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Passive swab versus grab sampling for detection of SARS-CoV-2 markers in wastewater

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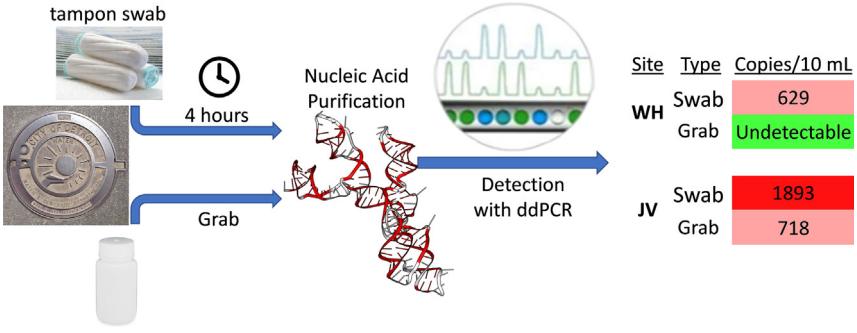
HIGHLIGHTS

- Tampons, designed for absorbency, were tested in sewers for capture of SARS-CoV-2.
- Tampon swab sampling (4 h immersion) detects SARS-CoV-2 missed by grab samples.
- Swabs captured 2–3 times more viral markers, on average, than paired grab samples.
- Improved detection of SARS-CoV-2 with swabs varied significantly among sewersheds.
- Swabs are especially advantageous at small sewersheds that often are more variable.

GRAPHICAL ABSTRACT



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ABSTRACT

Early detection of the COVID-19 virus, SARS-CoV-2, is key to mitigating the spread of new outbreaks. Data from individual testing is increasingly difficult to obtain as people conduct non-reported home tests, defer tests due to logistics or attitudes, or ignore testing altogether. Wastewater based epidemiology is an alternative method for surveilling a community while maintaining individual anonymity; however, a problem is that SARS-CoV-2 markers in wastewater vary throughout the day. Collecting grab samples at a single time may miss marker presence, while autosampling throughout a day is technically challenging and expensive. This study investigates a passive sampling method that would be expected to accumulate greater amounts of viral material from sewers over a period of time. Tampons were tested as passive swab sampling devices from which viral markers could be eluted with a Tween-20 surfactant wash. Six sewersheds in Detroit were sampled 16–22 times by paired swab (4 h immersion before retrieval) and grab methods over a five-month period and enumerated for N1 and N2 SARS-CoV-2 markers using ddPCR. Swabs detected SARS-CoV-2 markers significantly more frequently ($P < 0.001$) than grab samples, averaging two to three-fold more copies of SARS-CoV-2 markers than their paired grab samples ($p < 0.0001$) in the assayed volume (10 mL) of wastewater or swab eluate. No significant difference was observed in the recovery of a spiked-in control (Phi6), indicating that the improved sensitivity is not due to improvements in nucleic acid recovery or reduction of PCR inhibition. The outcomes of swab-based sampling varied significantly between sites, with swab samples providing the greatest

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improvements in counts for smaller sewersheds that otherwise tend to have greater variation in grab sample counts. Swab-sampling with tampons provides significant advantages in detection of SARS-CoV-2 wastewater markers and are expected to provide earlier detection of new outbreaks than grab samples, with consequent public health benefits.

1. Introduction

Detecting new outbreaks of COVID-19 in the community has become increasingly difficult as more people conduct non-reported home tests, or because of mild symptoms or other reasons, decide not to test at all. Some individuals remain asymptomatic during infection (Long et al., 2020; Oran and Topol, 2020) but nevertheless can spread infections to others (Pei et al., 2022). Yet, the detection of COVID-19 infections in the community remains important as thousands of people are still being hospitalized and dying from the disease (Shah et al., 2022). Early awareness of new outbreaks can be important to trigger mitigation strategies to slow the spread of disease in the community and to prepare staff and medical supply chains for potential systemic stresses. A possible solution to providing such early warning is through Wastewater-Based Epidemiology (WBE) in which markers of SARS-CoV-2, the virus that causes COVID-19, are measured in community wastewater (National Academies of Sciences Engineering and Medicine, 2023).

Although the most notable symptoms of COVID-19 are related to the respiratory system, COVID-19 is also recognized as a multi-organ disease (Gupta et al., 2020), and SARS-CoV-2 has been observed in respiratory fluids, urine, and feces (Peng et al., 2020; Wu et al., 2020a; Wu et al., 2020b; Wyllie et al., 2020). Both symptomatic and asymptomatic individuals shed SARS-CoV-2 in fecal specimens (Han et al., 2020), which means that wastewater can be an effective medium to track this viral material. Sampling wastewater for SARS-CoV-2 is far less intrusive than sampling individuals, and the sampling area can be scaled to appropriate levels, from a single congregate living facility such as a nursing home (Spurbeck et al., 2021), to a neighborhood (Layton et al., 2022), to an entire city (Hopkins et al., 2023). Comparing WBE monitoring of SARS-CoV-2 with clinical testing and hospitalization rates has shown WBE strategies are able to predict surges in caseloads days to weeks before individuals require medical intervention (Karthikeyan et al., 2021; Peccia et al., 2020; Zhao et al., 2022).

The goal of the present study was to develop a more sensitive WBE strategy through the use of a simple passive sampling device for collecting viral material from sewers over several hours. Wastewater collection strategies can be placed into three categories: grab, composite, and passive sampling. Grab samples collect a volume of wastewater at a single timepoint, making logistical implementation very straightforward; however, the presence of SARS-CoV-2 in wastewater may be sporadic, dependent not only on the health of individuals but also on when the infected individuals defecate into the sewage system, relative to the sampling time. Thus, grab samples may miss legitimate signals (Gerrity et al., 2021). Composite sampling, typically with an expensive automated sampler preprogramed to pump and combine sewer water samples taken at specific intervals addresses this shortcoming, Yet, autosamplers do not typically sample continuously and may still miss the dynamic SARS-CoV-2 signal. Additionally, autosamplers are not deployable at all sites of interest and may increase logistical level of effort (e.g., transport to location, security in place, maintenance of cooling, and retrieval). Passive sampling offers an interesting middle ground between the two and typically uses some form of absorbent material that can capture material continuously by filtration, adsorption, or a combination of these mechanisms. A classic example is the so-called Moore Swab, which is pleated cotton fabric secured with a string or wire and hung in the wastewater flow for minutes to hours (Sikorski and Levine, 2020). This allows sampling anywhere a grab sample can be taken, but the swab remains in the wastewater flow for some duration of time, often between 1 and 24 h (Jones et al., 2022).

To study the effectiveness of passive versus grab sampling methods we utilized a form of commercially available swabs that are prepackaged, sterile, and have relatively uniform properties, a tampon. Over the course of five months (mid-April 2022 to mid-September 2022), we collected weekly grab samples and tampon samples at six independent sites across the City of Detroit. This study analyzes paired observations to determine (a) how the two methods compare in detecting a positive signal when the levels of SARS-CoV-2 markers are near the detectable limit (sensitivity) and (b) how the two methods compare quantitatively as the presence of SARS-CoV-2 in wastewater rose to high levels and subsided at various times during the sampling period. This paper reports that passive sampling of wastewater with a tampon had a higher probability than paired grab samples at detecting SARS-CoV-2 and that the significant quantitative advantage of passive sampling overall varied from site to site.

2. Methods

2.1. Sample collection

Six sites across the City of Detroit were selected for paired methodological comparison, based on results obtained in previous studies (West et al., 2022). The six sites include three dormitory building sewersheds (AS, UC, and WH, with residents numbering <500 each during most of the sampling period), two sewersheds that include a long-term care facility (LTCF) and surrounding neighborhood (CS and JV, with estimated sewershed populations of 2000 and 500, respectively), and SG ("ZIP Code"-sampling sewershed with an estimated population of 12,000 people). Once per week at each site a grab sample of approximately 200 mL was collected between 7 am and 9:30 am, placed into a 250 mL high density polyethylene bottle, transferred to a Ziplock bag with paper towel, and transported on ice in a cooler to the lab within 2 h. Immediately after collection of the grab sample an o.b.® Fluid-Lock® regular tampon (Playtex Manufacturing, Inc., Dover, DE), referred to in this paper as a swab, was enclosed in a wire metal cage and lowered into the wastewater stream. Upon retrieval 3 to 4 h later, swabs were placed into a Whirl-Pak bag (Nasco, ID#B01062), placed into a Ziplock bag with paper towel, and transported on ice in a cooler to the lab for analysis. Field blanks were collected each week at two of the sites, randomly chosen, at the time of retrieving the sample by decanting approximately 100 mL of deionized water into high density polyethylene bottles, inserting a sterile tampon into the field blank water, and transporting the blank on ice back to the lab in the same cooler as the wastewater samples. The present study analyzes data from 126 paired collections of grab and swab samples over a five-month period, from mid-April 2022 to mid-September 2022.

Sample collection information (location, date-time of sample collection, sample type [grab or. swab] and transfer of sample custody) from the field sampling crew to the laboratory were recorded on Chain-of-Custody forms. An accompanying data form also included water quality parameter data, including pH, temperature, dissolved oxygen, and specific conductance, measured during collection of the grab samples at each site using a YSI ProDSS with GPS (YSI, Yellow Springs, OH). A digital record of each Chain-of-Custody form and YSI data sheet has been preserved and archived with the project records.

2.2. Extraction and purification of viral RNA

Grab samples were processed as described in previous publications (Vasquez et al., 2021; West et al., 2022). Briefly, 45 mL of the collected sample in a 50 mL polypropylene tube was centrifuged at 3996 rpm (5000 $\times g$) at 4 °C for 15 min; 10 mL of supernatant was transferred to another tube for purification; samples were "spiked" with phage Phi6 (internal RNA virus

recovery control, added as 10 μL of 10^6 pfu) and then treated with lysis buffer and Proteinase K at 55 °C for 30 min; and then processed via an automated magnetic bead-based nucleic acid extraction system (Chemagic 360™) to yield 85 μL of purified nucleic acids in a PCR-compatible elution buffer. Phi6 recovery from the grab sample was compared to recovery from the similarly spiked field blank and also to an "eluate control" in which 10 μL of 10^6 pfu Phi6 had been added to 75 μL of elution buffer, as in West et al. (2022).

For swab samples, soluble materials and extractable particulates were extracted from each swab after adding 20 mL extraction buffer (0.5 × TE + 0.075 % Tween-20) to each Whirl-Pak bag. After adding the extraction buffer, the bag was sealed, and the swab was manipulated and squeezed in the bag by hand for 30 s to 1 min. The swab and the added suspension buffer were then held at 4 °C with agitation (orbital shaker set to ~250 rpm) for up to 40 h before further processing.

To extract nucleic acids after the agitation period, the swab was again manipulated and squeezed to release up to 30 mL of fluid (consisting of suspension buffer plus water that had been absorbed by the swab when it was in the sewer). The swab was then removed from the Whirl-Pak bag while being squeezed to get as much fluid out of it as possible, and the resultant fluid was transferred into a 50 mL polypropylene tube, out of which 10 mL was processed according to the identical procedures used to extract nucleic acids from grab samples (Vasquez et al., 2021; West et al., 2022), described above. The swab in the field blank bottle was similarly processed, pouring off the field blank water, adding 20 mL of the swab suspension buffer, agitating and squeezing the swab in the buffer before removing it, and then processing the fluid by the same extraction procedure as described for the sewer swabs and grab samples. Extracted nucleic acids and remaining centrifuged swab suspension buffer were stored at −80 °C for long term storage.

2.3. ddPCR analysis

The number of copies of SARS-CoV-2 markers N1 and N2 per sample was measured using digital droplet PCR with Primers and TaqMan® probes (Table 1), as described previously (Vasquez et al., 2021; West et al., 2022). To assess nucleic acid recovery efficiency, Phi6 in the final nucleic acids extract was also assayed, with the primers and probes shown in Table 1.

The ddPCR mix (22 μL/reaction) contained a final concentration of 1 × Supermix, 20 U/μL reverse transcriptase, 15 mM DTT, 900 nmol/μL of gene target primers, 250 nmol/μL of gene target probe, RNAse-free water, and 5.5 μL of template nucleic acids purified by Chemagic™. ddPCR reactions for each sample were run in triplicate along with quality controls (field blank, positive, no-template, and eluate controls). Each reaction mix was partitioned into 10,000–20,000 droplets/reaction in an Automated Droplet Generator (Bio-Rad, CA, USA), thermocycled (40 cycles of 95 °C denaturation for 30 s. and annealing/extension at 55 °C for 1 min) on a Bio-Rad C1000 Thermo Cycler, and droplet fluorescence read on a Bio-Rad QX200 Droplet Reader. Droplet data were analyzed with Bio-Rad QuantaSoft software package version 1.7.4.0917.

Table 1
Primer and probe sequences used for ddPCR.

Target	Primer/probe	Sequence	Reference
SARS-CoV-2	2019-nCoV_N1-F	5′-GACCCCAAAATCAGCGAAAT-3′	Lu et al. (2020)
	2019-nCoV_N1-R	5′-TCTGGTTACTGCCAGTTGAAT CTG-3′	
	2019-nCoV_N1-P	5′-FAM-ACCCCGCATTACGTTTGGT GGACC-BHQ1-3′	
	2019-nCoV_N2-F	5′-TTACAAACATTGGCCGCAAA-3′	
	2019-nCoV_N2-R	5′-GCGCGACATTCCGAAGAA-3′	
	2019-nCoV_N2-P	5′-HEX-ACAATTTGCCCCCAGCGCT TCAG-BHQ1-3′	
Phi6	Phi6-F	5′-TGGCGGCGGTCAAGAGC-3′	Gendron et al. (2010)
	Phi6-R	5′-GGATGATTCTCCAGAAGCTGC TG-3′	
	Phi6-P	5′-6FAM-TCCGCCTGGCACGGTA CTCCCT-BHQ1-3′	

For grab samples, results were calculated in terms of number of copies of PCR targets per 10 mL of the wastewater sample supernatant from which the nucleic acids were extracted by the lysis and magnetic bead purification procedure described above. For swab samples, we comparably calculated the number of copies per 10 mL of the supernatant of the swab suspension fluid. The total amount of viral markers in the swab were an estimated two to three times as large as this since the total suspension fluid (including the water that the swab absorbed in the sewer) varied up to 30 mL after the swab was removed from the suspension fluid. We also calculated the percent recovery of the spiked in Phi6 RNA for each sample compared to the recovery of the spike from the field blank sample and compared to the Phi6 spiked into the eluate control.

2.4. Statistics

For quantitative comparisons between viral copies/10 mL between the grab and swab samples, the counts of the N1 and N2 markers were averaged and statistical methods compared the averages. Seventy copies/10 mL was considered the limit of detection of the digital droplet measurement (corresponding to 2 positive droplets out of a typical 15,000 total droplets/reaction). Two positive droplets have been shown to be the limit of detection on the BioRad platform by Dong et al. (2021). Various statistical tests implemented using either GraphPad Prism software (GraphPad Software LLC, Version 9.4.1) or the data analysis add-on of Excel were used, as specifically identified in the Results, to analyze and compare the grab and swab measurements, with $p < 0.05$ indicating statistically significant differences.

3. Results

3.1. Frequency of detecting SARS-CoV-2 above the limit of detection

To determine if swabs would enable detection of a new outbreak even when grab samples might not, the simplest analysis was to compare the proportion of samples that were above and below the limit of detection for the two methods. The number of times for all sites at which each method was able to detect SARS-CoV-2 (i.e. >70 copies/10 mL) or failed to detect (<70 copies/10 mL) viral RNA was compared. Of the 126 pairs of observations made with both grab and swab methodologies, the grab samples detected SARS-CoV-2 77 times and failed to detect 49 times. In comparison, the swabs detected SARS-CoV-2 102 times and failed to detect only 24 times (significantly higher proportion than grab samples, Fisher exact test, two-tailed $p = 0.0008$).

3.2. Quantitative differences between amount of SARS-CoV-2 detected

To put this comparison on a more quantitative basis than just "detect/non-detect," we compared the average number of copies/10 mL between grab and swab samples. For this comparison, we first tested whether the data were normally distributed. The data range from <70 copies/10 mL (i.e., undetectable) to 250,000 copies/10 mL for swabs and 58,000 copies/10 mL for grabs, and were not normally distributed. We tested various transformations to normalize the data (e.g., with log-transformed data), the swab sample data were normally distributed (Lilliefors $p = 0.1487$, KS $p = 0.5607$); however, the grab sample data were not (Lilliefors $p = 0.0003$, KS $p = 0.0701$). Therefore, the paired grab and swab samples were compared with non-parametric tests, after deleting 17 instances for which both methods were non-detect and could not be rank ordered. A Wilcoxon matched-pairs signed rank test indicates that the two methods were significantly different (two-tailed $p < 0.0001$), and that the swab samples typically detect more signal than the grab samples (Fig. 1). The median amount detected by the swabs is 661 copies/10 mL, while the grab sample median was 236 copies/10 mL ($n = 107$ each). Considering the ratio of swab counts to grab counts for all swab:grab pairs, the median ratio of

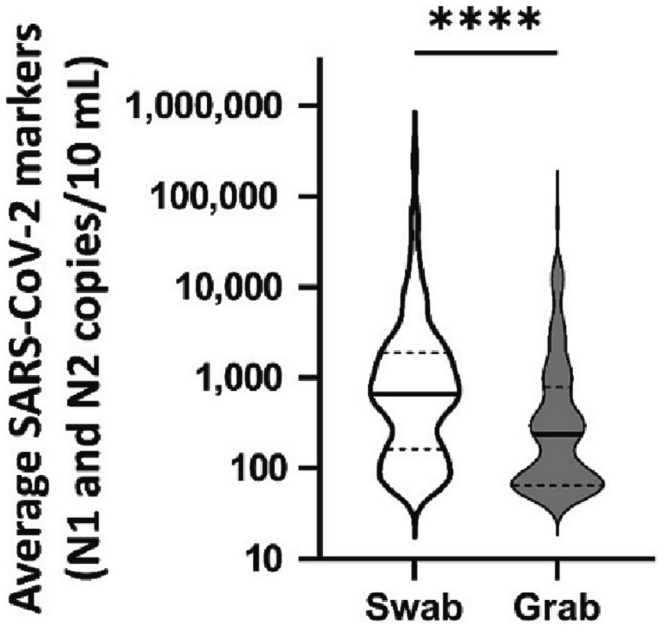


Fig. 1. Distributions of the count of SARS-CoV-2 markers (average of N1 and N2 markers in each sample) measured in swab versus grab samples of wastewater. Horizontal black bars represent the median of the counts of each distribution and the dashed lines the quartiles; width of the violin plots is proportional to the number of observations with the indicated counts. The median for the swab samples is significantly greater than for grab samples (107 paired samples; Wilcoxon matched-pairs signed-rank test; two-tailed $p < 0.0001$).

swab to grab was 2.22 (quartile range swab:grab ratio was 0.713514 to 9.0). A linear regression of swab samples compared to paired grab samples had a significant relationship ($p < 0.0001$) with $R^2 = 0.30$ (Fig. 2).

3.3. Site-specific differences in detection of SARS-CoV-2

To determine whether the increased counts obtained from swabs compared to grab samples was site-specific, we grouped the swab and grab samples by site and analyzed the effect of site with a mixed-effects non-parametric statistical model. Fig. 3 illustrates that sampling site had a significant differential effect on the swab v grab sample counts

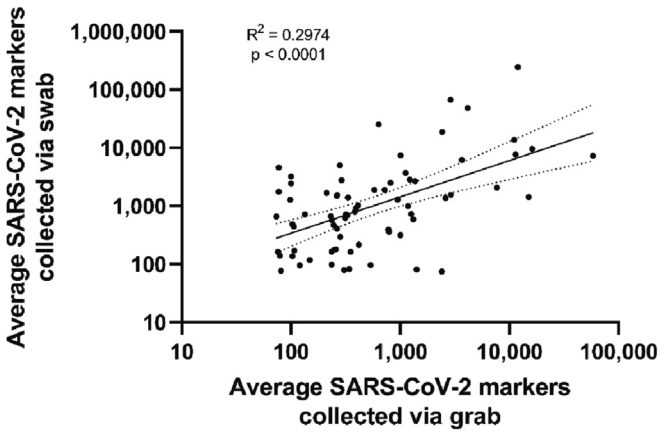


Fig. 2. Linear regression of SARS-CoV-2 markers observed in paired swab and grab samples. Each dot represents the average of N1 and N2 counts for a swab sample (vertical axis) and the average N1 and N2 counts for a grab sample (horizontal axis) collected from the same site on the same day. The black line represents the least squares regression; dotted lines represent 95 % confidence intervals. Regression includes only those pairs ($n = 72$ pairs) for which both sampling techniques measured above the limit of detection (70 copies/10 mL).

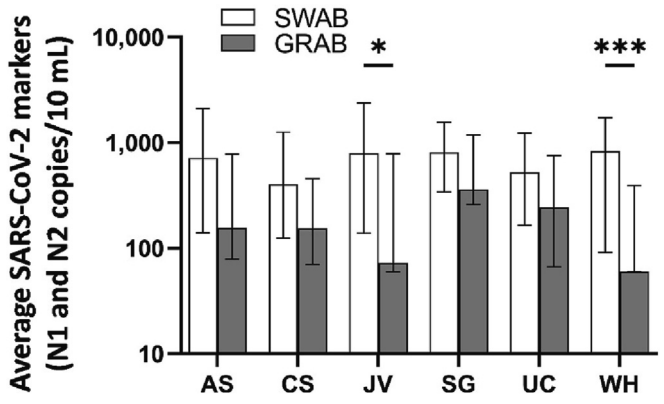


Fig. 3. Comparison of SARS-CoV-2 marker counts (represented by the average of N1 and N2 counts for each sample) detected in swab and grab samples for each site. Each site is represented by a two letter sewershed identification label. Bars represent the median of the marker counts, and error bars display the quartiles. A mixed-effect analysis indicated a significant difference overall in copies/10 mL ($n = 107$ pairs, $p < 0.0001$) and differences detected with Sidak's post-hoc multiple comparisons test for swab v. grab samples at sites JV ($p = 0.0268$, *) and WH ($p = 0.0004$, ***).

($p < 0.0001$). Although the swabs detected more SARS-CoV-2 on average at each site, this improvement was statistically significant only at sites JV and WH ($p = 0.0268$ and $p = 0.0004$, respectively, using Sidak's multiple comparisons post-hoc test).

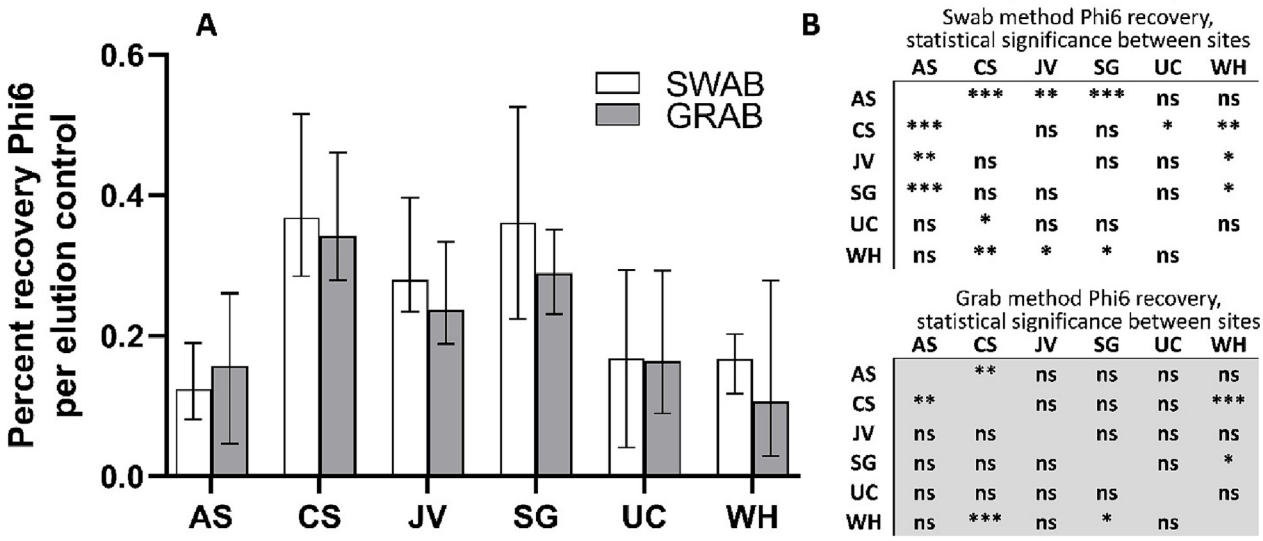
3.4. RNA recovery

In order to determine whether the higher counts observed for the swabs at some sites was due to better recovery of RNA from swab samples, we compared recovery of the spiked-in Phi6 internal control for both the grab and swab samples. We calculated the recovery of spiked-in Phi6 as a percent of the Phi6 measured in both the field blank control and the elution control. While some groups yielded normally distributed data, some distributions failed Shapiro-Wilks and/or Kolmogorov-Smirnov tests for normality. Therefore, this analysis used non-parametric methods. On a Wilcoxon matched-pairs signed ranks test on paired data from all sites, the percent recovery of Phi6 did not differ between paired swab and grab samples (as percent of elution control, medians, 24.7 % for swabs; 24.2 % for grab samples, $p = 0.357$; as percent of field blank control, medians, 42.7 % for swabs; 39.7 % for grab samples, $p = 0.049$).

Because previous studies (West et al., 2022) had indicated that extraction of RNA from grab samples collected at sites WH, AS, and UC had significantly worse RNA recovery than from some other sites, we also compared recovery of Phi6 from samples taken from different sites. Comparing swab and grab samples on a site by site basis we observed no significant differences in Phi6 recovery between the sampling methodologies at any of the sites in this study (Fig. 4; $n = 111$ pairs, Mixed-effect analysis, $p > 0.5$). However, site-to site comparisons within each method (i.e. comparing grab sample Phi6 recovery between sites; and comparing Phi6 swab sample recovery between sites) revealed that the recovery of Phi6 from wastewater of the university sites (WH, UC, and AS) was significantly lower than the Phi6 recovery from the other sites. This was especially apparent in comparisons of the Phi6 recovery to sites CS and SG (generally $p < 0.02$; see p values graphically represented in Fig. 4B).

4. Discussion

This study shows that extraction of nucleic acids from tampon swabs that had been placed in wastewater streams for approximately 4 h was significantly more likely to detect SARS-CoV-2 viral markers than grab samples taken from the same wastewater stream. On average, quantitatively more SARS-CoV-2 markers were measured in tampon extracts than



Swab	AS	CS	JV	SG	UC	WH
AS		***	**	***	ns	ns
CS	***		ns	ns	*	**
JV	**	ns		ns	ns	*
SG	***	ns	ns		ns	*
UC	ns	*	ns	ns		ns
WH	ns	**	*	*	ns	

Grab	AS	CS	JV	SG	UC	WH
AS		**	ns	ns	ns	ns
CS	**		ns	ns	ns	***
JV	ns	ns		ns	ns	ns
SG	ns	ns	ns		ns	*
UC	ns	ns	ns	ns		ns
WH	ns	***	ns	*	ns	

Fig. 4. Paired comparison of Phi6 recovery as a percent of the elution control. The bars represent the median, and error bars display the quartiles. (A) No significant difference in Phi6 recovery is present between grab and swab sampling methods. (B) Statistical significance comparing different sites for swab (upper) and grab (lower) methods (n = 111 pairs, ns – not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

corresponding grab samples. The advantage of this passive collecting method varied from site to site. The higher, more sensitive detection with swabs was due to a higher amount of the markers captured by the sampling method and not due to improved recovery of the RNA when purifying nucleic acids from the swabs, as spiked-in viral markers were recovered with approximately the same effectiveness from swab collectors as from grab samples.

One of the problems of sampling congregate living facilities such as dormitories and long-term care facilities is that inputs to the wastewater are from only a small number of people and therefore more dependent on the behaviors of individual people in relation to collection time than a larger sewershed with inputs from many people. For a small sewershed, if a grab sample is taken at a time prior to the input of fecal matter from an infected person into the system or too long after, the fecal "signal" in the wastewater will be missed. For a larger sewershed, a larger number of inputs into the system are more likely to occur at any given time and, also, given the larger and varied distances over which the fecal matter travels and mixes in the larger system, the "pulse" of virus would likely spread out over larger volumes and time of the waste stream.

The hypothesis that SARS-CoV-2 markers in wastewater samples may be more consistently found in samples from large sewersheds than from medium-sized sewersheds is supported by a number of previous observations. Weidhaas et al. (2021) reported finding a higher frequency of detecting SARS-CoV-2 molecular signals in wastewater from large sewersheds than from medium- or small-sized sewersheds. Similarly, in other studies from our laboratory (unpublished), we found that grab samples from three ZIP Code-sampling sewersheds, with average population sizes of 14,000, had significantly higher frequencies of detecting SARS-CoV-2 markers and a significantly lower week-to-week variation than did samples from 8 LTCF sewersheds, with average population sizes of 1000 (ZIP Code sites, SARS-CoV-2 detections in 91 % ± 4 % of the samples, and LTCF sewersheds in 66 % ± 14 % of the samples, $p < 0.001$, unpaired *t*-test with Welch's correction for unequal variances; median week-to-week percentage change was 240 % for the ZIP Code sewersheds and 350 % for the LTCF sewersheds, $p < 0.0001$, Mann-Whitney test, for grab samples collected October 2021 to August 2022). In the present study, the swab appears to have improved the detectability of the SARS-CoV-2 signal, especially for the smaller sewersheds, which includes sites WH (a dormitory sewershed) and JV (an LTCF sewershed) in comparison to the ZIP Code site SG, for which little change in detectability was seen, as illustrated in Fig. 3.

Smaller sewersheds have more factors that reduce recovery of RNA from the wastewater sample. This study reproduced the observation that recovery of virus from different sites varies consistently (West et al., 2022), with the recovery of viral markers from some sites, such as WH in the present study, exhibiting consistently lower recovery of the markers than other sites. The factors in the wastewater that cause this difference, such as salt, pH, dissolved carbon, particulates, etc., have not been identified, but are clearly not removed by the swab-sampling method. A study by Hayes et al. (2022b) indicated that total suspended solids in wastewater reduced adsorption and viral recovery from an electronegative membrane passive sampling device.

Several previous studies have reported using various types of swabs as passive collecting technologies for SARS-CoV-2 detection. Table 2 summarizes some of their findings to which the present study may be compared. Additional passive sampling papers are reviewed by Shakallis et al. (2022). These previous studies reinforce our choice of tampons as our passive sampling device since several of them (Jones et al., 2022; Kevill et al., 2022; Li et al., 2022) concluded that tampons were better capture devices than several other alternatives, considering effective capture, cost, and convenience. Several studies that analyzed the kinetics of uptake of viral markers by various swab devices concluded that uptake begins to taper off within 4–8 h (Hayes et al., 2022a; Jones et al., 2022; Li et al., 2022). Three to 4 h of passive collecting seems to us to be an optimal time for gathering significant signal (as in Bivins et al. (2022)) and yet still be able to do same-day processing in time to give a rapid warning of a rise of markers of infections. Along with testing a plethora of different elution methods and media, several studies (Bivins et al., 2022; Hayes et al., 2021) found that the inclusion of Tween-20 in their elution medium, as we have also done, facilitated effective recovery of viral markers without interference with subsequent PCR. In pilot tests prior to the present study, we found that eluting in a larger volume (100 mL) and without Tween-20 yielded no quantitative advantage over paired grab samples; Tween-20 made a large difference in the amount of material visibly eluted from the tampons, and the 20–30 mL elution volume yielded a more concentrated eluate for purification.

The present study is unique in analyzing the swabs with ddPCR, as all of the swab studies summarized in Table 2 used either RT-PCR or RT-LAMP; ddPCR is a more sensitive technique, assisting in our goal of improving the sensitivity of detection for wastewater epidemiology of SARS-CoV-2. The number of sites and the large number of paired samples over several months in the present study, compared to most previous swab studies,

Table 2
Survey of passive sampling studies of wastewater for SARS-CoV-2 monitoring.

Reference	Swab type(s); grab or composite comparison?	Immersion time (h)	Extraction		Detection method	Comment
			Volume[a]	Method[b]		
This paper	Tampon; grab 6 sites; 126 pairs	4	~30 mL	Tween-20	ddPCR	Swab > grab, especially in small sewersheds
(Hayes et al., 2021)	1 site: cheesecloth & grab; 1 site: electronegative membrane (ENM)	24, 48, 72	6 mL	Tween-20; & compared to others	RT-qPCR	Tween-20 eluant better than others; cheesecloth > grab detection; ENM ok
(Rafiee et al., 2021)	Cotton Moore swab; grab; composite	16	~250 mL	Tween-80	RT-qPCR	Swab > grab; swab ~ =composite
(Corchis-Scott et al., 2021)	Tampon; grab	20	squeezed content (~20 mL)	CP Select column	RT-qPCR	Tampons detected; grabs did not
(Schang et al., 2021)	Gauze swab; ENM; cotton buds	24	10 mL	Tween-80 (gauze); bead-beating (others)	RT-qPCR	Passive > grab; ENM often best
(Acer et al., 2022)	Tampon	24	200 mL per 2 tampons	Milli-Q water + tampon fluid	RT-qPCR	Pos. cases vary with tampon data; no grab compared
(Bivins et al., 2022)	Tampon; 24 h composite	3	50 mL	Tween-20; & compared to others	RT-LAMP	Tween-20 best; predicts cases; no paired composite
(Jones et al., 2022)	Tampon; cotton Moore swab; 6 others	0.5, 1, 3, 6, 24	2 mL	Nuclisys lysis buffer	RT-qPCR	Tampons #1; Moore swabs #2; optimal at 3–6 h
(Kevill et al., 2022)	Tampon fragment; Whatman ion exchange	1, in wastewater in lab;	20 mL	Phosphate buffered saline	RT-qPCR	Tampons better than Whatman ion exchange
(Liu et al., 2022)	Cotton Moore swab; grab compared at only 1 site	Mostly 24–48, some 72	~250 mL	Tween-80	RT-qPCR	26 paired swab v grab; swab 24+/26 trends better than grab 18+/26
(Hayes et al., 2022a)	Granular activated charcoal (GAC); ENM; 2 others	In field, 24, 48, 72, 96; in lab, 2, 4, 8, etc. up to 96	6 mL	Tween-20	RT-qPCR	GAC > ENM; initial adsorption up to ~50 % max in 8 h; grab not compared
(Li et al., 2022)	Tampon; swab; ENM; gauze; grab	0.25, 0.5, 0.75, 1, 4, 8, 24, 48	For tampon: squeezed content (~25 mL)	Tampon, Centricon filtrate; gauze, Tween-80; others as in Schang et al., 2021	RT-qPCR	Tampon best; half-time ~ 2.5 h; suggests most effective time = 8 h
(Wilson et al., 2022)	Tampon; gauze	24, tested in lab	~50 mL (squeezed, plus elution buffer)	Tampon, Centricon; gauze, Tween-80	RT-qPCR	Tampon better than gauze
(Wilson et al., 2022)	Cotton swab; ENM (1 site, 15×); grab (4×) or composite (11×)	48 to 144	10 mL	Same as Schang, et al., 2021	RT-qPCR	Cotton & ENM slightly higher detection than grab/composite
(Vincent-Hubert et al., 2022)	Nylon membrane	24	8 mL	NucliSENS (BioMerieux)	RT-qPCR	Composite trends > than membrane

[a] If shown with ~, the volume includes the swab's fluid and the added elution solution. Otherwise, the volume is the added elution solution; the final solution would also include the swab's fluid.
[b] Tween, if listed, is typically 0.01–0.1 % (see citations).

enabled us to identify statistically significant paired comparisons of swab v grab samples that several studies could only indicate as trends. For example Liu et al. (2022) reported 24/26 positive for swab compared to 18/26 for the paired grab samples from Emory Hospital sewershed (for which we calculate a Fisher exact probability of 0.075); however, the study of Rafiee et al. (2021), comparing 34 paired swab and grab samples showed a statistically significant advantage of their cotton swab method (34/34 positive for 16 h cotton swabs v 24/34 for grab samples, $p < 0.001$; samples from 17 sites on two dates). As in the present study, the "advantage" of the swab over the corresponding grab sample seemed to vary from site-to-site in the study by Rafiee et al. (2021); however, the number of samplings at each site was only two, so a statistical conclusion on site-to-site differences was not possible.

Composite sampling can integrate samples into one over the course of a day and has been shown to recover SARS-CoV-2 more consistently and sometimes in higher amounts than paired grab samples (Augusto et al., 2022). The swab method is similar to a composite method in collecting viral markers over an extended period of time. Therefore, comparisons of swabs to paired composite samples collected by autosampler from the same sites over the same time period is of interest. In previous studies (Table 2), three of the publications made comparisons to composite samples: Rafiee et al. (2021) observed a slight advantage of the swabs (34/34 samples positive) over paired composite samples (29/34 positive); Wilson et al. (2022) found no difference between swab and composite samples (both had 10/11 positive detections of SARS-CoV-2); and Vincent-Hubert et al. (2022) reported a non-significant trend towards higher counts for composite compared to a membrane passive sampler. Given these nearly equivalent results of passive samples to composite samples, the lower demands in terms of labor and equipment of swab samples compared to autosampler composite methods clearly favor the swabs. Additional considerations in the present study are that some sites are structurally not suitable for automated composite sampling even if autosampling equipment and staff were available to do it. Specifically, (a) site WH is a small sewershed in which the flow is shallow and sometimes intermittent and would lead to suction failure, and (b) site SG is too deep (>25 ft below the manhole) for reliable automated pumping. Therefore, for testing the sensitivity of marker detection with a swab method, comparison to grab samples was preferred over composite samples.

One problem with the technique used in the present study is that extraction from the swab includes a long period of agitation (up to 40 h, as described in the methods), which might limit rapid reporting of results. Sometimes the agitation time was as short as 15 h, dependent on time and personnel available to process the grab samples, which had first priority in our state-approved sampling plan. Although we have not done a systematic test of shorter swab agitation times, we have recently used a faster alternative method with good results, in which swabs are held at 4 °C until processing and the extraction from the swab consists of adding 20 mL Tween-containing extraction medium and manually manipulating the swab for just a minute, and then assaying 10 mL of the resultant extract. With this method for one site not in the present study, we've had 5-fold higher counts (median for 7 weeks) from swabs than their paired grab sample. Also, on two recent dates at site WH (one of the small sewersheds in this paper) using this alternative method, the grab samples were below the limit of detection (70 copies/10 mL) and the swabs measured 280 and 490

copies/10 mL, respectively. These alternative methods have not been used systematically enough yet for a critical statistical analysis, as described for the methods in this paper, but they indicate that shortening the hold and agitation time is possible and potentially beneficial for rapid reporting of swab results.

Several caveats regarding this study should be kept in mind: (1) Only the recovery of SARS-CoV-2 markers from swabs was studied; other pathogens might or might not be captured or eluted as effectively (Vincent-Hubert et al., 2022). Nevertheless, comparable swab methods have been used for other human viruses with success (Mejías-Molina et al., 2023). Prior to depending solely on swab samples, each study should test and compare the advantage (or not) of sampling via swab, not only because different agents may be involved, but also because we've shown that with the current methods, sometimes swab sampling does not provide an advantage. (2) The comparison and advantage described here for swabs is for comparisons to comparable grab samples; it is not known how the swabs would compare to composite samples taken over the same immersion time period.

5. Conclusion

A four-hour tampon-based passive sampling of wastewater, combined with eluting viral markers with a Tween-20 containing solution, improved the detectability of SARS-CoV-2 viral markers, especially near the limit of detection. The improvement varied from site-to-site, and was better in samples taken from small, highly variable sewersheds, as compared to larger sewersheds for which no significant improvement in detectability or sensitivity was observed. We suggest that passive swab sampling with tampons is a more effective strategy for monitoring SARS-CoV-2 viral markers in small sewersheds than the use of grab samples. Our results can be used to improve early-warning and response to prevent or mitigate against outbreaks across a diversity of communities.

CRediT authorship contribution statement

Nicholas W. West: Conceptualization, Methodology, Investigation, Writing-Original Draft, Data Analysis, Visualization **James Hartrick:** Methodology **Md Alamin:** Methodology, Writing-Review & Editing, Data Analysis **Adrian A. Vasquez:** Data Curation **Azadeh Bahmani:** Methodology, Data Curation, Project Administration **Carrie L. Turner:** Methodology, Project Administration **William Shuster:** Conceptualization, Funding Acquisition, Project Administration **Jeffrey L. Ram:** Conceptualization, Methodology, Writing-Review & Editing, Data analysis, Visualization, Supervision, Validation, Funding Acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

Jeffrey L. Ram reports financial support was provided by Michigan Department of Health and Human Services. Jeffrey L. Ram reports a relationship with Michigan Department of Health and Human Services that includes: funding grants.

Since March 8, 2023, Nicholas West is employed by BioBot Analytics, 501 Massachusetts Ave., Cambridge, MA 02139.

Carrie Turner and James Hartrick are employed by LimnoTech, a company that designed collection procedures and collected samples for the project.

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