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Protocols

Effect of not vortexing nasopharyngeal and throat swabs on SARS-CoV-2 nucleic acid detection: A pilot study

Jamil N. Kanji [a,b,c,d,*], Kanti Pabbaraju [a], Anita Wong [a], Candace Beitku [e], Ashwin Deo [e], Seema Kailey [e], Raymond Ma [e], Graham Tipples [e,f,g], Nathan Zelyas [d,e]

[a] Public Health Laboratory, Alberta Precision Laboratories, Calgary, AB, Canada
[b] Division of Infectious Diseases, Department of Medicine, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[c] Department of Pathology & Laboratory Medicine, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[d] Department of Laboratory Medicine & Pathology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada
[e] Public Health Laboratory, Alberta Precision Laboratories, Edmonton, AB, Canada
[f] Department of Medical Microbiology & Immunology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada
[g] Li Ka Shing Institute for Virology, University of Alberta, Edmonton, AB, Canada

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ABSTRACT

The processing of swabs for respiratory virus detection involves vortexing while still in the viral transport medium (VTM). The effect of not vortexing swabs prior to analysis has not been studied extensively for SARS-CoV-2 detection, and presents an opportunity to improve pre-analytic laboratory workflow. We aimed to assess the impact of not vortexing nasopharyngeal/throat swabs submitted in VTM for SARS-CoV-2 testing. To assess the impact of not vortexing swabs, 277 swab samples were tested for SARS-CoV-2 RNA in paired vortexed and non-vortexed aliquots using eight routine nucleic acid amplification assays. We compared the qualitative (positive/negative) and semi-quantitative (cycle threshold, Ct) results. Following discordant analysis, all but one non-vortexed sample had the same qualitative result as the vortexed sample. 27.4 % of samples were SARS-CoV-2 positive. Comparison of Ct values revealed an apparent reduction in human cellular nucleic acid in the non-vortexed samples (mean Ct values of 24.0 and 26.5 for vortexed and non-vortexed samples, respectively, $p < 0.0001$) and increased Ct values for non-vortexed samples using a laboratory-developed SARS-CoV-2 assay (mean Ct values of 4.1 and 4.2 for vortexed and non-vortexed samples, respectively; $p < 0.0001$), but this was not observed for a more automated commercial SARS-CoV-2 assay (mean Ct values of 15.2 for both vortexed and non-vortexed samples, respectively; $p = 0.68$). While vortexing swabs appears to improve the recovery of cellular material, it does not have an appreciable impact on the qualitative sensitivity of SARS-CoV-2 nucleic acid tests, which may support omission of this step and simplification of front-end sample processing.

1. Introduction

The coronavirus infectious disease (COVID)-19 pandemic has put an immense strain on clinical laboratories with the need to process high volumes of samples for the detection of severe-acute respiratory syndrome virus 2 (SARS-CoV-2) (Pabbaraju et al., 2020). Laboratories have faced challenges with shortages in specimen collection and transport materials, molecular extraction and testing reagents, as well as human resources, all the while coping with increasing volumes of COVID-19 testing (Tsai et al., 2021).

Real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) testing for SARS-CoV-2 is a multistep process that involves a complex workflow to ensure high quality results and minimize contamination (Carter et al., 2020). A common initial step in the processing of samples for SARS-CoV-2 rRT-PCR includes vortexing swabs in viral transport medium (VTM) post-collection to ensure viral particles and human cells containing virus nucleic acid entrapped in mucus or swab material are dispersed into the medium. While each sample only requires vortexing for several seconds, the cumulative time in COVID-19 specimen processing that could be saved with removal of the vortexing step may be considerable.

We conducted a pilot study to evaluate the effect of removing the

* Corresponding author at: Public Health Laboratory, Alberta Precision Laboratories, Foothills Medical Centre, 3030 Hospital Drive NW, Calgary, AB, T2N 4W4, Canada.
E-mail address: jamil.kanji@ahs.ca (J.N. Kanji).

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swab vortexing step on the SARS-CoV-2 rRT-PCR result. We hypothesized that, given the high sensitivity of rRT-PCR assays, specimens processed without vortexing may have less cellular material, but the overall qualitative rRT-PCR result would likely not be affected.

2. Materials and methods

2.1. Setting

A large proportion of COVID-19 testing in the province of Alberta, Canada (population 4.4 million) is carried out at the Public Health Laboratory (Alberta Precision Laboratories), which consists of two testing sites, one in each of the cities of Calgary and Edmonton, Alberta. The laboratory utilizes a number of PCR-based assays to test for the presence of SARS-CoV-2 (Table S1). Testing is conducted on individuals with symptoms concerning for COVID-19 or for asymptomatic contacts of those involved in outbreaks, as outlined by the Ministry of Health (Government of Alberta) (Government of Alberta, 2022). Sample processing of all specimen types first includes vortexing the closed specimen container for 5–7 seconds, followed by a settle time of 5−15 min to minimise aerosols when container caps/lids are opened. All processing, regardless of specimen type, is carried out in an appropriate biosafety cabinet. The specimen is then processed as per the requirements of the SARS-CoV-2 PCR assay being used (Table S1), which may require a separate extraction procedure (in the circumstance that a laboratory-developed test [LDT] is used). Commercial assays were all performed as per manufacturer instructions.

2.2. Study procedure

Between April 15, 2021 – May 15, 2021, an average of 9–10 nasopharyngeal (NP) and/or throat swab specimens in viral transport media (VTM) received for SARS-CoV-2 testing daily were randomly selected from the two laboratory sites to be tested both with and without a vortexing step on a daily basis. All specimens included in this study first underwent removal of a 1000 μL VTM aliquot prior to vortexing, which was then stored at −70 °C. Each aliquot tube of the primary specimen was then labeled to indicate inclusion in the study protocol. After removal of the aliquot, the remaining volume in specimens were processed as per routine where they underwent the 5–7 seconds of vortexing as described above.

After vortexing, specimens included in this study were processed and tested using the SARS-CoV-2 envelope (E) gene LDT assay (Pabbaraju et al., 2021) at the Calgary laboratory site or the cobas SARS-CoV-2 test (Roche Diagnostics, Basel, Switzerland) at the Edmonton site. Non-vortexed aliquots were batched and tested on the same assay as the vortexed portion. Due to reagent shortages, not all non-vortexed aliquots could be run on the same assay as the vortexed portion, and thus were then run on other available SARS-CoV-2 assays currently in use in the laboratory (Table S1). Clinical testing results were reported based on test results from the vortexed protocol, as this was the routine testing protocol. Non-vortexed samples in total were subjected to one freeze-thaw cycle (FTC) and frozen for up to 48 h prior to being thawed for testing. Aliquots of vortexed samples were stored at −70 °C as per standard laboratory operating procedure.

To evaluate how the presence or absence of vortexing affected the amount of human cellular material tested, all vortexed and non-vortexed samples tested using the E gene LDT also underwent testing for detection of beta-2-microglobulin (B2M) RNA using an RT-PCR assay (Lupberger et al., 2002).

2.3. Evaluation of discordant testing results

To evaluate the effect of the one FTC on non-vortexed samples that displayed discordant qualitative SARS-CoV-2 rRT-PCR results compared to the vortexed sample, an aliquot of the stored vortexed sample was thawed and subjected to repeat testing. The intent was to evaluate if the FTC was responsible for the discordant results.

2.4. Data analysis

Qualitative test results (positive or negative for the presence of SARS-CoV-2 RNA) were recorded for all assays used in this study. The cycle threshold (Ct) values for specimens tested on the E gene LDT and two targets on the Cobas assay (Orf1 a/b and E genes) were also recorded. Ct values for specimens tested on any other assays were not used for Ct value comparison. Comparison of vortex and non-vortexed specimens was done in both a qualitative and semi-quantitative manner. Quantitative comparison was carried out only between the same assay type (which was done for the E gene LDT and Cobas assay). All other comparisons were qualitative in terms of the final result (positive versus negative for SARS-CoV-2 RNA).

Quantitative values were summarized using basic summary statistics. Ct values for the same targets on each of the LDT E gene, cobas, and B2M assays were compared using the Wilcoxon matched-pairs signed rank test (non-parametric). All statistical analysis was carried out using GraphPad Prism (version 9.2.0; GraphPad Software Corporation, San Diego, USA).

3. Results

Over the course of the study period, 277 specimens submitted for SARS-CoV-2 testing (193 NP swabs and 84 throat swabs) were included in the study (Table 1). Of these, 219 (79.1 %) and 58 (20.9 %) were tested initially on the E gene LDT and cobas assay respectively, and assigned as either positive or negative based on this initial test result. Some specimens were run on multiple assays. The overall test positivity was 27.4 %. The test positivity of testing on the individual assays varied based on the number of specimens run on each (Table 1). B2M RT-PCR was conducted on 178 specimens also run on the E gene LDT.

From a qualitative perspective, the final testing results (positive or negative for the presence of SARS-CoV-2 RNA) were 100 % concordant for all 277 vortexed specimens run on the E gene LDT, and the non-vortexed specimens on a series of other assays (BioFire, Panther, E gene LDT, SC2/Flu LDT, Xpress SARS-CoV-2, and Xpress SARS-CoV-2/Flu/RSV) (Table 2). For 58 vortexed specimens run on the cobas assay, 100 % concordance was seen for 33 specimens non-vortexed specimens run on the BioFire and 29 run on the Panther. Concordance was seen for 34/35 (97.1 %) and 33/35 (94.3 %) of the 58 specimens tested non-vortexed using the cobas and Simplexa assays respectively (Table 2).

Further evaluation of these discordant results (termed samples A, B, and C; Table S2) from Table 2, demonstrated that two of three discordant results were likely due to the effect of the FTC on the non-vortexed sample (Tables S3; samples A and B). This is evidenced by the vortexed sample demonstrating the same result (after one FTC). The Ct values of

Table 1
Number and positivity rates of specimens tested on each assay.

Assay[a]	Total Specimens Tested (%)	Number Positive (%)
E gene LDT	219 (79.1)	38 (17.4)
cobas	58 (20.9)	38 (65.5)
BioFire	30 (10.8)	20 (66.7)
Panther	30 (10.8)	20 (66.7)
Simplexa	30 (10.8)	20 (66.7)
SC2/Flu	39 (14.1)	24 (61.5)
Xpress SARS-CoV-2	34 (12.3)	22 (64.7)
Xpress SARS-CoV-2 / Flu / RSV	34 (12.3)	22 (64.7)
Total specimens received[b]	277	76 (27.4)

[a] For details regarding assays, please refer to Table S1.
[b] Some specimens were tested on multiple assays.

Table 2
Qualitative agreement (positive and negative) in COVID-19 test results between various assays when the same specimens were processed pre- and post-vortexing.

	Non-Vortexing[a]							
Vortexing[a]	cobas	BioFire	Panther	Simplexa	E gene LDT	SC2/Flu	Xpress SARS-CoV-2	Xpress SARS-CoV-2/FLU/RSV
E gene LDT	–	2/2	2/2	–	217/217	39/39	34/34	34/34
cobas	34/35[b](97.1)	33/33	29/29	33/35[b](94.3)	–	–	–	–

[a] For details regarding assays, please refer to Table S1.
[b] The three samples where vortexed and non-vortexed samples did not have the same qualitative result, were termed discordant, and evaluated using a discordant analysis (see Materials and Methods).

all three discordant results rRT-PCR targets were ≥ 34 cycles.

The distribution of the Ct values of 217 samples tested vortexed and non-vortexed on the E gene LDT assay ranged from 0 to 35.4 cycles (where 0 refers to samples from which SARS-CoV-2 RNA was not detected) (Fig. 1; Table S4). The same is shown for 187 samples run on the E gene LDT assay that were also tested using the B2M RT-PCR assay (median Ct values 22.8 and 25.9 vortexed and non-vortexed, respectively). Using the E gene LDT assay, the Ct values for vortexed and non-vortexed samples were found to be significantly different (mean Ct difference of 0.09 cycles; $p < 0.0001$). Vortexing was also found to result in significantly more cellular material in the VTM sample being detected compared to non-vortexed samples (mean B2M Ct difference between the two groups was 2.5 cycles; $p < 0.0001$) (Fig. 1A). Interestingly, the distribution of Ct values for non-vortexed and vortexed samples tested on the cobas were not found to be significantly different for either the orf1 a/b target or the E gene target of the assay ($p = 0.99$ and$p = 0.68$, respectively; mean Ct difference for both orf1 a/b and E gene targets was zero cycles) (Fig. 1B).

4. Discussion

In the present study, we demonstrate that removal of the initial vortexing step of NP and throat swabs submitted for SARS-CoV-2 rRT-PCR testing has a minimal effect on the qualitative (positive/negative) result of the test. Reduced human cell recovery due to lack of vortexing also did not translate into a reduced rate of detection of SARS-CoV-2 RNA. This finding has the potential to help improve workflow efficiency in clinical laboratories and contamination from potential aerosols, as these two swab types constitute the majority of specimens received for COVID-19 rRT-PCR testing.

We have been unable to find any large-scale studies that evaluated absence of vortexing or swab agitation step prior to removal of VTM for further processing. In one study evaluating pre-analytical variables of concern with regards to SARS-CoV-2, researchers found that two

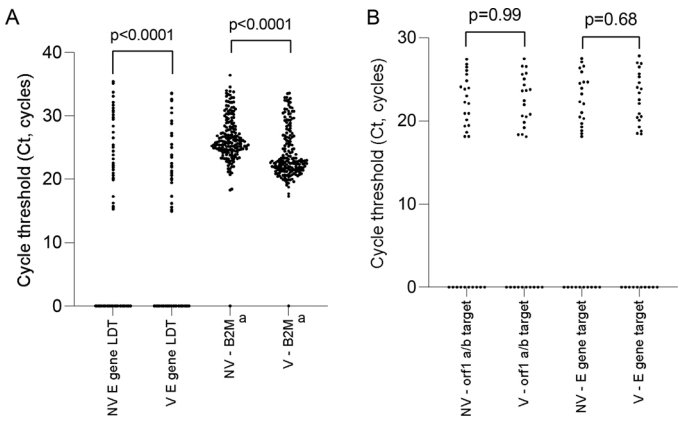

Fig. 1. Comparison of Ct values for various rRT-PCR targets when evaluating non-vortexed (NV) and vortexed (V) specimens using the A) E gene LDT and B2M[a] assays; B) cobas assay.
[a]B2M was tested only on specimens tested on the E gene LDT, but not the cobas.

samples processed with and without vortexing did not differ with regards to the qualitative result. The Ct difference between the two procedures ranged from 0.38−0.58 cycles (Basso et al., 2020).

Vortexing respiratory swabs in VTM is generally recommended to release all cell-associated virus (Carroll et al., 2019). However, the findings of our study likely suggest there are sufficient concentrations of SARS-CoV-2 nucleic acid in the extracellular space that can be detected by diagnostic rRT-PCR assays, and that not all virus is cell-associated. This is possibly explained by the release of modified extracellular vesicles by SARS-CoV-2 infected cells that can contain viral RNA molecules which are then detected by diagnostic assays (Machhi et al., 2021). This is in addition to the continuous budding of SARS-CoV-2 virions from infected cells producing a more constant level of virus particles that transit through the extracellular space to infect other cells (Brahim Belhaouari et al., 2020; Mendonça et al., 2021).

The effect of removal of vortexing on Ct values, based on our study, is not entirely clear. While the Ct values with and without vortexing on the E gene LDT were significantly different, the same was not seen with Ct values for the orf1 a/b and E gene targets on the cobas assay. This observation is likely due to the cobas assay requiring more specimen volume (600 μL vs 200 μL for the E gene LDT) and also being a fully automated assay (as the E gene LDT has multiple manual steps). Despite the Ct value differences noted, the absence of vortexing did not affect the qualitative result. This is more applicable to daily COVID-19 testing in a clinical laboratory where Ct values are not reported, and advised not to be used routinely in clinical management of patients with COVID-19 (Association of Public Health Laboratories (APHL), Government of Canada, Infectious Diseases Society of America (IDSA)).

The major strengths of this study include the large number of samples evaluated using a vortex and non-vortex methodology as well and the variety of SARS-CoV-2 rRT-PCR assays used (which collectively include multiple different gene targets). The results are limited by the inclusion criteria being only nasopharyngeal and throat swabs. However, these comprised the majority of specimens received for testing and where removal of the vortex step could have the largest impact on laboratory workflow. We also did not evaluate rapid point-of-care PCR or antigen testing platforms. Furthermore, we only evaluated the impact of not vortexing on SARS-CoV-2 detection, and not other respiratory viruses. Other respiratory viruses that may be more cell-associated compared to SARS-CoV-2 could be impacted.

In conclusion, the benefit of vortexing nasopharyngeal and throat swabs received in VTM for SARS-CoV-2 rRT-PCR appears to have limited effect on the qualitative result that is reported. This finding has the potential to help reduce the processing time for testing routine samples for SARS-CoV-2. Given the pilot nature of this study, ongoing efforts to replicate the findings are important, especially to evaluate the effect of not vortexing samples with higher Ct values.

Ethics approval

Presentation of the data included in this manuscript was approved by the Human Research Ethics Board at the University of Alberta (Edmonton, Alberta, Canada; study identifier Pro00115280).

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

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Contributions

Jamil N Kanji – formal analysis, visualisation, writing – original draft, writing – review and editing.

Kanti Pabbaraju – Data curation, investigation, methodology, validation, project administration, writing – review and editing.

Anita Wong – Data curation, investigation, project administration, writing – review and editing.

Candace Beitku – Data curation, investigation, writing – review and editing.

Ashwin Deo - Data curation, investigation, writing – review and editing.

Seema Kailey – Resources, supervision writing – review and editing.

Raymond Ma - Data curation, investigation, writing – review and editing.

Graham Tipples – Conceptualisation, funding acquisition, project administration, resources, writing – review and editing.

Nathan Zelyas – Conceptualisation, formal analysis, methodology, project administration, resources, validation, writing – original draft, writing – review and editing.

Declaration of Competing Interest

None of the authors have conflicts of interests to declare with regards to this manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2022.114468.

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