salmonella Typhi and ParaTyphi A. Given its simplicity and affordability, the Moore swab method is well suited for COVID-19 wastewater surveillance of buildings or communities where COVID-19 prevalence is relatively low and wastewater surveillance is feasible through a sewage network. The identification of positive signal in a building or community may trigger epidemiological investigation to identify those who are infected and alert the residents in the building to prevent further transmission. Our results showed that the Moore swab method was sensitive enough to identify 1–2 COVID-19 cases in the quarantine building where all of the residents were confirmed or suspected cases, as well as indicate the presence of COVID-19 cases in residence halls where the actual number of cases was difficult to determine. This alert enabled campus health authorities to recommend diagnostic testing for the students in the residence halls with positive wastewater samples.

In addition to the low-cost, Moore swabs allowed greater flexibility in that they could be placed in manholes that had low or intermittent sewage flow where it was not possible to collect grab samples. In this study, the sewage flows were very low for most of the residence halls (due to the reduced student population on campus during the study period), and it was not possible to collect grab samples from most of the manholes downstream of these buildings. Furthermore, Moore swabs did not require electricity or pose a risk of theft like automated composite samplers. Although there are advantages to using the Moore swab method, the method also has several limitations. First, this method only provides results on the presence or absence of the SARS-CoV-2 RNA, so quantitative estimates of virus titer in wastewater is limited. Second, our limit of detection experiments in Moore swab indicated that we could detect between 5 and 50 ECG/mL of seeded inactivated SARS-CoV-2 viruses in distilled water and surface water, but the limit of detection for the Moore swab may be different for wastewater with different characteristics, such as turbidity. Third, each Moore swab sample requires two trips per site to place and later retrieve the swab, while grab sampling only requires one trip. Finally, this study was designed as a proof of concept, and we note that positive PCR results were not observed in a few instances when there were confirmed COVID-19 cases in the buildings. This may reflect variability in swab sample processing, our use of different RNA extraction methods depending on availability of reagents, unrecognized PCR inhibitors, or other unknown factors that may have affected the results.

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Credit authorship contribution statement

Pengbo Liu: grant writing, conceptualization, methodology, experiment design, writing manuscript, editing, supervision.

Makoto Ibaraki: sample collection, laboratory testing, data collection, writing manuscript.

Jamie VanTassell: sample collection, data collection, writing manuscript.

Kelly Geith: sample collection, laboratory testing, data collection, writing manuscript.

Matthew Cavallo: sample collection, laboratory testing, data collection, writing manuscript.

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Lizheng Guo: laboratory testing.

Christine L. Moe: grant writing, conceptualization, methodology, experiment design, editing, supervision.

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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