ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at:www.ijmacr.com

Volume – 6, Issue – 1, February - 2023, Page No. : 01 - 06

A study on evaluation of extraction free SARS-CoV-2 RT PCR as an alternative to conventional RT- PCR.

[1]Dr Sarita Yadav, Professor, Deptt of Microbiology, BPS, Govt. Medical College for Women, Khanpur Kalan, Sonepat, Haryana, India

[2]Ms. Kajol Verma, Research, Assistant, VRDL, Deptt. Of Microbiology, BPS, Govt. Medical College for Women, Khanpur Kalan, Sonepat, Haryana, India

[3]Dr.Surinder Kumar, Professor, Deptt of Microbiology, BPS, Govt. Medical College for Women, Khanpur Kalan, Sonepat, Haryana, India

[4]Dr.Naveen Sharma, Associate Professor, Deptt of ENT, BPS, Govt. Medical College for Women, Khanpur Kalan, Sonepat, Haryana, India

[5]Dr.Seema Garg, Associate Professor, Deptt of Microbiology, BPS, Govt. Medical College for Women, Khanpur Kalan, Sonepat, Haryana, India

Corresponding Author: Dr. Sarita Yadav, Professor, Deptt of Microbiology BPS, Govt. Medical College for Women Khanpur Kalan, Sonepat, Haryana, India.

How to citation this article: Dr Sarita Yadav, Ms. Kajol Verma, Dr. Surinder Kumar, Dr. Naveen Sharma, Dr.Seema Garg, "A study on evaluation of extraction free SARS-CoV-2 RT PCR as an alternative to conventional RT- PCR", IJMACR- February - 2023, Volume – 6, Issue - 1, P. No. 01 – 06.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Objectives: The COVID-19 pandemic created a great demand for molecular tests that are based on viral RNA extraction and detection by RT-PCR. This required long turnaround time, high cost and skilled laboratory personnel. We evaluated the performance of a dry swab-based method without a conventional RNA extraction step as compared to traditional RT-PCR.

Methodology: Two Nasopharyngeal swabs were collected from COVID -19 suspected patients. One swab was transported as dry swab and other swab was transported in Viral Transport Media (VTM). The dry swab was subjected to treatment by Tris-EDTA-Proteinase K(TE-K) buffer and heat inactivation at 98°C for 6 minutes. The elute thus obtained and the RNA extracted from VTM were subjected to RT-PCR and cycle threshold (CT) values were compared.

Results: We observed strong correlation between CT values of VTM extracted RNA and TE-K buffer elutes. Both the methods yielded same results for 97 samples while the results differed for six samples.

Conclusion: The dry swab method can increase the throughput of a laboratory and simplify the sample collection and transportation of samples especially in rural peripheral health centres in developing countries.

Introduction

The clinical spectrum of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) infections can range from asym pato matic/mild, moderate,severe to critical [1,2]. The relevance and importance of mass diagnosis is widely recognized as a mandatory tool to reinforce the control measures for monitoring virus circulation and reduce the spreading of SARS CoV-2. [3,4]In view of the increasing demand for SARS-CoV-2 testing in less time and to examine sensitivity and specificity comparative to gold standard RTPCR method, the need to find alternatives with low cost that reduces the time of analysis was felt[5] The conventional tests used to detect viral RNA involve purification step to extract viral RNA from patient samples(Nasopharyngeal swab, sputum or other) followed by quantitative reverse transcription PCR(RT PCR) assay to detect SARS CoV-2 RNA[6]. Despite being a superior method,RT-PCRdemands significant amount of time,laborious and expensive RNA isolation step, skilled personnel and high cost,thus, increasing turnaround time and impacting public health management[4,5]Along with this multiple challenges such as shortage of RNA isolation reagents, plasticware, sample transport facilities and limited human resources were also being faced during the pandemic[7,8]To overcome the lack of reagents and to increase the capacities for SARS CoV-2 testing various approaches like pooling strategies, various buffer solutions, alternate transport media, addition of detergents in RT PCR reaction, direct RT PCR without extraction step using primary material or isothermal methods were developed and evaluated by different researchers[6,7,8,9]Direct RT-PCR offers advantages of simplified detection and reduced turnaround time[10,11]We conducted a protocol previously described using Tris EDTA Buffer(Ethylenediamine tetraacetic acid) and Proteinase K treatment of dry nasopharyngeal swab samples followed by heating at 98° C[11,12,13] We evaluated an alternative RNA extraction free direct RT-PCR protocol for diagnosis of SARS-CoV-2 at Viral Research and Diagnostic Laboratory (VRDL) in comparison to conventional VTM based RNA extraction and amplification by RT-PCR.

Methods

Collection of Clinical Samples and Transport

The study was conducted in the Indian Council of Medical Research (ICMR) approved Virology Research and Diagnostic Laboratory (VRDL), Department of Microbiology at Bhagat Phool Singh Government College for Women, Khanpur Kalan, Sonepat. Two Nasopharyngeal swab samples were collected from each patient and one was transported as dry swab in falcon tube and another in 3 ml of Viral Transport Media (VTM) respectively. The samples were refrigerated at 4°C till further analysis.

Processing of Samples [11,12,13]

Processing of all SARS-CoV-2 samples was done under Biosafety Cabinet Class 2A by following procedures:

Processing of Dry Swab Tubes

400 µl of TE- Proteinase K buffer (10mM Tris, pH 7.4, 0.1mM EDTA and 2mg/ml proteinase K) was added to dry swabs. The samples containing this solution were incubated at room temperature for 30 minutes. 50µl of TE-P extract was transferred into a separate Eppendorf tube. All aliquots were heated at 98°C in dry bath for six

minutes. These inactivated samples were subjected for direct RT-PCR analysis.

RNA Isolation from VTM

RNA isolation was performed from the samples received in VTM in Automated RNA Extraction Machine using HiPurA®Super 11 Magnetic Pre-filled kit (HIMEDIA HIGENO-MB) according to the protocol of manufacturer. 50µl of extracted RNA elute was subjected for RT-PCR analysis.

RT-PCR Analysis

The SARS-CoV-2 was detected using GENES2ME VIRALDTECT- II, Multiplex Real Time PCR Kit for COVID-19. The assay targeted primers for E gene(FAM probe), RdRp gene (Texas Red probe) and N gene(Cy5 probe) of SARS-CoV-2. Target HEX channel is used for detection of RNase P control. The results were analyzed by CT values of specific target genes of SARS-CoV-2 and RNase P control. Each reaction plate included positive and negative controls provided with the kit.All the samples were tested in duplicates and the results are presented as mean CT value. A target was considered as "positive" with CT values lower than 37.

Statistical analysis

Microsoft Excel was used for statistical analysis and plotting.

Ethical consent

Although all samples were collected as part of routine clinical care,informed consent was taken from all the participants.

Results

A total of 103 nasopharyngeal swabs were processed by using the conventional gold standard RT PCR method and TE-K buffer method. Twenty-nine samples were found to be positive for SARS-CoV-2 RNA with VTM based RT-PCR method (Table 1)

The samples were considered positive when CT values were less than 37, for the target E gene and target RdRp gene or N gene. We observed strong correlation between C_T values of VTM extracted RNA and TE-K buffer elutes (Figure 1, 2 and 3). Both the methods yielded same results for 97 samples while the results differed for six samples. Four samples were found to be negative by dry swab but were considered positive by traditional RT PCR. Two samples were considered negative by RT PCR whereas the same samples show positive results by dry swab method. (Table 1)

Considering conventional VTM based RNA extraction and RT PCR as gold standard, the dry swab method demonstrated a sensitivity of 86.2% with a specificity of 97.29%.

Discussion

During the initial waves of the Covid-19 pandemic, a rapidly increasing demand for molecular testing was experienced on global scale. Our ICMR approved VRDL has tested near about 10 lakh samples till August 2022. The samples were collected from the peripheral health centres and transported to the laboratory.Many a times the laboratory personnel faced difficulties like spilled VTM and soaked caps of falcon tubes posing a infection risk to them.Moreover,when a large number of tests were to be performed per day, the issues like shortage of reagents, plastic ware, diagnostic kits and skilled manpower were also observed. Therefore, the need to simplify procedure, reduce turnaroundtime, costs per test and increase throughput per day was noted.

In the present study,we evaluated performance of 103 nasopharyngeal samples collected within a dry tube and then subjected them to elution method utilizing Tris EDTA Proteinase K buffer and heat inactivation at 98°C for 6 minutes.[11,12]The method was adopted as per the

Advisory proposed by ICMR.[13]The results revealed that the performance of dry swab TE-K buffer extracts in direct RT-PCR and the currently used standard method of detection from VTM samples were comparable.Overall, both the methods showed consistent results for 97 samples out of 103 samples (72 negatives, 25 positives) and differed for 6 samples. Our results are in accordance with earlier reports of RNA extraction free RT PCR studies. [9,10,11,12]

Although, in the present study, the dry swab method was associated with some reduction of the sensitivity of the test. However, the reduction was moderate and observed in those samples whose CT values were very high (>35). Sing Anaya gam and other authors [7,14] suggested that these cases are known to exhibit lower infectivity and are often associated with late stages of the infection. Jefferson et al observed a cut off RT-PCR CT value of >30 was associated with non-infectioussamples. [9,15]

We could find only a limited number of studies in India regarding the evaluation of dry swab-based TE-K buffer method. [11,12]Go kulan and colleagues proposed dry swab method as a viable and economical alternative for the existing gold standard PCR method.[11] The direct PCR can be used be useful where repeated and frequent testing is required.[16]

It is anticipated that compliance with covid 19 testing recommendations will increase with the use of simple tests that will augment the testing capabilities to improve patient outcomes specifically in low resource settings where testing components are in limited supply.[19]The dry swab method is an affordable, quick and efficient method that can be incorporated into alternative approaches of detection utilising PCR and increase the throughput of a lab.[10,16,18]The usability and feasibility of dry swabs is expected to hold similar advantages for detection of other viral pathogens that are diagnosed using RT-PCR as well.[10,16]

One limitation of this study is that we assessed only a limited number of samples and conducted the study when the pandemic had stabilised to a great extent.The heavy workload on the laboratory and the urgency to provide the results in time limited the evaluation study.

India is a rural country and many health centres being situated in peripheral areas,the utilization of dry swabs has the potential to simplify specimen collection, allow better accessibility to patients and diminute the safety risks during transportation while preserving the accuracy of testing.

Conclusion

We suggest sample collection using dry swab approach which not only eliminates the need for VTM, but also makes the sample handling, transporting and testing more convenient and safer for the frontline healthcare workers and technicians.

Acknowledgement

All the authors acknowledge the support received from ICMR, India.

References

1. Wang Y, Wang Y, Chen Y, Qin Q. Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. J Med Virol 2020;92(6):568-576.

2. Garnett L, Bello A, Tran KN, Audet J, Leung A, Schiffmann Z, et al. Comparative analysis of different swabs and transport mediums suitable for SARS-CoV-2 testing following shortages. J Virol Methods 2020; 285:113947

3. Ulloa S., Bravo C., Parra B., Ramirez E., Acevedo A., Fasce R., Fernandez J. A simple method for SARS-

CoV-2 detection by rRT-PCR without the use of a commercial RNA extraction kit. J Virol Methods. 2020;285 doi: 10.1016/j.jviromet.2020.113960.

4. Vandenberg O., Martiny D., Rochas O., van Belkum A., Kozlakidis Z. Considerations for diagnostic COVID-19 tests. Nat Rev Microbiol. 2020:1–13. doi: 10.1038/s41579-020-00461-z.

5. Mallman L, Hermann BS, Schallenberger K, Demo liner M, Eisen AKA, Heldt FH, et al.Proteinase K treatment in absence of RNA isolation classical procedures is a quick and cheaper alternative for SARS-CoV-2 molecular detection. J Virol Methods 2021; 293:114131.

6. Chomczynski P, Chomczynski PW, Kennedy A, Rymaszewski M, Wilfinger WW, Heiny JA, et al. Rapid processing of SARS-CoV-2 containing specimens for direct RT-PCR. Plos One 2021;16: e0246867.

7. Morecchiato F, Coppi M, Baccani I, Maggini N, Ciccone N, Antonelli A, et al. Evaluation of extraction-free RT-PCR methods for faster and cheaper detection of SARS-CoV-2 using two commercial systems. Int J Inf Dis 2021; 112:264-268.

8. CameronA,Pecora ND, Pettengill MA. Extraction-Free Methods for the Detection of SARS-CoV-2 by Reverse Transcription-PCR: a Comparison with the Cepheid Xpert Xpress SARS-CoV-2 Assay across Two Medical Centers. JCM 2021;59(2): e02643-20.

9. Visseaux B, Collin G, Fidouh NH, Hing rat QL, Ferre VM, Damond F. Evaluation of three extraction-free SARS-CoV-2 RT-PCR assays: A feasible alternative approach with low technical requirements. J Virological Methods 2021; 291:114086.

10. Srivatsan S, Han PD, Raay KV, Wolf CR, McCulloch DJ, Kim AE, et al. Preliminary support for a "dry swab,extraction free" protocol for SARS-Cov-2 testing via RT-qPCR.

11. Go kulan CG, Kiran U, Kuncha SK, Mishra RK. Temporal stability and detection sensitivity of the dry swab-based diagnosis of SARS-Co-V-2. J Biosci 2021; 46:95

12. Kiran U, Go kulan CG, Kuncha SK, Vedagiri D, Chander BT, Sekhar AV, et al.Easing diagnosis and pushing the detection limits of SARS-CoV-2. Biology Methods Protocols 2020;1-5.

13. ICMR 2021.Advisory on use of Dry Swab RNA extraction free RT-PCR Method. http://www.icmr.gov.in/pdf/covid/techdoc/Advisory_Dry_Swab_RNAExtraction_20042021_pdf

14. Singanayagam A, Patel M, Charlett A, Lopez BJ, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold value in cases of COVID-19, England, January to May 2020. Eurosurveillance 2020; 25.doi:10.2807/1560-7917.ES.2020.25.32.2001483.

15. Jefferson T, Spencer EA, Brassy J, Heneghan C. Viral cultures for COVID-19 infectious potential

16. Smyrlaki I, Ekman M, Lentini A, De Sousa NR, Papanicolaou N, Vondracek M. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. Nature Communications 2020; 11:4812.

17. Parikh BA, Wallace MA, McCune BT, Burnham CAD, Anderson NW. The effects of "Dry Swab" incubation on SARS-CoV-2 molecular testing. JALM 2021;1281-1286.

18. Srivatsan S, Heidl S, Pfau B, Martin BK, Han PD, Zhong W, et al. SwabExpress: An end-to-end protocol for extraction-free COVID-19 testing. Clinical Chemistry 2022; 68:143-152.

19. Padgett LR, Kennington LA, Ahis CL, Samarasinghe DK,Tu YP, Wallander ML, et al. Polyester nasal swabs collected in a dry tube are a robust and inexpensive, minimal self-collection kit for SARS-CoV-2 testing. Plos ONE 2021;16(4): e0245423.

Legend Figures

Figure 1: C_T values of E gene of VTM extracted RNA &TE-K buffer elutes.

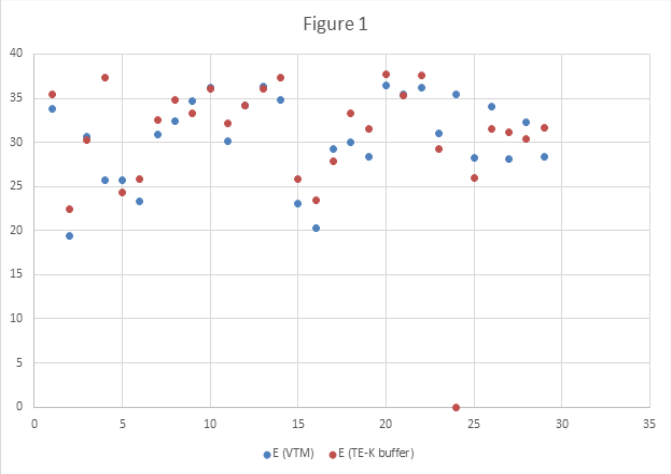

Figure 2: C_T values of RdRp gene of VTM extracted RNA &TE-K buffer elutes.

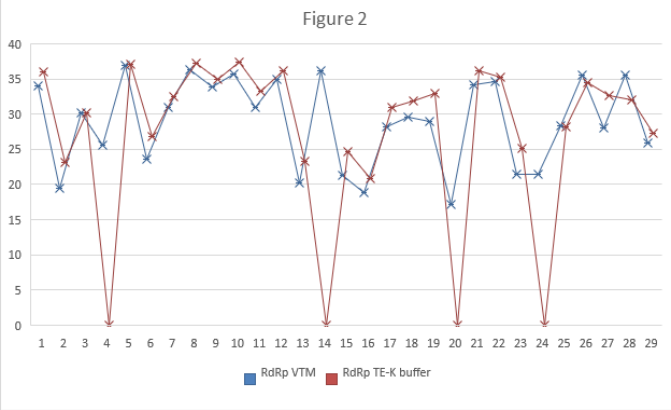

Figure 3: C_T values of N gene of VTM extracted RNA &TE-K buffer elutes.

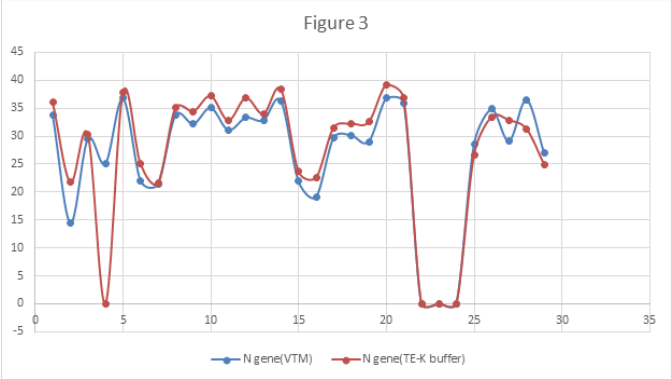

Table 1:

	Positive by RT PCR (True positives)	Negative by RT-PCR (True Negatives)
Positive by Dry swab method	25	2
Negative by Dry swab method	4	72
	29	74