

RESEARCH ARTICLE

Validation of saline, PBS and a locally produced VTM at varying storage conditions to detect the SARS-CoV-2 virus by qRT-PCR

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Abstract

Coronavirus Disease-2019 tests require a Nasopharyngeal (NP) and/or Oropharyngeal (OP) specimen from the upper airway, from which virus RNA is extracted and detected through quantitative reverse transcription-Polymerase Chain Reaction (qRT-PCR). The viability of the virus is maintained after collection by storing the NP/OP swabs in Viral Transport Media (VTM). We evaluated the performance of four transport media: locally manufactured (“REVITAL”) Viral Transport Media (RVTM), Standard Universal Transport Media (SUTM), PBS and 0.9% (w/v) NaCl (normal saline). We used laboratory cultured virus to evaluate: i) viral recovery and maintaining integrity at different time periods and temperatures; ii) stability in yielding detectable RNA consistently for all time points and conditions; and iii) their overall accuracy. Four vials of SARS-CoV-2 cultured virus (2 high and 2 low concentration samples) and 1 negative control sample were prepared for each media type (SUTM, RVTM, PBS and normal saline) and stored at the following temperatures, -80°C, 4°C, 25°C and 37°C for 7 days. Viral RNA extractions and qRT-PCR were performed at 1, 2, 3, 4 and 7 days after inoculation with the cultured virus to assess virus stability and viral recovery. Ct values fell over time at 25°C and 37°C, but normal saline, PBS, RVTM and SUTM all showed comparable performance in maintaining virus integrity and stability allowing for the detection of SARS-CoV-2 RNA. Overall, this study demonstrated that normal saline, PBS and the locally manufactured VTM can be used for COVID-19 sample collection and testing, thus expanding the range of SARS-CoV-2 viral collection media.

Introduction

During the initial stages of the Coronavirus Disease-2019 (COVID-19) pandemic, caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), public health

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authorities across the globe resorted to mass testing as a strategy to contain the fast-spreading disease [1]. A standard COVID-19 test requires a Nasopharyngeal (NP) and/or Oropharyngeal (OP) specimen from the upper airway, from which virus RNA is extracted and detected through quantitative reverse transcription-Polymerase Chain reaction (qRT-PCR) [2, 3]. Additionally, the accuracy of a test result is dependent on the integrity of the pre-analytical step, which involves sample collection, storage, transport, and maintenance in cold chain. The viability of the virus is maintained after collection by storing the NP/OP swabs, in Viral Transport Media (VTM), and cold chain shipment to the laboratory testing station. Several types of VTM exist and have been used to transport samples for the diagnosis of other infectious agents such the H1N1 strain of Influenza A, Herpes simplex and adenovirus [4]. For COVID-19 diagnosis, the United States Centre for Disease Control gave recommendations for the components of an appropriate VTM. These include a Sterile Hanks Balanced Salt Solution (HBSS), heat-inactivated Fetal Bovine Serum (FBS) and a suitable antibiotic or anti-fungal agent [5]. However, the magnitude of the pandemic necessitated an increase in the testing capacity of diagnostic laboratories, leading to marked disruptions in the supply chain of reagents, including VTM [6].

Different VTM are therefore currently available commercially, including DNA/RNA Shield™, the IMPROVIRAL™ NAT medium, Minimum Essential Medium (MEM), 1X Phosphate-buffered Saline (PBS) and normal saline (0.9% (w/v) NaCl). Although PBS and normal saline are considered non-conventional transport media and may not be optimal for the storage of NP/OP samples [6], they have been shown to perform well as alternatives to the commercially available mainstream transport media [7, 8]. For instance, Penrod et al. (2021) evaluated five transport media (VTM (containing 29.5g/l tryptose phosphate, 5g/l gelatin, 10,000U/l penicillin, 10,000U/l streptomycin, 25µg/l Fungizone), Copan Universal Transport Medium™, Becton Dickinson Liquid Amies Elution Swab (Eswab) Collection/Transport System, Remel Microtest™ M4RT® Multi-Microbe Media, and sterile 0.9% (w/v) sodium chloride) and reported that they all had equivalent sensitivity/specificity in COVID-19 diagnosis. Similar findings were obtained by several other studies which confirmed that PBS and normal saline not only maintained the stability and integrity of viral RNA (for up to 1 month when stored at -80°C and 7 days at 4°C) but also provided results that matched those obtained from other media [3, 7, 9, 10]. On the contrary, a different study demonstrated conflicting results of viral samples in 0.9% (w/v) saline not performing as well as commercially available transport media over a 72-hour storage period, at room temperature. Although, comparisons in this study were of samples during stock outs of VTM with those where VTM was available, rather than for split or standardized samples [11]. Furthermore, a recent report showed that PBS was less sensitive compared to other media such as DNA/RNA shield™, highlighting the need for further research to ascertain the stability of this alternative transport medium [3].

Kenya, like several countries during the COVID-19 pandemic, suffered from disruptions in the supply of reagents required for diagnosis. It was therefore imperative to seek alternatives that would cushion diagnostic centres from unforeseen shortages. Consequently, the present study evaluated the performance of four transport media, i.e., a locally manufactured (i.e. "REVITAL") Viral Transport Media (RVTM), Standard Universal Transport Media (SUTM), PBS and 0.9% (w/v) NaCl as plausible options for the transportation and storage of NP/OP samples. We used a laboratory cultured virus to evaluate: i) viral recovery and maintaining integrity at different time periods and temperatures; ii) stability in yielding detectable RNA consistently for all time points and conditions; and iii) their overall accuracy. These were all assessed against the Standard Universal Transport Media (SUTM).

Methods

Virus inoculation

Cultured SARS-CoV-2 was used to prepare virus stocks in minimum essential medium (MEM 2%) (Gibco, Thermo Fisher Scientific) containing 2% fetal bovine serum (FBS) (Gibco, United States). We first generated a 10-fold dilution series (viral dilutions 1 to 10, [S1 Table](#)) that were used to identify the high concentration (virus dilution 3) cycle threshold (Ct) value of 19 and low concentration (virus dilution 6) Ct of 30 (approximately 1,000,000 to 1,000 nucleic acid copies per μl , respectively). We inoculated the high and low concentration viral dilutions in standard universal transport medium (SUTM, Copan Diagnostics, Italy), a locally produced viral transport medium (REVITAL Healthcare, Kenya) (RVTM), 1X Phosphate Buffered Saline (PBS) (OXOID, Hampshire, England) and 0.9% (w/v) sodium chloride (NaCl) (SIGMA). Five vials (2 high concentration SARS-CoV-2 positive samples, 2 low concentration SARS-CoV-2 positive samples and 1 negative control [no-viral inoculum sample]) were prepared for each media type and stored at the following temperatures, -80°C , 4°C , room temperature (25°C) and 37°C for 7 days ([Fig 1](#)). Viral Ribonucleic acid (RNA) extractions and qRT-PCR were done on the following days after inoculation with the cultured virus, days 1, 2, 3, 4 and 7 to assess virus stability and viral recovery, generating a total of 80 vials per viral concentration and negative control. All in-house laboratory prepared buffers and media were filtered and autoclaved before use.

RNA extraction

Viral RNA was extracted from serially diluted and the 4 different media SARS-CoV-2 samples using the QIAamp Viral RNA Mini Kit (QIAGEN GmbH, Hilden, Germany). Briefly, a $140\mu\text{l}$ sample was mixed with $280\mu\text{l}$ of viral lysis buffer and $2.8\mu\text{l}$ of carrier RNA. Thereafter, $280\mu\text{l}$ of

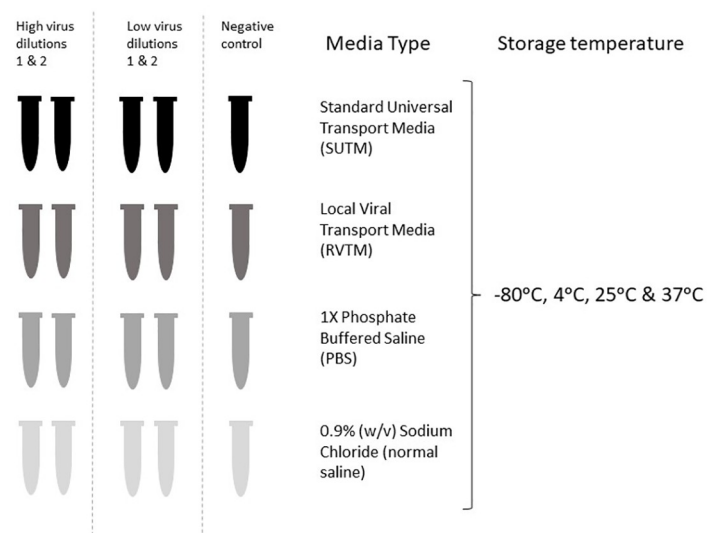


Fig 1. An illustration showing virus dilutions in different media types and different virus inoculation temperatures. Each media type was made up of 2 tubes inoculated with high virus concentrations, 2 tubes of low virus concentrations and 1 negative control (no viral inoculum) tube, generating a total of 5 tubes stored at the following temperatures: -80°C , 4°C , 25°C (room temperature) and 37°C and subsequently used to extract RNA for qRT-PCR on days 1, 2, 3, 4 and 7. Thus, each media type consisted of a total of 80 tubes, that were subsequently extracted for RNA to conduct the qRT-PCR.

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absolute ethanol was added and the contents were briefly vortexed before transferring into QIAamp Mini Spin Columns. The spin columns were spun at 6,000 x g for 1 minute, the elute was discarded and 500µl of buffer AW1 was added followed by centrifugation at 6,000 x g for 1 minute. The flowthrough was discarded and 500µl of buffer AW2 was added followed by centrifugation at 16,000 x g for 3 minutes. The elute was discarded and the spin columns allowed to air dry for 5 minutes before elution with 60µl buffer AVE.

Quantitative reverse transcription PCR assays

For the qRT-PCR assay, the European Virus Archive–GLOBAL (EVAg) primer/probe set containing 1.75µl primer probe mix, 3.75µl Nuclease free water, 2.5µl TaqMan™ Fast Virus master mix and 2.5µl of template RNA was used [12]. Cycling conditions were 50°C for 5min, 95°C for 20sec then 40 cycles of 95°C for 3sec and 58°C for 45sec on the QuantStudio 5 and 7 qRT-PCR systems (Applied Biosystems, CA). The negative sample (the no viral inoculum sample, 80 vials) and non-template control (NTC) samples (no RNA template was included in 15 samples across the ten PCR plates ran across the five days of sampling) were included in each run. All samples were done in duplicates.

Ct values obtained for each sample were analyzed to assess the impact of the four media types and storage condition on Ct values. A test was considered successful if the negative and NTC samples did not have Ct values. Furthermore, data obtained from the PCR analyser were in Ct values. These were reviewed and interpreted using a Ct cut-off of 36, as determined by Mohammed et al. (2020), interpreting those values of less than and greater than the cut-off as positive and negative test results, respectively [12]. The qRT-PCR machine also gave an ‘undetermined’ result among the Ct values, and these were interpreted as negative test results.

Statistical analysis

All analyses were performed using Stata version 15 (Stata Corp., College Station, TX). Graphical presentations were produced in R version 3.6.1 [13]. First, we note that there were 128 negative qRT-PCR samples defined as “undetermined” that were replaced with an arbitrary high Ct for negative (i.e., 37) to uncover the bias that might occur due to their exclusion during statistical analysis. Temporal (in days) and temperature trends on viral detection across the four media types were assessed. A Spearman’s correlation coefficient was obtained between the SUTM and the other 3 media types and a line of best fit was estimated using a Deming regression model [14]. Accuracy of the media types was determined by estimating the positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) [15]. Finally, Ct values were compared using a multiple linear regression model with main effects for media type, temperature, concentration (low/high/negative control), day and the interactions for media type and day, and temperature and day as covariates. Overall effects were tested using the Wald test. All tests were performed at a 5% significance level.

Results

qRT-PCR

In total, 811 qRT-PCR assays were conducted for all 4 media types at all 4 temperatures over a 7-day period. A total of 15 non-template controls were incorporated for all the run days and these yielded negative results as expected. However, there were 16 (<2%) negative control (no inoculum) samples from the total of 80 negative controls run in duplicate qRT-PCRs that had

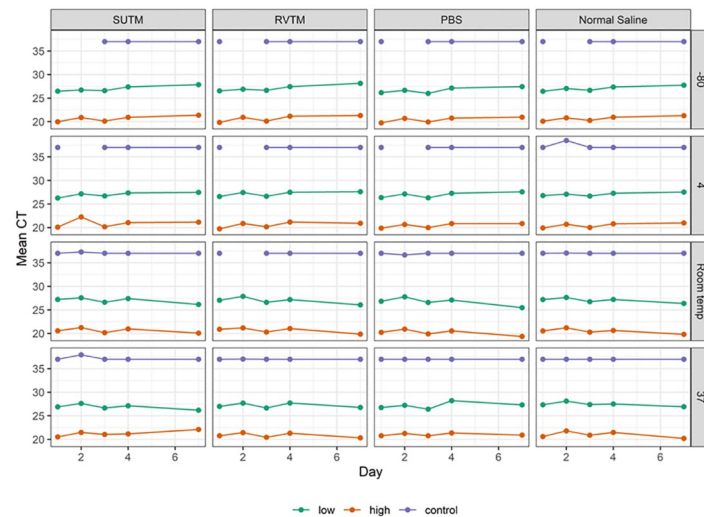


Fig 2. Mean Ct values for the four media types over time (in days) at -80°C , 4°C , room temperature (25°C) and 37°C stratified by concentration. A $\text{Ct} > 36$ or 37 indicates a negative result. The gaps in temperatures -80°C and 4°C indicate the negative samples, these were samples with $\text{Ct} < 36$, which were excluded from the analyses. Each data point is the mean of 4 samples, the 2 high and 2 low concentration samples assayed in duplicate and the negative control sample data points are the mean of the duplicates.

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a $\text{Ct} < 36$, this indicates a contamination rate of the COVID testing process from sample storage to qRT-PCR amplicon generation in a lab routinely conducting testing. Furthermore, on a retrospective examination of the data, most (14/16) of the negative control samples were from day 2, highlighting the day-to-day and plate-to-plate variation in the qRT-PCR. These were considered as contaminated and hence excluded from any further analysis. They were primarily observed at -80°C and 4°C across all media types (S2 Table and Fig 2). Of the remaining 780 samples there were 194 (24.9%) for SUTM, 195 (25%) for PBS, 193 (24.7%) for RVTM, and 198 (25.4%) samples for normal saline. Of these, 640 (82%) gave positive test results while 140 (18%) gave negative test results as expected.

Comparisons between the four media types

For all temperature levels, media types and days, the Ct values were consistently highest in the low concentration samples (between Cts 25 and 28) and they were significantly higher than those for high concentration samples (between Ct 18 and 23) (Tables 1 and 2, Fig 2). The Ct values varied over time in the four temperature levels examined (Table 2). Ct values generally increased over time (day) in the media stored at -80°C and 4°C , decreased and remained the same at room temperature and 37°C , respectively (Fig 2). The multiple linear regression analysis demonstrated there were no statistically significant differences in Ct values across the media types (Table 2; Fig 1). The correlations of Ct values between the 4 media types were linear indicating a similarity in the media types in preserving the virus for testing at both low and high viral concentrations (Fig 3A and 3B). The overall accuracy of RVTM, PBS and saline compared to SUTM was 100% (Table 3).

Discussion

Normal saline, PBS and the locally manufactured VTM showed comparable performance in maintaining virus integrity and stability allowing for the detection of SARS-CoV-2 RNA. All

Table 1. Mean Ct values for the high and low concentrations and negative controls.

	Concentration		Negative controls N = 140
	Low N = 320	High N = 320	
Media			
SUTM	26.97 (0.53)	20.86 (0.91)	37.07 (0.35)
RVTM	27.10 (0.57)	20.68 (0.57)	37.02 (0.10)
PBS	26.89 (0.68)	20.51 (0.64)	36.98 (0.12)
Normal Saline	27.15 (0.51)	20.66 (0.54)	37.08 (0.35)
Temperature			
-80	26.97 (0.60)	20.61 (0.55)	37.00 (0.00)
4	27.04 (0.47)	20.60 (0.69)	37.08 (0.37)
Room temperature	26.93 (0.63)	20.49 (0.54)	37.02 (0.21)
37	27.17 (0.60)	21.02 (0.82)	37.05 (0.29)
Concentration			
Low	27.03 (0.58)		
High		20.68 (0.69)	
Control			37.04 (0.26)
Days			
1	26.74 (0.41)	20.26 (0.47)	37.00 (0.00)
2	27.35 (0.46)	21.14 (0.57)	37.30 (0.69)
3	26.62 (0.33)	20.28 (0.41)	37.00 (0.00)
4	27.38 (0.32)	21.00 (0.36)	37.00 (0.00)
7	27.04 (0.80)	20.71 (0.94)	37.00 (0.00)

The data are means (standard deviations), the contaminated negative controls are not included in this table.

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the media types supported the recovery of SARS-CoV-2 to as low as 1000 viral nucleic acid copies per μl . Furthermore, even at temperatures above 25°C virus was detected over the 7-day sampling frame.

There were variations in Ct values based on temperature storage conditions and day of testing, notably in the samples stored at -80°C and 4°C, which could suggest a reduction in viral load over time. Similar findings have been reported previously, including in other respiratory viruses such as Herpes simplex, Influenza, enterovirus and adenovirus [4, 10]. Viral recovery appeared more stable at 37°C than at room temperature. Additionally, normal saline appeared to have lower Ct values post-day 4 at 37°C compared to SUTM. A previous study described better sensitivity to detect Ct values in samples in saline across 4, 25 and 37°C, low Ct values were also detected in samples stored at 37°C [16]. The reliability of Ct results on an assessment of the media types revealed consistency at high RNA concentrations. This is expected, since at low viral loads there is an increased signal to noise ratio as the limit of detection of the qRT-PCR test is reached and taking into consideration the variability between qRT-PCR kits.

There were some contaminated negative control samples (Ct<36) in <2% of the samples examined. The contamination may have occurred during extraction or the compilation of qRT-PCR reagents [17] due to amplicon carry-over given the high number of SARS-CoV-2 amplicons being generated in the lab. It may also be a reflection of the high signal-to-noise ratio at high Ct values. We had mitigated against contamination by incorporating proper disinfection and use of dedicated working spaces ensuring minimal viral contamination [17].

Table 2. Multiple linear regression analysis to compare the differences between the media.

Variable	Estimate (SE [†])	p value
Media		
SUTM	Ref.	
RVTM	0.04 (0.10)	0.683
PBS	-0.18 (0.10)	0.075
Normal Saline	0.13 (0.10)	0.192
Temperature		
-80	Ref.	
4	0.27 (0.1)	0.007
Room temperature	1.05 (0.1)	<0.001
37	0.95 (0.1)	<0.001
Concentration		
Low	Ref.	
High	-6.35 (0.04)	<0.001
Negative controls	9.98 (0.05)	<0.001
Day	0.19 (0.02)	<0.001
Day x Media		
Day x SUTM	Ref.	
Day x RVTM	-0.02 (0.02)	0.443
Day x PBS	-0.004 (0.02)	0.883
Day x Normal Saline	-0.04 (0.02)	0.121
Day x Temperature		
Day x -80	Ref.	
Day x 4	-0.06 (0.02)	0.010
Day x Room temperature	-0.32 (0.02)	<0.001
Day x 37	-0.20 (0.02)	<0.001

Adjusted R² = 0.99;

[†]SE stands for Standard error.

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These results based on laboratory cultured samples stored at a range of temperatures suggest that samples swabbed directly from patients could be transported in the different media types examined in this study, given the comparable ability to detect the viral RNA. Furthermore, the data suggest that storage at 25°C or 37°C, over 7 days can still yield detectable viral RNA, potentially limiting the need for transportation of clinical samples in cold chain in settings where this could be a challenge. However, we did not test the viability of clinical samples under these test conditions and considering the variability in clinical viral concentrations, sample collection and handling procedures, and whether the different media types would support downstream culturing of clinical isolates, cold chain remains the recommended method for sample shipment and storage.

The cultured virus we used may have been at a higher concentration than samples taken in the field, and storage conditions during transport may be more variable. However, we did not observe evidence of any differences in RNA levels based on the PCR results by VTM, including when the virus was stored at room temperature.

Overall, this study has demonstrated that normal saline, PBS and the locally manufactured VTM can be used for COVID-19 sample collection and testing, thus expanding the range of

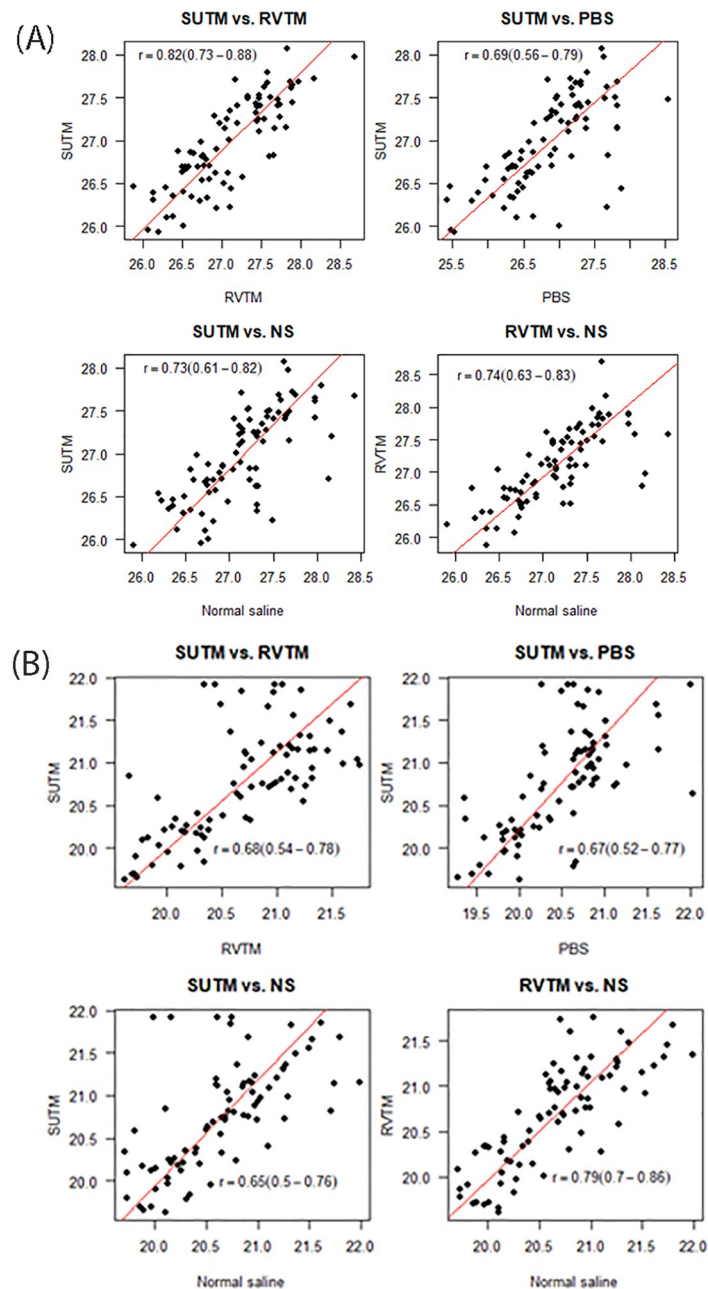


Fig 3. Correlations between Ct values from the 4 media types. (A) Scatter plots for Ct values at low concentration. (B) Scatter plots for Ct values at high concentration. The line of best fit was estimated using a Deming regression model.

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SARS-CoV-2 viral collection media. However, these results are based on a laboratory cultured virus, and further research using field isolates is required to validate the findings. It is recommended that clinical samples be stored at 4°C or frozen if they are not tested within 24 hours of sample reception.

Table 3. Overall agreement between RVTM and SUTM for all time points and conditions*.

RVTM (Comparator)	SUTM (Reference)		PPA	NPA	OPA
	Positive	Negative			
Positive	160	0			
Negative	0	33			
Total	160	33	100 (97.6–100)	100 (89.6–100)	100 (98–100)
PBS (Comparator)					
Positive	160	0			
Negative	0	34			
Total	160	34	100 (97.7–100)	100 (89.9–100)	100 (98.1–100)
Saline (Comparator)					
Positive	160	0			
Negative	0	35			
Total	160	35	100 (97.7–100)	100 (90.1–100)	100 (98.1–100)

PPA = Positive percent agreement; NPA = Negative percent agreement; OPA = Overall percent agreement.

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Supporting information

S1 Table. SARS-CoV-2 cultured virus dilution series and corresponding Ct values. Virus dilution 3 was used for high concentration while virus dilution 6 for low concentration. (DOCX)

S2 Table. Numbers of negative samples with a Ct<36 for each media type and at each temperature tested. (DOCX)

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Methodology: Caroline Ngetsa.

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Supervision: Lynette Isabella Ochola-Oyier.

Validation: Victor Osoti, Faith Marura.

Visualization: Benedict Orindi.

Writing – original draft: Caroline Ngetsa, Victor Osoti, Dorcas Okanda, Benedict Orindi.

Writing – review & editing: Philip Bejon, Lynette Isabella Ochola-Oyier.

References

1. Shen M, Xiao Y, Zhuang G, Li Y, Zhang L. Mass testing—An underexplored strategy for COVID-19 control. *The Innovation*. 2021 May 28; 2(2):100114. <https://doi.org/10.1016/j.xinn.2021.100114> PMID: 33942035
2. Kevadiya BD, Machhi J, Herskovitz J, Oleynikov MD, Blomberg WR, Bajwa N, et al. Diagnostics for SARS-CoV-2 infections. *Nature Materials*. 2021; 20(5):593–605. <https://doi.org/10.1038/s41563-020-00906-z> PMID: 33589798
3. Barrera-Avalos C, Luraschi R, Vallejos-Vidal E, Figueroa M, Arenillas E, Barría D, et al. Analysis by real-time PCR of five transport and conservation mediums of nasopharyngeal swab samples to COVID-19 diagnosis in Santiago of Chile. *J Med Virol*. 2022 Mar 1; 94(3):1167–74. <https://doi.org/10.1002/jmv.27446> PMID: 34755352
4. Druce J, Garcia K, Tran T, Papadakis G, Birch C. Evaluation of swabs, transport media, and specimen transport conditions for optimal detection of viruses by PCR. *J Clin Microbiology*. 2012 Mar; 50(3):1064–5. <https://doi.org/10.1128/JCM.06551-11> PMID: 22205810
5. CDC. Centers for Disease Control and Prevention. Preparation of viral transport medium [Internet]. 2020 [cited 2022 May 10]. <https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf>
6. Penrod Y, Garcia D, Dunn ST. Evaluation of transport media for laboratory detection of SARS-CoV-2 in upper respiratory tract swab specimens. *J Med Virol*. 2021 May 1; 93(5):2774–81. <https://doi.org/10.1002/jmv.26643> PMID: 33128389
7. Rodino KG, Espy MJ, Buckwalter SP, Walchak RC, Germer JJ, Fernholz E, et al. 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; 58(6):1–2. <https://doi.org/10.1128/JCM.00590-20> PMID: 32229604
8. Borkakoty B, Jakharia A, Bali N, Sarmah M, Hazarika R, Baruah G, et al. A preliminary evaluation of normal saline as an alternative to viral transport medium for COVID-19 diagnosis. *Indian Journal of Medical Research*. 2021; 153(5):684–8. https://doi.org/10.4103/ijmr.IJMR_4346_20 PMID: 34643568
9. Rogers AA, Baumann RE, Borillo GA, Kagan RM, Batterman HJ, Galdzicka MM, et al. Evaluation of transport media and specimen transport conditions for the detection of SARS-CoV-2 by Use of real-time reverse transcription-PCR. *J Clin Microbiol*. 2020 Jul 23; 58(8):1–5. <https://doi.org/10.1128/JCM.00708-20> PMID: 32341141
10. Perchetti GA, Huang M-L, Peddu V, Jerome KR, Greninger AL. Stability of SARS-CoV-2 in phosphate-buffered saline for molecular detection. *J Clin Microbiol*. 2020 Aug; 58(8):1–2. <https://doi.org/10.1128/JCM.01094-20> PMID: 32414839
11. Garnett L, Bello A, Tran KN, Audet J, Leung A, Schiffman Z, et al. Comparison analysis of different swabs and transport mediums suitable for SARS-CoV-2 testing following shortages. *J Virol Methods*. 2020 Nov 1; 285: 113947. <https://doi.org/10.1016/j.jviromet.2020.113947> PMID: 32781008
12. Mohammed KS, de Laurent ZR, Omuoyo DO, Lewa C, Gicheru E, Cheruiyot R, et al. An optimisation of four SARS-CoV-2 qRT-PCR assays in a Kenyan laboratory to support the national COVID-19 rapid response teams. *Wellcome Open Res*. 2020 Jul 7; 5:162.
13. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [Internet]. 2019 [cited 2022 Jul 21]. <https://www.R-project.org/>
14. Konings H. Use of deming regression in method-Comparison studies. *Survey of Immunologic Research*. 1982 Dec; 1:371–4. <https://doi.org/10.1007/BF02918550> PMID: 6764850
15. Clinical and Laboratory Standards Institute. User protocol for evaluation of qualitative test performance, 2nd Edition [Internet]. 2008 [cited 2022 Jul 21]. <https://clsi.org/standards/products/method-evaluation/documents/ep12/>
16. Sinha P, Jain DK, Gupta S, Gupta M, Gupta M, Agarwal A, et al. Evaluation of saline as potential alternative to viral transport media for COVID-19 samples stored at different temperatures. *Journal of clinical and diagnostic research*. 2021; 15(4):19–21.
17. Borst A, Box ATA, Fluit AC. False-positive results and contamination in nucleic acid amplification assays: Suggestions for a prevent and destroy strategy. *European Journal of Clinical Microbiology and Infectious Diseases*. 2004; 23(4):289–99. <https://doi.org/10.1007/s10096-004-1100-1> PMID: 15015033