Swab-free transport as an optimized pre-analytical workflow for SARS-COV-2 amplification

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Impact statement

Here, we validate the use of swab-free transport of samples being evaluated for SARS-CoV-2 nucleic acid using the Hologic Panther Fusion amplification assays. We show that the virus elutes quickly into the transport media and that the swab can be discarded after provider-supervised, self-collected nasal samples are used to briefly inoculate the transport media. We estimate that adopting this method could save 1FTE shift/day/500 samples.

Abstract

Introduction: Efficient detection of SARS-CoV-2 will continue to be an invaluable tool for pandemic control. Current instructions specify that the collection swab should be transported within its collection media to the laboratory. Developing a process whereby this swab is removed before transport to the lab would allow for improved automation and decreased manual manipulation of samples.

Methods: A proof of principle approach was taken by eluting viral particles from flocked swabs into collection buffer with and without a mucus background. Paired swab-free and swab-containing samples were transported to the laboratory and evaluated for SARS-CoV-2 (n=28) or RNaseP (n=6). SARS-CoV-2 amplification was performed using the Hologic Panther Fusion Aptima and RT-PCR assays.

Results: SARS-CoV-2 was detected in all proof of principle samples with Ct values indicative of dilution. The rare exception was for a few samples where the dilution pushed the viral load below the LOD. Paired samples were 100% concordant for SARS-CoV-2 and RNaseP detection.

Conclusion: Discarding the swab after inoculating the transport buffer is an appropriate pre-analytical modification. Adopting this approach can save up to 1 minute/sample. For labs processing more than 500 samples/day this equates to one full time equivalent shift/day.

Introduction

Nasal and nasopharyngeal swabs are currently the preferred specimen types for SARS-CoV-2 RNA detection (1). Collection instructions specify that the swab should be transported within a viral or universal transport media (VTM, UTM) to the laboratory. Additional transport media such as saline or buffer have also been validated (2). Transporting the swab within the media has two main purposes. First, discarding the swab at collection might be considered a hazard in the absence of designated biohazard disposal and second, releasing the virus from the swab into the media may require time to elute. Unfortunately, the swab poses an operational barrier for high volume laboratory workflows because it needs to be removed before any automated liquid handling or manual aliquoting of the sample can proceed. There are alternatives to this collection method, including "dry" nasal swabs (no transport media) or saliva, but both of these have their own operational limitations prohibiting them from being an optimal specimen types (3).

For the Hologic CoV-2 TMA and RT-PCR assays, an acceptable transport media is the assay "lysis buffer", which contains detergent and is known to inactivate the virus (4). Use of an inactivating buffer is an operational advantage because the swabs can be discarded at collection with little risk to the healthcare workers (particularly with additional precautions such as sheathed swabs and secure biohazard bin lids). Additionally, lateral flow antigen tests being performed in CLIA-waived laboratories discard the swabs at collection, indicating the risk of swab disposal at the collection

site is manageable. The question of viral elution from the swab still remains; there have been no studies evaluating how quickly the virus is transferred to transport media once the swab is submerged. The purpose of this study was to evaluate if discarding the nasal swab at collection, after inoculating the transport media, would be an acceptable collection strategy for SARS-COV-2 detection using the Hologic Panther Fusion reverse transcription PCR (RT-PCR) and transcription mediated amplification (TMA) assays.

Methods

Specimen collection, processing, and SARS-COV-2 detection

Sarstedt (part #60.550.050) pre-filled with 2.5mL lysis buffer or Aptima MultiTest Tubes (MTS; Hologic part number PRD-03546) pre-filled with 2.9 mL lysis buffer (Hologic part #PRD-04423) were inoculated with flocked swabs (Puritan part #25-1506). The swab either remained in the tube until analysis in the laboratory or was removed at the collection site quickly after inoculation. Two sample sources were used. The first was residual UTM with detectable SARS-CoV-2 that had been submitted to the laboratory for routine analysis. The second was self-collected nasal swabs placed directly into the lysis buffer-filled collection tubes. For asymptomatic samples, the collection was unsupervised because all asymptomatic volunteers were medical professionals; symptomatic samples were all health care provider observed collections.

Before analysis, samples were vortexed for 30 seconds to reconstitute any mucus particles and then centrifuged at 2000g for 4 minutes to eliminate bubbles. SARS-CoV-2 RNA detection was performed on the Hologic Panther Fusion using both the TMA and RT-PCR assays per manufacturer specifications. Results are provided in Ct value (RT-PCR; semi-quantitative) and as an endpoint RLU (TMA; qualitative). All samples were run on both assays unless otherwise indicated.

This study was evaluated by the KPWA IRB and determined to be a quality improvement project.

Proof of Principle

For each amplification a single swab was submerged into residual SARS-CoV-2 positive UTM for ~45 seconds. The UTM-coated swab was gently rotated in lysis buffer for 10 seconds before removing and discarding. This was performed in 3 different matrices as described below and illustrated in Figure 1.

Matrix 1: Residual UTM from 9 unique patients with SARS-CoV-2 Ct values ranging from 20.4-35.4 were used to saturate a swab. The swab was used to inoculate lysis buffer in a Sarstedt tube.

Matrix 2: Healthy volunteers (n=20) self-collected paired nasal swabs that were gently rotated into lysis buffer (Sarstedt tube) for 10 seconds and discarded. The first tube was used as a negative control to ensure that the volunteer was not an

asymptomatic carrier. The second tube was inoculated with SARS-CoV-2 positive UTM using a second swab, as described above. This allowed us to assess viral elution when mucus was present in the buffer. The UTM samples (n=5 unique patients) used for inoculation had SARS-CoV-2 Ct values ranging from 18.8-37.0 when initially analyzed in the clinical laboratory. Samples were evaluated by SARS-CoV-2 amplification on day zero, stored at 4 degrees, and re-evaluated on day 4.

Matrix 3: Residual samples collected in MTS tubes negative for SARS-CoV-2 with high mucus content (n=20 unique patients combined into 5 individual pools and divided into 15-2.5mL Sarstedt tube aliquots) were used to additionally challenge viral elution. These high mucus samples gave an invalid/clot error on the instrument when originally evaluated for routine analysis, and hence are known to be more analytically difficult samples. After aliquoting, the samples were inoculated with SARS-CoV-2 positive UTM-coated swabs, as described, using 5 unique patients' samples with initial Ct values of 17.6-31.6. Each patient sample was evaluated in triplicate.

In order to calculate the expected dilution, dry swabs (n=5) were weighed before and after submerging in blank UTM for 45 seconds to determine the average volume of media absorbed by the swab.

Elution Time course

A single swab was submerged into residual SARS-CoV-2 positive UTM for ~45 seconds. The swab was removed and gently rotated in lysis buffer (Sarstedt tube) for 5, 10, 15, 20, or 25 seconds before removing and discarding. Each time point was performed using the same residual UTM sample and evaluated in triplicate using a fresh swab and lysis buffer tube for each replicate. Samples were run on the RT-PCR assay only.

Paired patient samples

Volunteer symptomatic patients (n=28) receiving routine SARS-CoV-2 testing self-collected two nasal swab samples: one sample where the swab was transported in the lysis buffer (MTS) and one where the patient was instructed to gently rotate the swab for 10 seconds in the lysis buffer (MTS) and then to discard the swab. Both samples were evaluated in tandem for SARS-CoV-2 nucleic acid detection.

RNase P Detection in paired samples

Healthy volunteers (n=6) self-collected paired nasal swabs in lysis buffer (Sarstedt). For one sample the swab was transported in lysis buffer and for the second the swab was rotated for 10 seconds in the lysis buffer before discarding. Both samples were evaluated in tandem with an LDT that contains a custom HEX fluor-labeled IDT RNase assay. RNase P reactions are multiplexed with two different SARS-CoV-2 targets and serve as an internal control to assess sample collection sufficiency. Each SARS-CoV-2 target is measured in duplicate, producing a total of 4 RNase P

amplifications for each sample. Samples with detectable Ct values for RNase P amplification are considered acceptable for determination of SARS-CoV-2. Across >700 samples measured during nucleic acid extraction validation, observed RNase P Ct values ranged from 17.6 – 34.3 with a mean Ct of 24.5 and standard deviation of 2.6 cycles.

Statistics

Calculations were performed in GraphPad Prism. Statistical significance was evaluated using a paired t-test. To correct for the dilution, 3.2 Ct was added to each of the original values when compared to the inoculated Ct.

Results

Proof of principle

Weight analysis

The average weight of dry and saturated swabs was 472.6 mg (SD=7.5 mg; 1.6%CV) and 514.2 mg (SD=11.8; 2.3%CV), respectively. The average weight of liquid absorbed was 41.6 mg (SD 8.1, 19.4%CV) indicating that the average volume of UTM transferred from the swab to the lysis buffer tube was 41.6 microliters. Typically, 500 microliters of UTM is aliquot into the lysis buffer tube indicating that if 100% of the viral particles eluted from the swab we would expect to see an ~10-fold (~3.2 Ct) increase in values between the "proof of principle" samples and the clinical result.

Lysis buffer only

Table 1 lists the Ct and RLU values for each of the nine samples (matrix 1). The average difference between the original Ct value and the Ct value from inoculating with swab alone was 2.7 (SD = 1.6), which was equivalent to the increase in Ct expected from dilution alone (p=0.8). There was negligible difference in Ct values between the day 0 and day 4 amplification reactions, with the day 4 results showing a slight increase in signal (mean Ct decrease = 0.3; SD = 0.7; p=0.89). The TMA assay gave one false negative result, but the RT-PCR Ct values for that sample were 35.4-38.1, indicated that the sample was below the claimed LOD of the assay (Ct 35.6).

Lysis buffer with mucus background

All volunteers had undetectable SARS-CoV-2. Similar to lysis buffer alone, the average difference between the original Ct value and the Ct value from swab inoculation was equivalent to the difference that would be expected based on dilution alone (average delta Ct = 3.3; SD=0.5; Table 1, matrix 2; p=0.29). The UTM sample with an initial Ct of 37 did not have detectable SARS-CoV-2 results using the inoculation method indicating the sample was diluted beyond the LOD. Similarly, for the sample with a starting Ct of 31.6 one of the TMA amplifications did not detect virus. For the subset of samples re-evaluated by RT-PCR on day 4, there was negligible difference compared to the day 0 results (mean delta Ct = 0.2; SD = 0.5 p=0.56).

Mucus rich samples

Inoculation of mucus rich samples with a UTM saturated swab gave similar delta Ct value compared to the other matrices (mean delta Ct = 4.3; SD = 0.7; p=0.60).

Elution time course

There was no change in the SARS-CoV-2 Ct value for samples where the swab was rotated in the lysis buffer for 5 seconds (mean Ct= 26.0; SD=1.1), 10 seconds (mean Ct= 26.0; SD=0.7), 15 seconds (mean Ct=24.6; SD= 0.1), 20 seconds (mean Ct=25.4; SD= 0.6), or 25 seconds (mean Ct=25.9; SD= 0.1).

Paired patient samples

Results for the paired collection were 100% concordant. Of the 28 paired patient samples, 2 had detectable SARS-CoV-2. The SARS-CoV-2 Ct values for these two patient samples were 13.7 and 31.2 with the swab transported in lysis buffer and 16.2 and 28.3, respectively with the swab discarded after inoculation.

RNase P detection in paired samples collected from healthy volunteers

Table 2 shows that there was little difference in RNase P detection between samples transported with the swab compared to those where the swab was discarded after inoculation. This remained true regardless of which collection strategy was performed first.

Discussion

Efficient detection of SARS-CoV-2 will continue to be an invaluable tool to control the spread of infection. Unfortunately, most laboratories are at their operational capacity, and would benefit from more streamlined workflows in order to improve their throughput. Swab disposal at the time of collection could save laboratories up to 1 minute for each SARS-CoV-2 sample. For laboratories processing ~500 samples/day this would be equivalent to one FTE shift. This savings is particularly true for the Hologic assay, where samples can be collected into the reaction buffer and the collection tube can be loaded directly onto the instrument. However, the approach could be adapted to diverse liquid handling solutions to conceivably provide equivalent time saving solutions across alternative platforms.

In addition to the time efficiency, implementing this collection strategy would significantly reduce repetitive movements for our laboratory employees. Submitting the sample without the swab eliminates at least 2 uncapping/recapping steps and potentially removes a manual pipetting step for each sample. This is an important workflow modification as mitigating these movements improves laboratory morale, while reducing repetitive strain injuries and overall burnout. Further, the less a sample is manually manipulated, the lower the risk of contamination, which has been suggested to be one of the most common mechanisms of false positive amplification results (5).

For our patient population, implementing this strategy is not expected to reduce the analytical sensitivity of SARS-CoV-2 detection. All of the validation experiments performed in this study suggest that the virus elutes from the swab very quickly, and that the variability observed was a function of dilution or analytical imprecision. This is further bolstered by the RNase P results, which indicated that the difference in RNase P detection between collection types was concordant with the expected assay imprecision, which averaged 0.7 PCR cycles with a coefficient of variation of 2.9% for the laboratory negative SARS-CoV-2 control across 47 independent, consecutive RNase P assays.

The strategy described here was designed for an integrated healthcare network, which has the benefit of being a closed system. Advantages of a closed system include standardization within the consumable supply chain, medical/laboratory assistant training, and amplification assays. A process like this would be more difficult to implement in a reference laboratory setting, where there is a diverse clientele, including lack of standardization in specimen collection, and oftentimes multiple manufacturer assays utilized to manage testing volumes/reagent allocations. The concept of swab-free transport could be modified to suit larger clients or regional medical centers.

Our laboratory is enthusiastic about implementing this approach; however, there are limitations to this study. First, our sample size was limited, particularly for the paired positive SARS-CoV-2 samples. We utilized several surrogates to improve our

confidence in the method, but these surrogates may not have been sufficient substitutes. Second, we only evaluated one type of swab, tube, and elution buffer system. Conceivably, the virus might elute differently into UTM. Further studies would be needed to validate if this approach could be extended to various collection consumables. The current landscape of supply chain disruptions and shortages underscores the need for flexibility and bridging studies to support different media and collection swabs. Third, collection media such as UTM does not inactivate the virus. Therefore, discard bins would need to be optimized to minimize unintentional aerosolization of the waste. Lastly, we have not yet operationalized the strategy, and are therefore unable to provide foresight into how the medical and lab assistants will feel about swab disposal. We believe this strategy should take an identical amount of time to current practices at the clinic because besides a quick discarding of the swab, there are no additional capping/uncapping, or other steps that require fine motor skills. However, it is also possible that there may be other unknown operational barriers that arise.

In summary, this is the first publication to evaluate the ability to discard the swab at collection for respiratory viral detection. This process improvement could be adopted by a diverse array of laboratories and clinical settings. If validated and implemented properly, this can significantly reduce processing time, increase laboratory throughput, and decrease repetitive movements.

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Table 1: Proof of principle matrices and the Ct values observed after inoculating a Sarstedt pre-filled with 2.5mL lysis buffer. Abbreviations: transcription mediated amplification (TMA), cycle threshold (Ct), reverse transcriptase polymerase chain reaction.

Matrix	Original RT-PCR Ct	RT-PCR Ct (Day 0)	TMA (Day 0)	RT-PCR Ct (Day 4)	TMA (Day 4)	Delta Ct (Day 0-Original)
1	20.4	23.3	positive	23.2	positive	2.9
1	21.9	24.4	positive	24.3	positive	2.5
1	22.9	26.3	positive	26	positive	3.4
1	25.5	27.7	positive	28	positive	2.2
1	25.7	31	positive	31.2	positive	5.3
1	28.9	32.6	positive	32.2	positive	3.7
1	29.4	31.8	positive	32	positive	2.4
1	33.5	32.7	positive	32.1	positive	-0.8
1	35.4	38.1	positive	36.1	negative	2.7
2	18.8	22.7	positive	23	Not done	3.9
2	18.8	21.7	positive	Not done	positive	2.9
2	18.8	22.2	positive	22.1	Not done	3.4
2	18.8	22.4	positive	Not done	positive	3.6
2	18.8	22.4	positive	22.2	Not done	3.6
2	24.7	27.4	positive	Not done	positive	2.7
2	24.7	27.6	positive	27.5	Not done	2.9
2	24.7	28.5	positive	Not done	positive	3.8
2	24.7	29.1	positive	29.2	Not done	4.4

2	24.7	27.3	positive	Not done	positive	2.6
2	31.6	34.9	positive	36.1	Not done	3.3
2	31.6	34.9	positive	Not done	positive	3.3
2	31.6	34.8	negative	Not done	negative	3.2
2	31.6	35.8	positive	Not done	negative	4.2
2	31.6	35	positive	35	Not done	3.4
2	37	negative	positive	Not done	negative	-
2	37	negative	negative	negative	Not done	-
2	37	negative	negative	Not done	negative	-
2	37	negative	negative	negative	Not done	-
2	37	negative	negative	Not done	negative	-
2	31.6	34.2	positive	Not done		2.6
2	31.6	34.4	positive			2.8
2	31.6	34.4	positive			2.8
2	31.6	35.3	positive			3.7
2	31.6	35.3	positive			3.7
3	17.7	21.1	positive			3.4
3	17.7	21.0	positive			3.3
3	17.7	21.0	positive			3.3
3	17.6	22.2	positive			4.6
3	17.6	22.2	positive			4.6
3	17.6	22.2	positive			4.6
3	21.6	26.5	positive			4.9
3	21.6	27.3	positive			5.7
3	21.6	26.0	positive			4.4
3	27.0	32.1	positive			5.1

3	27.0	30.8	positive		3.8
3	27.0	31.6	positive		4.6
3	31.6	35.1	positive		3.5
3	31.6	35.5	positive		3.9
3	31.6	36.5	positive		4.9

Table 2: RNAse P recovery between paired nasal swabs remaining in the transport media compared to those discarded immediately after inoculation. Samples are given a unique number (1-6); "a" indicates that this collection strategy was performed first and "b" indicates that the collection strategy was performed second.

Sample ID	Collection method	RNaseP 1	RNaseP 2	RNaseP 3	RNaseP 4	Average RNaseP	Delta Ct
1a	swab in	22.7	22.6	22.8	22.7	22.7	0.5
1b	swab out	23.2	23.2	23.2	23.2	23.2	
2a	swab in	23.9	23.9	24.2	24.0	24.0	0.1
2b	swab out	23.9	24.0	24.4	24.2	24.1	
3a	swab in	25.7	25.5	25.8	25.9	25.7	0.6
3b	swab out	26.1	26.3	26.4	26.5	26.3	
4a	swab out	26.6	26.8	27.2	27.0	26.9	1.1
4b	swab in	25.7	25.7	26.0	25.9	25.8	
5a	swab out	29.2	29.6	29.7	29.7	29.5	0.9
5b	swab in	28.5	28.5	28.6	28.9	28.6	
6a	swab out	28.7	28.8	28.7	29.1	28.8	1.4
6b	swab in	30.1	30.2	30.3	30.2	30.2	

Figure Legends

Figure 1: Flow diagram illustrating the matrices used for the proof of principle verification experiments