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A Study on Requirement of Cold Chain Maintenance for Reliable Testing of SARS-CoV-2 Samples

Microbiology Section

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ABSTRACT

Introduction: Coronavirus Disease 2019 (COVID-19) has been haunting the world since December 2019 and has grown to pandemic proportions from March 2020. Even after a full year of research and study, the most effective way to control the spread of this infection is early diagnosis and isolation of the cases. Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) is considered the standard test all over the world for the diagnosis of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) infection. All the sample collection guidelines have recommended stringent maintenance of the cold chain for the sample transport. However, it is not possible for the resource constrained developing countries with inadequate infrastructure to comply with these guidelines all the time.

Aim: To determine necessity of stringent transport criteria and the effect of temperature on the clinical sensitivity of a RT-PCR assay for diagnosis of SARS-CoV-2 infection.

Materials and Methods: In this prospective experimental study conducted in November 2020, 49 positive samples were kept at ambient room temperature and were tested everyday with RT-PCR for the detection of SARS-CoV-2 Ribonucleic Acid (RNA). The samples were also kept under refrigeration at 4°C and were also tested by RT-PCR and the results were compared with their respective counterparts kept at room temperature till nine days. Python Jupiter notebook SciPy and Anaconda software was used for statistical analysis.

Results: It was observed that the positivity of the RT-PCR results were not deteriorated till five days and there was no significant deterioration even after nine days of samples being stored at room temperature suggesting that even if the viral RNA itself is not stable outside strict temperature control but small fragment or target genetic sequences are enough for detection of virus by RT-PCR.

Conclusion: It is possible to keep samples at this ambient temperature for five days without any loss of positivity in RT-PCR.

Keywords: Ambient temperature, Coronavirus disease 2019, Developing countries, Nucleic acid, Reverse transcription polymerase chain reaction, Viral transport medium

INTRODUCTION

COVID-19 pandemic caused by SARS-CoV-2 virus since its emergence in December 2019 in Wuhan [1], China has taken the world with surprise and has been raging war against humanity with unprecedented ferocity. Total 93,698,260 cases have occurred worldwide till 14 January 2021 [2] which has led to a global catastrophises in financial losses and loss of more than 2 million lives directly due to COVID and unaccounted indirect loss of lives due to post COVID complications. Coronaviruses comprise a unique clade under the subgenus Sarbecovirus [3] and consists of a group of SARS-like bat coronaviruses, including both SARS-CoV-1 and SARS-CoV-2. Earlier epidemics like the Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) had raised awareness regarding timely diagnosis to prevent rapid transmission of such viral infections, but COVID-19 had spread uncontrollably beyond all restrictions. In spite of all understanding we got about this virus in a span of almost one year, best effective tool to control the spread of infection is early diagnosis and isolation of the cases [4]. In such a scenario inability to detect the positive samples may lead to missing out an important source of infection in community undiagnosed. Out of various modalities for diagnosis the most reliable and gold standard method for detection of SARS-CoV-2 viral infection is real-time RT-PCR which is the most sensitive and specific assay [5]. Diagnosis of COVID-19 disease by real-time RT-PCR mainly requires the preservation of RNA instead of replication competent virus. It utilises the amplification of the viral nucleic acid present in the virus from the patient sample. Coronaviruses have a number of molecular targets like *E* (Envelope), *N* (Nucleocapsid), *Orf* (Open reading frame) and *RdRp* (RNA dependent RNA polymerase) gene within their positive-sense, single-stranded RNA genome that can be used for PCR assays [6].

Earlier, some studies have been conducted to understand the effect of temperature on virus viability [7,8], but very little is known about the effect of temperature on the results of RT-PCR for viral RNA detection which does not require viable virus. For screening or early diagnosis of coronavirus infectious disease, a nasopharyngeal or an oropharyngeal swab is the recommended specimen of choice [9,10]. After collection, these swabs are immediately put in Viral Transport Medium (VTM) before sending them to laboratory for testing. All the national [11] and international [12] sample collection and transport guidelines strongly recommend the maintenance of cold chain during transport and storage of samples. The outrageous increase in the number of cases has outreached the capacity of the healthcare system enormously and often it becomes very difficult to comply with these sample collection and storage guidelines especially in resource limited developing countries. Even the developed countries of the world had faced similar problem as none of them had expected and were prepared for such a massive outburst. In such a situation either these samples are rejected or even if tested, it raises a concern about the quality of testing and reliability of results obtained with such samples.

This study was undertaken to observe the effect of ambient temperature on the RT-PCR results of SARS-CoV-2 samples. It can serve as a guide to the degree of confidence with which the samples are exposed to the ambient temperature for a prolonged duration of time and can be proceeded for testing and the results generated be relied upon.

MATERIALS AND METHODS

This study was a prospective experimental study conducted within the month of November 2020, on the samples (nasopharyngeal and oropharyngeal swabs) received in Vitromed Healthcare, India (VTM) for diagnosis of the SARS-CoV-2 viral disease at the COVID-19 testing laboratory, Department of Microbiology, SMS Medical College, Jaipur, Rajasthan, India. As the study was conducted on the remanent samples which were received for the routine testing for COVID-19 infection after the implied consent of the patient and the study had no direct or indirect implication on the patient, so the ethical clearance for the study was not required.

Inclusion criteria

- Samples which were collected within the hospital premises.
- Samples which reached the laboratory within four hours of collection and were processed in one hour of receiving.
- Primary Ct (Cycle threshold) value between 16-34.
- Samples coming in same type of VTM.

Exclusion criteria

- Samples received after four hours of collection.
- Non compliance in maintenance of cold chain.

Sample size calculation: Sample size was calculated at 95% confidence level assuming 50% positivity till nine days while not maintaining cold chain (maximum variance sampling). At the relative allowable error of 25%, minimum 62 specimens were required as sample size. So initially, 62 samples were separated for research work but on continuous daily follow-up 13 samples were discarded due to various reasons like sample volume insufficient, leakage or some inconclusive result, so total 49 samples were analysed and presented in this study.

Study Procedure

All the samples were tested as per the routine laboratory testing protocol and RT-PCR test for SARS-CoV-2 detection was conducted for all the samples. All the samples were opened in a class II biosafety cabinet and followed by initial lysis in the lysis buffer (PerkinElmer, Germany). Then all the samples were further processed for nucleic acid extraction in an automated magnetic bead based nucleic acid extraction system (chemagic 360 by PerkinElmer, Germany). The extracted RNA was tested by RT-PCR with primers-probes directed at SARS Cov-2 *E* gene and *N* gene. It was a triplex PCR assay with Internal Control (IC) added as the amplification control in the master mix (Quantiplus Multiplex COVID-19 Detection Kit V2.0 from Huwel Lifesciences Pvt., Ltd., India). PCR for all the samples was carried out on Biorad CFX 96 platform. Cycling conditions were starting from 53°C for 10 minutes for cDNA synthesis followed by initial denaturation for 15 minutes at 95°C and then repeated cycling at 95°C and 60°C for 15 seconds and 30 seconds respectively for 40 cycles. The cut-off threshold was recorded for all the samples and Ct value of less than 36 was taken as positive as per the manufacturer's protocol. Real-time RT-PCR cycle threshold (Ct) values represent the number of amplification cycles required for the target gene to exceed a threshold level. Ct values are therefore inversely related to viral load and can provide an indirect method of quantifying the copy number of viral RNA in the sample [13]. Samples were reported as positive when both *E* and *N* gene were detected or even *N* gene was detected alone. The results were considered as inconclusive if only *E* gene was detected. If both target genes were not detected then the sample was reported as negative. The results were obtained in about five hours.

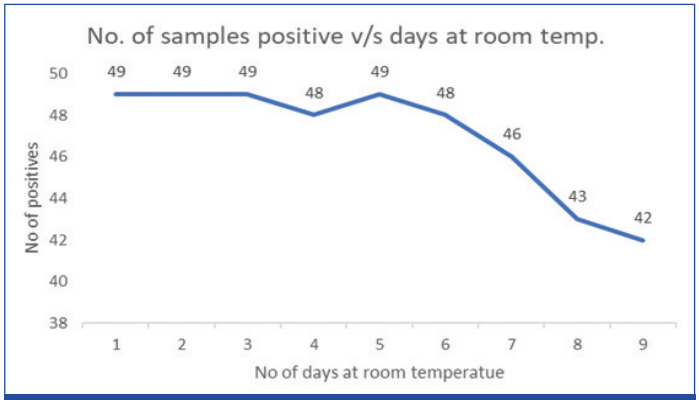
After analysing the results a set of positive samples with Ct values ranging from 17.5-33.5 were separated for the research work. The results of all samples were positive and the first day initial testing was considered as day zero for the study purpose. A 500 µL of each sample was separately aliquoted and kept in the refrigerator (4-8°C) at the beginning of the study as required for the short-term storage. The remaining samples from the study group were kept at the room temperature (15-25°C) for the assessment of the effect of the temperature on the integrity of the viral RNA. The room temperature and the temperature of the refrigerator were monitored on daily basis. The test samples were subjected to the same RT-PCR testing protocol repeatedly with 300 µL of sample on daily basis and the results compared (positive and negative controls included in each run). This study continued till the volume of the samples kept at room temperature got exhausted and was insufficient for further testing. As there was no initial dilution of the VTM samples so the available volume of most samples supported the study for nine days. On the last day of the study the part of the samples kept under refrigeration at the 4°C was also tested by RT-PCR and the results were compared with their respective counterparts kept at room temperature till nine days. Initially the samples were stored at 4-8°C as this temperature was considered adequate for short-term storage of 5-7 days. But longer duration of storage mandates lower temperature of -20°C or less.

STATISTICAL ANALYSIS

Statistical analysis was done by using python Jupiter notebook SciPy and Anaconda software and Chi-square test was applied for finding the independence between the original Ct value and test results.

RESULTS

In this study, it was observed that of the 49 positive samples (22 females and 27 males with mean age of 43 years), that were left at ambient room temperature for nine days, 42 samples remained positive by RT-PCR till the end of the study period. Of these 49 samples one sample became negative on day 6, two samples turned negative on day 7, three on day 8 and one sample turned negative on day 9 as shown in [Table/Fig-1,2]. So in total 42 out of 49 samples (85.71%) remained positive for SARS-CoV-2 by RT-PCR after nine days of keeping at room temperature. It was observed that all the samples stored outside refrigerator remained RT-PCR positive till five days. There was one sample which gave negative result on fourth day but again gave positive results from next day onwards, which may be due to a false negative result on day four due to some technical issues discussed further. The seven samples which gave negative result at room temperature by the end of nine days were found to show a strong concordance with the original Ct value of the samples as shown in [Table/Fig-3]. It was found that six out of seven samples were of Ct value of more than 30. Of these seven negative samples, only one was found negative when the test was conducted with its corresponding part kept in the refrigerator as summarised in [Table/Fig-4]. So, out of 49 only one sample gave negative results on keeping them at 4-8°C as compared to seven samples becoming negative on being stored at room temperature.

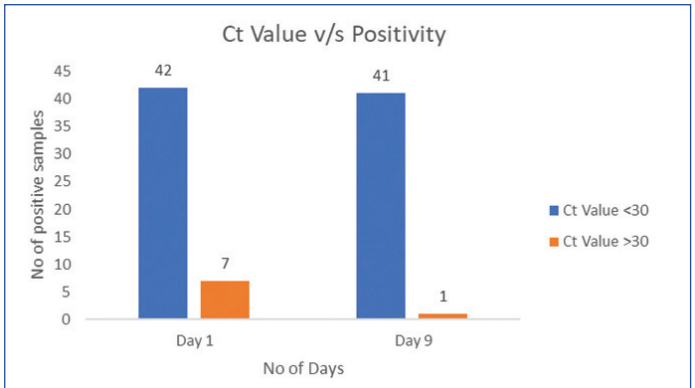


[Table/Fig-1]: No. of samples positive versus days at room temperature.

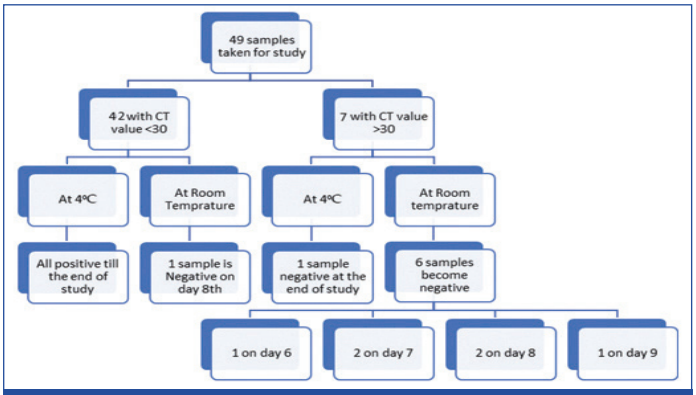
S. No.	Day 0 (E, N)	Day 1 (E, N)	Day 2 (E, N)	Day 3 (E, N)	Day 4 (E, N)	Day 5 (E, N)	Day 6 (E, N)	Day 7 (E, N)	Day 8 (E, N)	Day 9 (E, N)
1.	26.2, 28.1	26.4, 28.4	26.4, 28.8	26.7, 28.1	26.2, 28.3	26.5,28.6	27.1, 28.8	28.1, 28.9	28.2, 29.2	28.4, 29.9
2.	29.3, 30.4	29.2, 30.4	29.9, 30.6	29.3, 30.6	29.8, 30.7	29.7,30.8	30.6,31.6	Negative	Negative	Negative
3.	22.4, 24	22.4, 24	23.4, 24.5	22.8, 24	23.4, 25	23.6,25.0	23.6, 25.3	23.9, 25.8	24.2, 26.3	24.6, 26.7
4.	23.7, 25.1	23.7, 25.1	23.4, 25.2	24.1, 25.3	23.6, 25.4	23.6,25.8	23.7, 25.9	24.7, 26	24.9, 26.8	25.4, 27.2
5.	28.1, 29.4	28.2, 29.4	27.9, 28.6	28.2, 29.8	27.9, 28.6	27.9,29.1	27.4, 30.2	27.7, 30.5	27.9, 30.8	30.4, 31.5
6.	31.2, 32.9	31.4, 32.9	31.6, 31.9	31.8, 33.1	31.3, 31.8	31.4,32.2	31.4, 32.4	31.6, 32.4	31.4, 32.8	31.9, 33.3
7.	20.4, 21.2	20.5, 21.4	21.6, 23.1	21, 21.8	21.4, 23.1	21.9,23.8	22.4, 23.7	22.5, 23.9	22.6, 24.2	23, 24.8
8.	24.2, 25.7	24.4, 25.8	22.1, 23.9	23.8, 25.1	22.1, 23.9	22.9,24.1	22.9, 24.6	23, 24.8	23.3,25	23.9, 25.8
9.	22.1, 23.6	22.2, 23.6	22, 22.9	20.8, 22.6	20.5, 22.4	20.8,22.5	22.8, 23.9	23.8, 23.6	24.4, 23.8	24.6, 24.8
10.	30.5, 31.8	31, 32	32.3, 33.8	30.4, 31.6	32.3, 33.9	32.7,33.8	33.9, 35.3	Negative	Negative	Negative
11.	28.4, 29.9	28.6, 29.9	28.1, 29	28.5, 30	28.1, 29.3	28.9,30.0	30, 30.8	30.4, 31.6	Negative	Negative
12.	18.3, 19.5	18.4, 19.5	18.9, 22.4	18.6, 19.8	18.3, 19.8	18.7,20.3	19.2, 20.7	19.6, 20.9	19.9, 21.2	19.7, 21.3
13.	32.4, 33.9	32.6, 34	34.1, 35.1	33.4, 34.1	32.6, 33.9	33.3,35.2	33.8, 35.6	33.5, 35.8	Negative	Negative
14.	17.5, 18.9	17.6, 19	18.5, 19.9	17.8, 18.4	17.5, 19.1	17.7,19.4	18.2, 19.4	18.3, 19.6	18.9, 19.5	19.8, 20.6
15.	19.6, 20.8	19.8, 20.8	20.4, 20.9	19.8, 21	19.6, 21	18.2,21.6	18.4, 21.8	18.6, 21.9	18.9, 22.3	21.4, 22.9
16.	22.8, 24.1	22.6, 24.2	22.6, 23.9	23.1, 24.3	23.9, 25.2	24.2,26.1	24.3, 26.4	24.2, 26.8	25.7, 27.1	29.2, 30.4
17.	18.4, 20.1	18.6, 20.4	19.1, 20.7	19.2, 20.2	18.8, 20.1	18.9,20.8	19.2, 20.6	19.6, 21.4	20.1, 21.9	21.2, 23.1
18.	19.1, 21.6	20, 21.8	19.8, 21.1	19.9, 21.8	19.3, 21.8	19.7,22.1	19.3, 22.8	19.6, 22.9	20.4, 23.4	21.2, 24.2
19.	23.6, 25.1	24, 25.5	23.8, 24.9	23.9, 25.6	23.6, 25.4	23.8,25.8	24.2,26.1	24.6, 26.3	24.9, 26.8	25.7, 27.8
20.	20.1, 21.9	21, 22	18.6, 22.8	18.8, 22.9	18.4, 22.3	19.3,20.7	19.6, 20.8	20.2, 20.9	20.8, 21.6	21.3, 22.7
21.	22.4, 23.8	22.6, 24	23.2, 24.1	22.4, 23.9	23.2, 24.7	23.8,25.2	23.9, 25.8	24.1, 25.9	23.9, 25.2	24.2,25.9
22.	26.5, 28.1	26.5, 29	26.9, 27.8	27, 28.2	26.5, 28.6	26.6,28.9	26.7, 29.2	26.5, 29.3	26.7, 29.8	30.4, 31.2
23.	25.4, 26.9	25.4, 27	25.8, 26.6	25.7, 26.9	25.8, 27.4	26.1,27.8	26.4, 27.3	26.4, 27.7	26.8, 27.9	26.9, 28.1
24.	20.6, 21.8	20.8, 22	21.1, 21.9	20.3, 21.9	20.6, 22.3	20.8,22.6	21, 22.9	22.5, 23.2	22.9, 23.4	22.9, 23.4
25.	25.2, 26.7	25.4, 26.8	26.4, 27.3	25.5, 26.9	25.6, 26.9	26.4,27.2	26.8, 28.1	27.4, 28.9	26.4, 28.2	27.6, 29
26.	22.5, 23.6	22.5, 23.6	23.5, 24.2	23, 23.8	22.8, 24.1	23.2,24.8	23.3, 24.8	23.6, 24.9	23.3, 25.5	23.3, 25.5
27.	32.5, 33.8	33, 34	32.1, 33.6	32.7, 33.7	32.7, 34.1	32.8,34.5	33.4, 35.4	33.8, 35.6	Negative	Negative
28.	27.4, 28.9	27.8, 29	26.4,28.2	27.6, 29	Negative	28.1,29.7	28.7, 30.2	28.8, 30.4	28.9, 30.8	28.9, 30.8
29.	17.3, 19.1	17.6, 20.1	18.2, 19.4	17.4, 18.8	17.6, 19.6	17.6,19.8	18.2, 19.4	18.5, 19.8	18.7, 19.9	18.7, 19.9
30.	30.4, 31.9	31, 32	28.2, 31.6	28.2, 32	30.5, 32.2	30.7,32.1	30.8, 32.3	31.8, 32.7	32.4, 33.7	32.4, 33.7
31.	18.5, 19.9	18.6, 20	18.8, 19.5	18.5, 20	18.9, 20.3	18.3,20.7	18.3, 20.9	18.7, 21.3	18.6, 21.4	18.6, 21.4
32.	20.6, 21.8	20.8, 22	20.2, 21.6	20.8, 21.8	20.4, 21.8	20.4,21.9	20.6, 21.9	20.6, 21.6	20.8, 21.7	20.8, 21.7
33.	18.4, 20.5	18.4, 21.2	18.7, 20.9	18.8, 20.9	19.2, 21.3	19.5,21.4	19.5, 21.5	19.5, 21.6	19.3, 21.9	20.4, 22.6
34.	24.2, 26.1	24.4, 26.3	24.8, 25.7	24.3, 26.1	25, 25.9	25.2,26.3	25.3, 26.6	25.8, 26.9	26.2, 27.2	26.2, 27.2
35.	29.4, 30.8	29.6, 30.8	30.2, 30.7	29.4, 32	31.2, 31.9	31.9,32.4	32.9, 33.3	33.4, 35.3	33.9, 35.8	Negative
36.	22.5, 24.1	22.6, 24.2	22.9, 24.3	22.7, 24.5	23.1, 24.8	23.5,25.3	23.5, 25.5	23.5, 25.8	23.8, 26.1	23.8, 26.1
37.	21.7, 22.1	21.7, 22.1	21, 22.2	21.7, 22.7	21.4, 22.8	21.6,23.1	21.8, 23.2	21.7, 23.7	21.8, 23.9	21.8, 23.9
38.	29.1, 30.5	29.6, 31.5	29.4, 31	29.3, 30.6	29.8, 31.6	29.6,31.6	29.8, 31.3	29.9, 31.7	30.3, 32.3	30.3, 32.3
39.	33.2, 34.4	33.4, 34.4	33.5, 34.8	33.2, 34.8	33.5, 35.2	33.5,35.7	Negative	Negative	Negative	Negative
40.	20.4, 21.2	21.2, 21.4	22.4, 21.8	22.2, 21.8	20.6, 21.8	20.8,22.2	21.4, 22.6	21.8, 22.8	21.9, 23.1	22.5, 23.8
41.	16.2, 17.7	16, 17.5	16.8, 18.1	16.7, 17.6	16.8, 18.7	17.1,18.9	17.6, 19.2	17.8, 19.7	18.4, 20.2	18.4, 20.2
42.	22.4, 23.6	22.5, 23.6	22.7, 23.8	22.4, 24.1	22.8, 24	22.8,24.3	23.4, 24.8	23.2, 24.6	23.4, 24.9	23.4, 24.9
43.	20.8, 21.9	21.2, 21.8	21, 21.9	20.8, 22.1	21.3, 22.4	21.6,22.4	21.3, 22.7	22.3, 23.9	22.8, 24.1	23.8, 25.2
44.	23.2, 25.1	23.5, 25.2	23.8, 25.2	23.2, 25.3	23.9, 25.8	23.4,25.7	23.4, 25.8	23.9, 26	24.2, 26.4	24.2, 26.4
45.	22.4, 23.6	22.2, 23.4	23.1, 24.1	22.4, 23.8	23.3, 24.3	23.2,24.5	23.5, 24.6	23.7, 24.5	23.8, 24.9	23.8, 24.9
46.	20.6, 21.8	20.4, 21.6	20.7, 21.8	21.6, 22.9	20.8, 22.5	21.2,22.7	21.2, 22.8	22.3, 23.2	22.5, 23.7	23.5, 24.3
47.	22.9, 23.9	22.6, 23.4	22.7, 24.1	22.9, 24.3	23.1, 24.9	23.6,25.1	23.4, 25.6	23.8, 25.9	24.1, 26.3	24.8, 27.3
48.	19.4, 20.6	19.2, 20.5	19.7, 20.8	19.8, 21	19.7, 21.2	19.9,21.8	20.3, 22.4	20.3, 22.9	21.4, 23.8	22.3, 24.8
49.	24.5, 25.8	24.3, 25.6	24.7, 25.2	24.1, 24.6	24.8, 24.3	25.1,26.3	25.4, 26.8	25.9, 26.9	26.1, 28.6	27.4, 29.3

[Table/Fig-2]: Showing sample-wise RT-PCR results on each day.

All the results were statistically analysed by using python Jupiter notebook SciPy and Anaconda software and Chi-square test was applied for finding the independence between the original Ct value and test results. The p-value was found to be much below the significance level of 0.05 (0.00000282) suggesting that the negative results were highly dependent on the original Ct value of the sample and were strongly associated with Ct value of more than 30. While comparing the Ct values of the samples on various days it was observed that in most samples Ct value was increasing but there was no orderly trend and many a times Ct value also decreased. It was also noted that all the samples that turned negative by the end of study period were of Ct value of 28 and above.



[Table/Fig-3]: Association of Ct value with positivity.



[Table/Fig-4]: Flow chart of the results.

DISCUSSION

With the sudden upsurge of the coronavirus pandemic all over the world, healthcare system has come under tremendous pressure for establishing infrastructure and maintaining the continuous supply of all the diagnostic and treatment modalities. In such a scenario adherence to all the guidelines proposed by World Health Organisation (WHO) for sample transportation and storage often becomes difficult. This study has clearly shown that SARS-CoV-2 RNA in the samples kept properly in VTM at ambient temperature remains detectable till five days without any drop in detection rate. In this study, it was observed that the majority of the samples (85.71%) remain RT-PCR positive for detection of coronavirus RNA till the end of the study period i.e., day 9 of samples kept on room temperature. Few previous studies [8,14] of temperature also support the findings of present study and showed that the virus remains viable at room temperature till seven days. The results of this study was in contradiction of the study done by Kirkland PD and Frost MJ from Australia [15] which reported that PCR positivity was adversely affected at room temperature in commercial VTM and no viral RNA was detected after 48 hours. This was attributed to the presence of RNA in the commercial VTM used for sample collection by them. It suggests that composition of various VTM may affect the outcome of the test as it may vary between different manufacturers and individual studies comparing various commercial VTM would give a better insight. In this study, it was seen that such decay and positivity with RT-PCR was completely maintained till five days and fairly maintained at room temperature even after nine days of sample collection. It is to be noted that in this study none of the samples turned negative till first five days of being kept at room temperature so it gives us a sufficient pocket of time if samples are exposed to ambient temperature during transportation. One sample that turned out to be negative on day four also became positive next day onwards pointing out to be a false negative result due to some technical issue. This false negativity may be due to some technical faults like pipetting errors or improper vertexing leading to uneven composition of sample or some sensitivity issues.

Studies [16,17] have suggested that corona virus RNA may be degraded if the samples are heated till 56ºC, but in this study the temperature was never as high as 56ºC so this study recommends that the air conditioning temperature of 15-25ºC, temperate climate or the colder months of the tropical countries is good enough to get satisfactory results of RT-PCR for coronavirus testing without any attrition of positivity till five days. When the results were compared with the results of sample part kept in refrigerator the results of most of the samples was very comparable with that of day one with only one sample gave negative result. This also point towards possibility of sample storage at 4-8ºC as against recommendation of -70ºC for more than five days storage.

This study gives very encouraging results for the testing of COVID-19 samples kept at ambient temperature for long duration especially in pandemic like situation where all resources are on verge of collapsing even in storage and transportation of the samples to the accredited labs for testing. It can serve as a fair guide to analyse the effect of such a breach in compliance with temperature guidelines in COVID-19 testing. It will also help the medical fraternity in preparedness for any future pandemic situations. In few such cases Ct value may increase but detection of positive sample is still possible which makes it highly acceptable with limited resources. As most of the samples which turned out negative in this study were of Ct value 30 and above so this study suggest that there are few chances of missing out such low positive samples on being exposed to higher temperatures but minimal chances of missing out highly infectious samples with low Ct values. In this study, out of 49 samples, 42 were having Ct value less than 30 on day one and seven samples were of Ct value more than 30. Out of those all but one in the Ct value less than 30 turned negative.

Though coronavirus pandemic has astonished the entire world with its rampant course, but the scientific community has come out still stronger against the deadly pathogen making all the developing countries also well equipped with the state of the art advanced molecular diagnostic techniques for its accurate diagnosis. RT-PCR has been taken as the desirable test for diagnosis but the specifications of sample handling required with it points towards the need for the development of some newer diagnostic point of care testing modalities which would obviate the need for transportation of the samples to highly specialised labs under specified conditions and would also decrease the turn around time for earliest diagnosis and isolation of the cases from the suspects. This study also emphasises the fact that for testing of SARS-CoV-2 with RT-PCR, the prerequisite is the intact nucleic acid and not the viable virus so the sample transportation and storage guidelines also need to be revisited after carrying on larger studies.

Limitation(s)

This study has a limitation that the temperature of exposure in this study varied from 15-25ºC but more studies need to be done at a much higher temperature ranges as found in many tropical countries. Another limitation of the study was the small sample size of the study so larger studies would be required for better understanding.

CONCLUSION(S)

In this study, it was observed that the positivity of the RT-PCR results was completely intact till five days of the samples being stored at the ambient room temperature of 15-25°C and 85.71% of the positive samples were also found RT-PCR positive even after nine days of the exposure to ambient temperature of 15-25°C. Most of the samples which gave negative results in study period were of Ct value 30 and above. It was concluded that it is possible to keep samples at this ambient temperature for five days without any loss of positivity in RT-PCR but more rigorous studies with larger sample size at higher temperatures are required to be conducted.

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