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SHORT REPORT

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Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is influenced by the type of transport medium: Implications for diagnosis and monitoring

Nikhil Patkar | Sweta Rajpal | Nitin Shetty | Anant Gokarn | Sumeet Mirgh | Shesheer Munipally | Sudhir Nair | Amit Joshi | Vedang Murthy | Navin Khattry | Sudeep Gupta

ACTREC, Navi Mumbai, India

Correspondence
Navin Khattry, Medical Oncology, Advanced Centre for Treatment Research and Education in Cancer, Tata Memorial Centre, Kharghar, Maharashtra, Pin: 410210, India.
Email: nkhattry@gmail.com

Abstract

It is unclear if the use of a molecular transport medium (MTM) containing guanidine isothiocyanate (GITC) would be advantageous over the CDC recommended, commonly used viral transport medium (VTM). We retested 70 SARS-CoV2 cases by RT-PCR in varying stages of follow-up using MTM and VTM in parallel and found discrepant results of *RNase P*, *E* and *N* genes. Majority (81%) patients tested positive with MTM as compared with VTM (27.1%). Even patients who were sampled 3 weeks after diagnosis demonstrated a significant discrepancy in the positivity rates between MTM vs VTM raising concerns about the clinical utility of VTM.

1 | INTRODUCTION

A novel RNA betacoronavirus outbreak was reported in late last year from Wuhan, China in late 2019 called SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2).[1] This virus results in a disease called coronavirus disease 2019 (COVID-19) characterised by a wide spectrum of disease symptoms including pneumonia and multisystem damage.[2] At the time of writing this manuscript more than 105 million individuals have been affected globally with more than 2 284 000 deaths. Majority of the patients (~80%) have mild flu such as illness. Severe life-threatening disease (respiratory failure, septic shock, and/or multiple organ failure) is seen in a smaller percentage of patients.[1]

Several assays are available for the diagnosis of SARS-CoV2 such as serological and nucleic acid amplification-based tests. However, the diagnosis of SARS-CoV2 infection largely relies upon real-time PCR (RT-PCR)-based assays which target different regions of the SARS-CoV2 genome such as helicase (Hel), nucleocapsid (N), transmembrane (M), envelope (E) and envelope glycoproteins spike (S) and RNA-dependent RNA polymerase (RdRp) genes. Diagnostic or monitoring samples are obtained from sputum or oropharyngeal and/or nasopharyngeal swabs (OP + NP) or in rare cases, bronchoalveolar lavage and shipped to the lab in a viral transport medium (VTM).[3-5] It is unclear if the use of a molecular transport medium (MTM) containing guanidine isothiocyanate (GITC), a chaotropic RNA stabilising agent, would be advantageous over the CDC recommended, commonly used VTM, that incorporates Hanks Balanced Salt Solution.[6] In this article, we report that there is poor preservation of SARS-CoV-2 in the commonly used VTM when compared with parallel samples collected in MTM.

2 | METHODS

2.1 | Patients

We selected seventy consecutive patients who were previously confirmed to have SARS-CoV2 infection diagnosed by RT-PCR and at varying stages of follow-up after the initial diagnosis was made (interquartile range [IQR] 19.3-30 days, median 24 days).

2.2 | Sample collection and transport

We retested these patients by sampling OP + NP regions. Samples were simultaneously collected in a viral transport medium, (VTM,

HiViral Transport Kit, HiMedia Laboratories, Nashik, India) and a molecular transport medium (MTM, Huwel Lifesciences, Hyderabad, India) for all patients at the time of follow-up. These samples were transported to the laboratory at 4°C within 2 hours.

2.3 | Ethical consideration

The study was approved by the Institute Ethical Committee (ECR/149/Inst/MH/2013).

2.4 | RNA extraction and RT-PCR

Samples were stored at 4°C within the laboratory. RNA was extracted within 4 hours of receiving the sample using Maxwell Viral Total Nucleic Acid Purification Kit (Promega Inc, Madison, WI, USA) using the manufacturers' recommendations. This RNA was converted to cDNA and multiplexed RT-PCR for *E*, *N* and *RNase P* genes was performed using an US FDA approved assay (TRUPCR SARS-CoV-2 RT qPCR kit, 3B BlackBio Biotech, Bhopal, India) in a single procedure as per the manufacturer's recommendations. Cycle threshold (Ct) value >36 was interpreted as negative.

2.5 | Dilution experiment

We serially diluted two positive samples collected in VTM as well as MTM in samples (collected in respective transport media) which were negative for SARS-CoV-2.

2.6 | Statistical methods

A normality test was performed using D'Agostino & Pearson omnibus normality test. Since the data were found to be non-Gaussian in distribution, a Wilcoxon test was used to compare differences in cycle threshold (Ct) values for the three genes assayed (*E*, *N* and *RNase P*) using different transport media. Graphs were generated using GraphPad Prism v6. Chi-Squared test was performed to assess discrepancies in samples collected between the two media.

3 | RESULTS

3.1 | Control gene testing

Of the 70 patient samples accrued, the internal control gene *RNase P* was expressed in all samples irrespective of the transport medium. No sample was rejected based on high Ct values for *RNase P*. However, results were dissimilar for samples collected in different transport media. In general, samples collected in MTM (Ct value interquartile range [IQR]: 21.5-22.6, median Ct: 21.9) showed a lower Ct of *RNase P* when compared with VTM collected samples (Ct IQR—23.6-26.1, median Ct—24.5). Statistical analysis demonstrated a significant difference in Ct levels of *RNase P* between MTM and VTM ($P < .0001$; Figure 1A).

What's known

- An important component of the SARS-CoV-2 real-time polymerase chain reaction (RT-PCR) diagnostic assay is the medium in which swabs are transported to the testing lab, which is typically the viral transport medium (VTM) based on Hanks Solution, as recommended by the Centres for Disease Control.

What's new

- In this study, oropharyngeal and nasopharyngeal swabs for the SARS-COV-2 test were obtained in duplicate from 70 patients and transported to the laboratory in VTM and a molecular transport medium (MTM) containing a chaotropic agent. A significantly higher proportion of samples transported in MTM (81.4%) was positive for SARS-COV-2 compared with those transported in VTM (27.1%).
- This study highlights that the sensitivity of the RT-PCR test for SARS-COV-2 is critically dependent on the sample transport medium and is higher for MTM compared with VTM.

3.2 | RT-PCR for SARS-CoV2-specific genes

A majority (n = 57, 81.4%) of patients tested positive with MTM as compared with samples collected in VTM (n = 19, 27.1%). On pairwise comparison, 19 (27.1%) patients tested positive by both the methods. On closer analysis, a total of 52 patients (74.3%) tested positive for *E* gene using MTM (Ct IQR—26.9-30.1, median 29.17) as compared with 13 patients (18.6%) testing positive using VTM (Ct IQR—28.2-30.7, median 30.33). Similarly, 57 patients (81.43%) were positive for *N* gene using MTM (Ct IQR—28.8-31.8, median 30.78) as compared with 17 patients (24.3%) using VTM (Ct IQR—29.9-34.2, median 31.0; Figure 1B,C).

3.3 | Concordance between transport media at different time points after diagnosis

We grouped patients based on the week of follow-up after the original diagnosis was rendered. These patients were then grouped by *E/N* gene results and transport medium as seen in Figure 1D,E. In 42 patients who were sampled 21 days after an initial diagnosis was made, there was a significant discrepancy in the SARS-COV-2 positive status between MTM (37 positives, 88.1%) and VTM (10 positives, 23.8%, $P < .0001$).

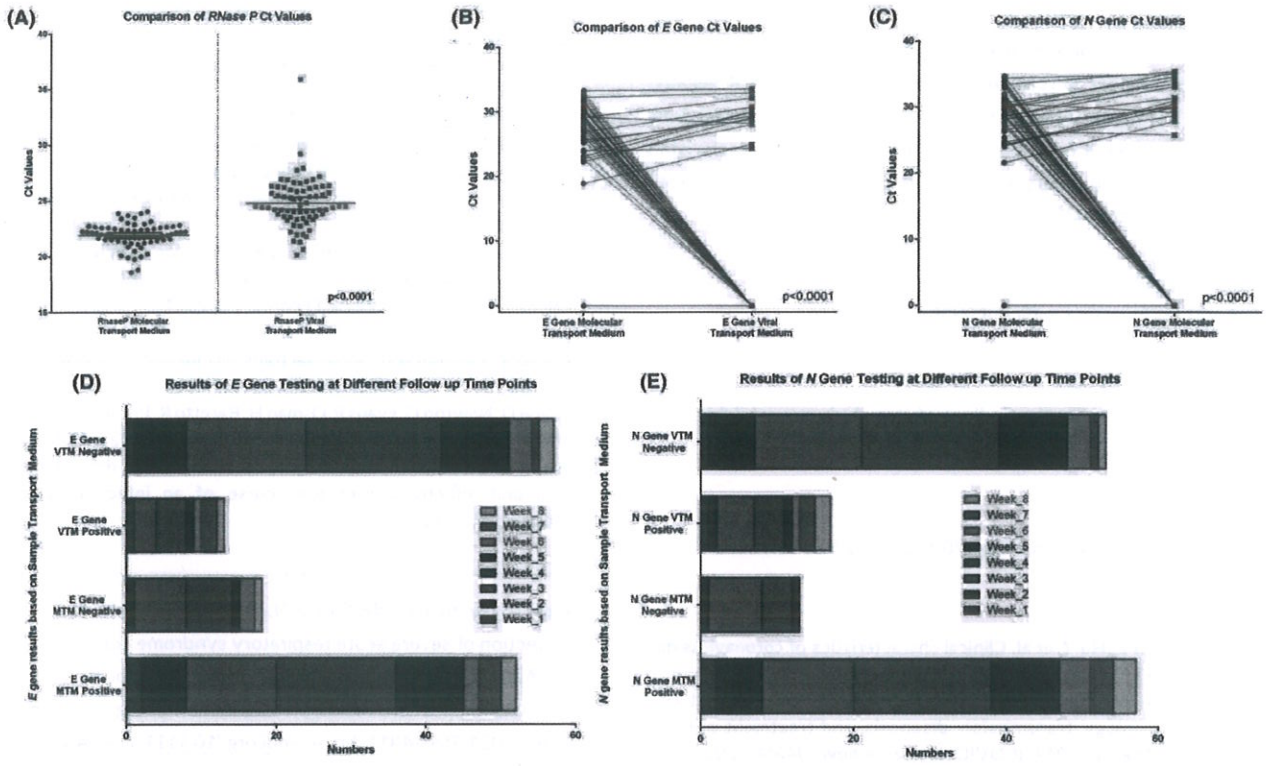

FIGURE 1 Comparison of Ct values for *RNaseP* (A), *E* gene (B), *N* gene (C) between samples collected in MTM and VTM, RT-PCR Results of *E* gene (D) and N gene (E) for patients at different stages of follow-up classified according to the transport medium. The blue lines in (B) and (C) join the corresponding Ct values obtained in MTM and VTM

TABLE 1 Comparison of Ct values of *N* gene and *E* gene on the dilution experiment of known positive patients' samples transported in both MTM and VTM

	MTM		VTM	
	N Gene	E Gene	N Gene	E Gene
Neat	25.88	25.9	26.78	25.69
Dilution 1	29.25	29.26	30.05	28.89
Dilution 2	32.05	31.72	33.07	31.72
Dilution 3	33.78	33.68	35.13	Not detected
Dilution 4	34.49	Not detected	Not detected	Not detected
Dilution 5	Not detected	Not detected	Not detected	Not detected

3.4 | Dilution experiment

Serial dilution of the samples collected in two transport media revealed better sensitivity with MTM with one log difference in the limit of detection for both *N* and *E* genes (Table 1).

4 | DISCUSSION

During the time of this pandemic, molecular diagnostic laboratories all over the world are tested to their limit. These labs are flooded with samples and are finding it difficult to report samples in a meaningful time frame. Any compromise in the cold chain, as well as delay of sample transportation, may lead to RNA degradation. This phenomenon is amplified in resource-constrained countries such as India with poor testing resources and suboptimal transport logistics. Similar analogies related to transportation in resource-constrained countries have been documented by Schlaudecker and colleagues.[7,8]

In addition, companies making the VTM are under pressure to meet high-demand supply chain. While most of the laboratories are using their local authority-approved commercial kits for the diagnosis of SARS-CoV-2, the validation of transport media is usually neglected. We obtained consistently higher Ct values of the control (RNase P) gene in VTM samples as compared with MTM samples. It is possible that the presence of the chaotropic agent in MTM prevented RNA degradation and therefore contributed to better sensitivity. Several studies have indicated that viral loads (measured by

RT-PCR) become negative within 21 days in majority patients after the onset of symptoms (median—14.5 days).[9] Our findings raise concerns with respect to recommendations that rely on PCR negativity from VTM sourced samples to release COVID-19 patients from isolation. Further, it is possible that transport media characteristics could contribute to discrepant results and this needs further investigation in a larger study.

DISCLOSURE

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Nikhil Patkar https://orcid.org/0000-0001-9234-2857

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