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Detection of SARS-CoV-2 is comparable in clinical samples preserved in saline or viral transport media

Running title: Saline media for transporting SARS-CoV-2

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Abstract

As the COVID-19 pandemic sweeps across the world, the availability of viral transport media (VTM) has become severely limited, contributing to delays in diagnosis and rationing of diagnostic testing. Given that SARS-CoV-2 viral RNA has demonstrated stability, we posited that phosphate buffered saline (PBS) may be a viable transport medium, as an alternative to VTM, for clinical qPCR testing. We assessed the intra- and inter-individual reliability of SARS-CoV-2 qPCR in clinical endotracheal secretion samples transported in VTM or PBS, evaluating the stability of the RT-qPCR signal for three viral targets (N gene, ORF1ab, and S gene) when samples were stored in these media at room temperature for up to 18 hours. We report that using PBS as a transport medium has high intra- and inter-individual reliability, maintains viral stability, and is comparable to VTM in the detection of the three SARS-CoV-2 genes through 18 hours of storage. Our study establishes PBS as a clinically useful medium that can be readily deployed for transporting and short-term preservation of specimens containing SARS-CoV-2. Use of PBS as a transport medium has the potential to increase testing capacity for SARS-CoV-2, aiding more widespread screening and early diagnosis of COVID-19.
Introduction

In December 2019, a novel coronavirus was recognized as causing a cluster of pneumonia cases in Wuhan, China (1). The infectious agent, an RNA virus, was termed acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and was identified as the cause of COVID-19, a clinical syndrome manifested by an influenza-like illness that can progress to acute lung injury or acute respiratory distress syndrome (ARDS) with substantial mortality (2). COVID-19 has affected more than 3.1 million people causing more than 220,000 deaths worldwide (https://coronavirus.jhu.edu/map.html, last accessed April 29, 2020).

SARS-CoV-2 detection using standard testing of upper airway secretions requires a nasopharyngeal (NP) or oropharyngeal (OP) swab that is transported to a clinical laboratory using viral transport media (VTM) (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2#offeringtests, last accessed April 29 2020). Recently, OP and saliva testing using VTM also were shown to be comparable to NP swabs for detection of the virus (3, 4). As the COVID-19 pandemic has swept across the world, availability of VTM has become severely limited, impairing local and regional capacity for diagnosis. Since SARS-CoV2 has capped RNA with a 5’ GTP resembling host RNA and that the virus sgRNA manifests remarkable stability (5), we posited that qPCR detection of SARS-CoV-2 in specimens preserved in phosphate buffered saline (PBS), which is readily available, would be comparable to those in VTM. Here, we report that sample preservation in PBS or VTM are comparably effective for the preservation of SARS-CoV-2 in endotracheal secretions.

Materials and Methods

Source materials. For transport media, we followed procedures outlined in standard references (5-8). We used phosphate buffered saline (PBS), a water-based salt solution containing disodium hydrogen
phosphate, sodium chloride, potassium chloride and potassium dihydrogen phosphate, pH 7.2 (Sigma
Aldrich, Saint Louis MO). Viral transport media (VTM) was derived according to the Centers for Disease
Control and Prevention (CDC) Coronavirus outbreak response (https://www.cdc.gov/coronavirus/2019-
cov/downloads/Viral-Transport-Medium.pdf, last accessed April 29, 2020). In brief, a solution with
Hanks Balanced Salt Solution (HBSS), heat-inactivated fetal bovine serum (final concentration 2%),
gentamicin 100µg /mL and amphotericin B 0.5 µg /mL was prepared and aliquoted into 2 ml screw top
vials (6). Tubes then were stored at 4°C until use.

Experimental Protocols: Respiratory secretions from 16 confirmed COVID-19 positive subjects were
collected over a four-day period from an intensive care unit at Robert Wood Johnson University Hospital
in New Brunswick NJ, according to a protocol approved by the Rutgers IRB (Protocol # Pro2020000800).
All subjects were mechanically ventilated for acute hypoxemic respiratory failure due to confirmed
COVID-19 pneumonia. Specimens were collected into a sterile container via closed circuit, in-line
catheter suction of respiratory secretions from the endotracheal tube (ET), as part of routine clinical
care. Swabs were then dropped into vials containing PBS or VTM and transported to the RUCDR
laboratory for analysis. To test intra- and inter-patient variation in efficacy of detecting SARS-CoV2
from ET-derived samples, three experimental procedures were performed. Eight samples from two
subjects (four from each subject) were harvested at the same time and transported in either VTM or
PBS. Samples were processed immediately (at 0 hours) or after 2 hours at room temperature (RT). Real
time-qPCR (RT-qPCR) was performed on 1 sample from each transport medium at each incubation time
(0 or 2 hours) and Ct values for the SARS-CoV-2 nucleocapsid (N), open reading frame 1ab ORF1ab, and
spike protein (S) genes were compared; bacteriophage MS2 (MS2) spiked into the samples was used as a
positive control. In a parallel experiment, the stability of detection of SARS-CoV2 sgRNA by RT-qPCR in
samples transported in PBS and VTM also was examined after remaining at room temperature for time
points ranging from 0 to 18 hours. These experiments mimicked field conditions, in which specimens
remain in transport at RT for periods up to 18 hours. Twenty samples from each of two subjects were kept for 0, 2, 4, 6 h or overnight at RT in VTM or PBS to mimic these real-world transport condition. Ct values were again compared for the RT-qPCRs for the three viral genes described above across the indicated time points. To examine inter-subject variance, we examined samples from an additional 12 patients whose ET secretions were transported in either PBS or VTM; RT-qPCR was performed immediately on arrival in the lab for these 24 samples, and Ct values were again compared between those transported in VTM or PBS.

**Viral RNA extraction.** After removal of the collection swab, 300 µl of VTM or PBS sample was transferred to a 96-well Deep Well Processing plate (PerkinElmer, Waltham, MA.), and extractions were performed on a Chemagic 360 instrument using the Chemagic Viral DNA/RNA 300 Kit H96 (PerkinElmer), as described by the manufacturer. This system eliminates manual sample handling, reduces risk of cross-contamination and ensures rapid and consistent processing. In brief, extraction included 4µl of Poly(A) RNA, 10 µl of Proteinase K, 300 µl of Lysis Buffer and 8 µl of MS2 phage Internal Control added to each sample. Then 300 µl of TaqPath™COVID-19C Positive Control and 300µl of nCoV Negative Control (Themo Fisher, Waltham, MA.) were added to designated wells to be extracted alongside the subject samples. Magnetic beads (150µl) were added, followed by 900 µL RNA binding buffer. The beads/RNA mixture was washed with 500 µL wash buffer 3, then with 500 µL wash buffer 4. Elution buffer was added, and residual beads washed in 500 µL water, followed by a second elution step to a final volume of 50 µl.

**SARS-CoV-2 assays.** Assays were conducted under FDA approved EUA#200090 at RUCDR Infinite Biologics® (Piscataway, NJ). 5 µL of each extracted RNA sample was aliquoted into a 384-well plate with 5 µL of positive and no template controls aliquoted into designated wells. Amplification was performed on the ABI QuantStudio 5 Real-Time PCR System using the TaqPath COVID-19 Combo Kit (Thermo Fisher) following the manufacturer’s directions. This assay detects the
SARS-CoV-2 ORF1ab (FAM-labeled), S (VIC-labeled), and N (ABY-labeled) genes with a MS2 Phage Control (JUN-labeled). Data were interpreted and Ct values generated using ABI QuantStudio5 PCR Real Time PCR software v1.3 (Thermo Fisher). Reactions were in 20 µL volumes and run using the cycling protocol: 25°C for 2 min, 53°C for 10 min; 95°C for 2 min; then 95°C for 3 sec; 60°C for 30 sec, collecting the fluorescence signal during the final 60°C step, and repeated for a total of 40 cycles. Assays were performed in triplicate, and there are positive and negative assay controls with the MS2 phage as a positive control of nucleic acid extraction and RT-PCR. The lower limit of SARS-CoV-2 detection is 200 copies/mL and the assay exhibits no cross-reactivity with 43 organisms and viruses that were tested.

**Interpretation.** The TaqPath COVID-19 Combo Kit provided negative, positive, and internal controls to monitor the reliability of the results for the entire batch of specimens from sample extraction to PCR amplification, according to the manufacturer’s instructions. MS2 Ct <37 was considered as positive, and N, ORF 1ab, and S Ct > 37 were considered negative.

**Statistical analysis.** Pearson correlation was used to quantify association between repeated samples within subject, between transport media, and between the three genes. Linear mixed effects models were used to address several of our research questions. These models were fitted separately for each of the 3 genes. The outcomes were the Ct values, while the predictors included the transport media (VTM versus PBS) and hours in storage (0, 2h, 4h, 6h, or 18h). The models included a random intercept for each subject, to account for repeated observations (within-subject correlation). We used lme4 and ggplot2 packages in R for the linear mixed effects models and plots, respectively (https://www.R-project.org)(9).
Results

Analysis of repeated samples from the same subject. From two unique subjects, we obtained paired identical swab specimens that were transported in PBS and VTM. A total of 39 samples were analyzed, representing 10 for each subject in PBS and 9 in VTM, and the variation in Ct plotted (Figure 1). As expected, SARS-CoV-2 was detected in all samples with Ct values significantly below the negativity cut-off of 37 in each assay. For each transport medium, there was strong correlation of values between the 9 or 10 replicates in each gene assay. These analyses indicate the consistency of the transport and measurements for samples that were obtained and stored in identical manners.

Comparison of VTM and PBS. First, we constructed scatterplots of the values from all 16 subjects tested, including all 5 times studied, for a comparison of 26 values for both PBS and VTM for each of the three genes studied (Figure 2). The correlations ranged from 0.83 for the ORF1ab gene to 0.93 for the N, and all were significant (p<0.05). Since some individuals contributed samples at multiple time points, there were repeated measures per person. To examine a single measurement per person, we restricted the analysis to the data obtained at time 0 only. The correlation for the three genes did not change substantially and remained significant. Across the time 0 samples from the 16 individual subjects, the standard deviations (SD) varied with respect to the gene studied, but did not vary significantly according to the transport medium. Thus, using PBS did not significantly skew the distribution of values in relation to the VTM (data not shown).

Next, we used a linear mixed effects model (see Methods) to compare the sensitivity of the two transport media in the timed samples, from 0 h to overnight (18h) storage. For all three genes, there were no significant differences, and in each case, the VTM values trended higher than for PBS. We next evaluated if across individual samples, the values obtained in testing one gene correlated with the
results for the other genes. Additionally, we tested whether the choice of transport medium made a difference. In total, for the 13 samples transported in VTM, the pair-wise correlations between the three genes ranged from 0.947 to 0.956. For the 14 samples transported in PBS, the correlations ranged from 0.963 to 0.991. Thus, the results for the three genes were highly correlated independent of transport medium type.

**Decay of the viral signal over time for specimens transported in the two media.** We next considered whether there was decay in viral signal over time, and whether it differed according to the transport medium used. From the prior analyses, the coefficient of hours of storage was estimated to be negative in the models of all three genes tested (approximately -0.03 for each gene) and was not significantly different from zero (all p-values >0.5). These data indicate that storage at room temperature for up to 18 hours had little effect on the values detected in the RT-qPCRs for the three SARS-CoV-2 genes tested. However, these models did not include an interaction term for the transport medium used for storage and time, and there could be differences between the media. To assess this possibility, we performed the same analyses as above, but included interactions between hours of storage and transport medium used. For each of the three genes tested, both the main effect of time and the interaction between time and the transport medium were not statistically significant (all p-values >0.5) and estimates were close to 0. Therefore, we did not detect decay in the viral signal or differential decay by transport medium over the time interval studied.

**Discussion**

During pandemics, molecular diagnostics are crucial to obtaining accurate and timely data to influence public health policy decisions in real time(10). However, mounting demand for testing has caused a depletion of the viral transport media needed to perform SARS-CoV-2 pCR testing (11). Thus, in the midst of the SARS-CoV-2 pandemic, the FDA has allowed laboratories to consider testing alternative
Our experiments using clinical samples demonstrate the efficacy of PBS as a transport medium and its applicability to clinically relevant conditions, such as overnight storage at room temperature. First, we determined that SARS-CoV-2 qPCR detection with PBS as a transport medium has high intra-patient reliability. Next, using PBS for transport, we demonstrated strong inter-patient reliability of SARS-CoV-2 qPCR. We also found strong correlation of Ct values from specimens transported in either PBS or VTM across multiple subjects with unknown viral loads. These results establish PBS as a dependable transport medium for use with clinical samples. Our data are consistent with the recent demonstration that PBS is equivalent to VTM when each medium is spiked with known quantities of SARS-CoV-2 (12).

With little decay in signal over storage times up to 18h, PBS also has utility for laboratories that test for several SARS-Cov-2 genes that have different specimen processing times. Testing can focus on any or all of the four SARS-CoV-2 structural proteins, including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins, or on any of their protein domains (13). That results for all three viral genes tested were strongly correlated across samples from multiple subjects, support the robustness of the entire testing pathway, including transport. Furthermore, delays in getting samples to the testing lab often occur in busy clinical settings (11). As such, our findings that PBS acts as a stable storage medium with lack of significant viral decay for up to 18 h at room temperature prior to qPCR is advantageous.

Our study is limited in that we used tracheal secretions from mechanically ventilated patients, and we do not know the extent to which our results can be extended to NP, OP swabs and/or saliva testing. Given the severity of illness in our subjects, they may have had higher viral loads than patients with milder disease in whom increased sensitivity of detection may be needed. However, the stability of the
signal, with minimal changes in intensity for 18 hours, indicates the robustness of the methodology. The stability of SARS-CoV-2 in the environment (5), which contributes to its widespread dissemination, may diminish the need for rapid transport of clinical specimens.

The extent to which clinical laboratories can respond to the COVID-19 pandemic is tied to the ability to develop and deploy proper diagnostic procedures (10). Early SARS-CoV-2 detection allows prompt treatment of infected patients and rapid implementation of control measures to limit viral transmission (14). Expanded testing capacity would also facilitate more widespread surveillance and containment of infectious transmission in communities, which could support policies to relax restrictions in work, travel, and social distancing. Our study establishes PBS as a clinically useful transport medium with the potential to increase viral detection capacity, thus improving clinical care and surveillance efforts.

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References.

1. Phelan AL, Katz R, Gostin LO. The Novel Coronavirus Originating in Wuhan, China: Challenges for Global Health Governance. JAMA, 2020. [Epub ahead of print]
   https://doi.org/10.1001/jama.2020.1097

2. Gralinski LE, Menachery VD. Return of the Coronavirus: 2019-nCoV. Viruses, 2020, 12 pii: E135.
   [Epub] doi: 10.3390/v12020135.

3. To KK-W, Tsang OT-Y, Chik-Yan Yip C, Chan K-H, Wu T-C, Chan JMC, Leung W-S, Chik TS-H, Choi CY-C, Kandamby DH, Lung DC, Tam AR, Poon RW-S, Fung AY-F, Hung IF-N, Cheng VC-C, Chan JF-W, Yuen K-Y. Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis, 2020. [Epub]
   https://doi.org/10.1093/cid/ciaa149

4. Wölfel R, Corman VM, Guggemos W, Selmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C. Virological assessment of hospitalized patients with COVID-2019. Nature, 2020. [Epub ahead of print] https://doi.org/10.1038/s41586-020-2196-x

5. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med, 2020, 382:1564–7

6. Leland D. Concepts of clinical diagnostic virology. Edited by Lennette EH. 2nd Edition. New York, NY, Marcel Dekker Inc, 1992

7. Johnson FB. Transport of viral specimens. Clin Microbiol Rev, 1990, 3:120–31
8. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, & National Institutes of Health. Biosafety in microbiological and biomedical laboratories (5th ed.). Edited by Chosewood LC and Wilson DE. 5th Edition. Washington, DC, U.S. Government Printing Office, 2007

9. R Core Team (2019) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

10. Nowak JA, Kaul KL. The role of community molecular diagnostics laboratories in the H1N1 pandemic. J Mol Diagn, 2009, 11:369–70

11. Babiker A, Myers CW, Hill CE, Guarner J. SARS-CoV-2 Testing. Am J Clin Pathol, 2020. [Epub ahead of print] https://doi.org/10.1093/ajcp/aqaa052

12. Rodino KG, Espy MJ, Buckwalter SP, Walchak RC, Germer JJ, Fernholz E, Boerger A, Schuetz AN, Yao JD, Binnicker MJ. Evaluation of saline, phosphate buffered saline and minimum essential medium as potential alternatives to viral transport media for SARS-CoV-2 testing. J Clin Microbiol, 2020. https://doi.org/10.1128/JCM.00590-20

13. Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, Zhou Y, Du L. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol, 2020. Epub: https://doi.org/10.1038/s41423-020-0400-4

14. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, Chen H, Mubareka S, Gubbay JB, Chan WCW. Diagnosing COVID-19: The Disease and Tools for Detection. ACS Nano, 2020. 14: 3822–3835
Figure Legends

**Figure 1. Ct counts from replicate samples from two individuals.** Black/gray points are from subject 1 and Dark blue/light blue are from subject 2. Dark points and light points indicate the two types of transport media, PBS and VTM, respectively. The horizontal axis, Comparative pair, refers to replicate samples (i.e. same person, same length of storage time).

**Figure 2. Comparison of Ct values in RT-qPCR assays for three SARS-CoV-2 genes for samples transported in VTM or PBS.** A total of 26 samples that were transported in the two media are shown; panels are for the N (A), ORF1ab (B), or S (C). The correlations are 0.93 for the N, 0.83 for ORF1ab, and 0.91 for the S, and all are significant (p<0.05).
Results from repeated identical sets from same subject

Results from repeated identical sets from same subject

Results from repeated identical sets from same subject

Subject #, Media
- 1, PBS
- 1, VTM
- 2, PBS
- 2, VTM

N gene Ct value

ORF1ab Ct value

S gene Ct value

Comparative Pair

Comparative Pair

Comparative Pair
