International Journal of Research in Medical Sciences
Jhajharia A et al. Int J Res Med Sci. 2024 Dec;12(12):4574-4579
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20243709

Original Research Article

Comparative analysis of different RNA extraction kits used in detection of COVID-19 virus

Abhishek Jhajharia[1]*, Riya Bhargava[2], Bharti Malhotra[3], Amit Tak[4], Parul Gupta[5]

[1]Sawai Man Singh Medical College, Jaipur, Rajasthan, India
[2]Department of Microbiology, Fortis Escorts Hospital, Jaipur, Rajasthan, India
[3]Department of Microbiology, Sawai Man Singh Medical College, Jaipur, Rajasthan, India
[4]Department of Preventive and Social Medicine, RVRS Medical College, Bhilwara, Rajasthan, India
[5]Department of Microbiology, Bharatpur Medical College, Bharatpur, Rajasthan, India

Received: 19 September 2024
Revised: 16 November 2024
Accepted: 18 November 2024

***Correspondence:**
Dr. Abhishek Jhajharia,
E-mail: 007abhishekjhajharia@gmail.com

ABSTRACT

Background: COVID-19, caused by SARS-CoV-2, was first reported in December 2019 and declared a Public Health Emergency of International Concern by the WHO. While various diagnostic methods exist, rRT-PCR remains the gold standard for detecting viral genes (N gene for SARS-CoV-2 and the E gene for all sarbecoviruses). Thus, ample RNA should be extracted for the efficient performance of the RT-PCR.
Methods: We compared the specificity, sensitivity, and efficiency of three automated nucleic acid extraction kits: Perkin Elmer®, HiPurA, and Zybio. A total of 96 known NP/OP samples from a biorepository were processed using these kits according to their respective protocols. Magnetic Bead method was employed for the same, followed by rRT-PCR using Quantiplus Multiplex COVID-19 detection kit. The JASP version 0.16.2 and MATLAB 2019a were used for statistical analysis.
Results: Both Perkin Elmer® and Zybio kits demonstrated 100% sensitivity, while the HiPurA kit showed 97.9% sensitivity compared to the original results. All three kits exhibited 100% specificity.
Conclusions: Based on the comparison of sensitivity, specificity, efficiency, cost, turnaround time etc. Perkin Elmer® followed by Zybio are recommended as the preferred extraction kits. Both Perkin Elmer® and Zybio performed well with 100% sensitivity. This study provides alternatives in high demands during surge and in places of limited resources.

Keywords: COVID-19, RT-PCR, Sensitivity, Specificity

INTRODUCTION

One of the largest pandemics ever after Spanish flu is COVID-19, caused by SARS-COV-2 virus. In December 2019, its first case was reported in Wuhan province of China having atypical pneumonia like symptoms with no known origin. Since then, it has spread worldwide taking the shape of a deadly pandemic.[1] As of May 2022, it has infected around 523 million people, with more than 6.2 million deaths worldwide and around 43 million infected cases with 0.52 million deaths in India alone based on the worldometers' statistics.[2] Later on, the etiological agent of the disease was identified to be a virus closely related to the earlier found SARS-CoV. In January 2020, WHO declared it a global 'public health emergency'. ICTV (International Committee on Taxonomy of Viruses) named the virus as Severe

Acute respiratory syndrome Coronavirus 2 whereas WHO announced the name of the disease to be "COVID-19" on 11th February 2020.[3] On March 11th 2020, WHO declared it as a global pandemic.[4] The virus, Sars-CoV-2, is

classified in the family Coronaviridae and genus betacoronavirus.[5] Earlier it was suggested that because of its close resemblance with Coronavirus found in Bats RaTG13 and Pangolin-Cov found in dead Malayan Pangolin lungs it might have its origin from bats and pangolins respectively. Later on this theory was disproved, as there was no sufficient similarity in their genomic sequence to prove them as direct progenitors.[6,7]

SARS-CoV 2 transmits via both direct and indirect modes, i.e., respiratory droplets as well as contaminated objects. Although, it mainly transmits through respiratory route but blood borne, urinary and feco-oral routes have also been reported. The source of infection is mainly the COVID-19 cases but pre-symptomatic and asymptomatic patients can also transmit it to healthy individuals. Pregnant women are at higher risks of getting infected so vertical transmission needs to be taken into consideration.[8,9] COVID-19 is mainly a respiratory disease having most common symptoms like fever, cough, shortness of breath, fatigue and difficulty in breathing. Other symptoms like chills, sore throat, myalgia, loss of smell and taste may also occur with minor frequency, besides gastrointestinal manifestations as diarrhoea, nausea and vomiting. In most severe cases the disease courses with severe pneumonia, acute respiratory distress syndrome, acute cardiac injury, and multi organ failure that usually leads to hospitalization and the need to use mechanical respirators. Age and coexisting illness play major role for the above signs and symptoms.[10,11]

Precautionary essential measures were required to control the pandemic as no vaccines were available initially: social distancing, use of masks, sanitisers and large-scale testing of the population. Due to high infected number, even testing of only symptomatic patients who looked for medical care was done as there was a huge sample volume for the diagnosis.[12] Thereby, alternative methods were required due to high demands. In this scenario, although rRT-PCR is the most valuable, computerized tomography (CT) scans of lungs have been used to overcome the shortening of rRT-PCR supplies.[13] However, COVID-19 diagnosis by CT scans is often difficult in asymptomatic patients and increases the risk of false-negatives. Therefor after several efforts being focused on fast development of novel rapid and reliable diagnostic tests, to date, real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) based assays on respiratory specimens is still considered the gold standard to detect SARS-CoV-2-infection.[14] rRT-PCR is highly sensitive and specific and, compared to other available viral detection methods (e.g., viral antigen detection, standard plaque assay, serology, electron microscopy) and significantly faster with a lower potential for contaminations and/or errors as it is carried out using primer probe sets which target the sequences of N gene and E(Envelope) gene.[15] Undoubtedly, rRTPCR performance can be greatly affected by the efficiency of the viral RNA extraction procedures.

Therefore, the present study is aimed at evaluating and comparing three different automated methods for SARS-CoV-2 RNA isolation and purification from nasopharyngeal and oropharyngeal swabs by rRT-PCR, marketed as commercial kits in India, in Advanced Diagnostic and Research Laboratory of Microbiology Department in Sawai Man Singh Medical College, Jaipur, Rajasthan.

METHODS

Study design

This was observational cross-sectional (descriptive and comparative) study.

Study universe

Clinical nasopharyngeal and oropharyngeal samples known to be positive or negative for SARS-CoV-2.

Study population

Total 48 known positive samples (Ct 20-32) and 48 known negative samples were taken.

Study period

This study conducted for 4 months from June 2022 to September 2022.

Inclusion criteria

Inclusion criteria were known positive and negative status based on COBAS SARS-CoV-2 test results; only NP/OP samples in VTM collected by Dacron swabs; samples transported within 2 hours under cold-chain conditions.

Exclusion criteria

Exclusion criteria were >32 cycle threshold (Ct) of the samples were not considered for the result interpretation, sample with indeterminate status, and samples transported for more than 2 hours.

Sample collection

For the analytical study, 48 known positive and 48 known negative samples were taken. The nasopharyngeal and oropharyngeal samples were collected by dacron swab and kept in Viral Transport Medium (VTM) and transported via triple layer packaging mechanism at suitable temperature as per the manufacturer's directions maintaining the cold chain. The samples were sent within 2 hours after collection to Advanced Diagnostic and research Laboratory of Microbiology Department of Sawai Man Singh Medical College, Jaipur. The positive samples identified from COBAS SARS-CoV-2 test (Roche diagnostics) were chosen based on cycle threshold values (Ct) being within range of 20-32.

For comparing the different extraction techniques, the samples were aliquoted separately for each one of them. The samples to be used for nucleic acid extraction were stored at 2-8℃ for less than 24 hours and at -20℃ for long term preservation. The samples were kept at room temperature prior to their use.

RNA extraction

RNA was extracted using 3 different automated RNA extraction kits, Perkin Elmer® (Lot No.-V300-172-21062), HiPurA^R Super 11 Pre-filled Extraction Kit for Insta NX^R Mag96 (Lot N0.- 0000520686), and Nucleic Acid Extraction Kit (Magnetic Bead Method) by Zybio (Lot No.- 5104042). The procedure of extraction kit involves 4 steps sequentially- sample lysis, nucleic acid binding to electromagnetic beads, washing and elution. The extracted RNA was transferred into 1.5ml centrifuge tubes at the end of the process.

rRT-PCR assay

For performing rRT-PCR assays, Quantiplus Multiplex COVID-19 Detection Kit was used. This assay detects 2 gene targets namely- E gene and N gene including Internal Control (IC). N gene is specific to SARS-CoV-2 whereas E gene is present in all sarbecoviruses.

Detector dye for targeting E gene, N gene and Internal Control (IC) were in FAM, VIC/HEX and Cy5 {Quantiplus^R Multiplex COVID-19 Detection Kit (Real-Time Qualitative PCR Kit)}.

16µl master mix and 10 µl of extracted RNA were added together. The total 26 µl of the preparation contains 13 µl core qPCR Mix, 2 µl multiplex PPM, 1 µl IC-B mix and 10 µl of extracted RNA. Reactions were incubated for 53℃ for 10 min., initial denaturation at 95℃ for 15 min., and PCR Cycling for 40 cycles were followed at 95℃ for 15s and 60 ℃ for 30s.

≤32 Cycle threshold (Ct) of the samples was considered for the result interpretation.

Statistical analysis

The tests are compared with golden standard tests and shown as confusion matrix. The performance metrics of the tests were expressed as accuracy, the area under the receiver operator characteristic (AU-ROC) curve, sensitivity, and specificity. The correlation between quantitative variables was found using Pearson's correlation. The JASP version 0.16.2 and MATLAB 2019a were used for statistical analysis. The significance level was considered at 5%.

Table 1: As per the manufacturer's instructions, the results interpretation.

E Gene (FAM)	N Gene (VIC/HEX)	Internal control (Cy5)	Interpretation	Conclusion
+	+	+	2019-nCoV RNA detected	Proceed for further analysis
-	+	+		
-	-	+	2019-nCoV RNA not detected	
+	-	+	Inconclusive B-beta CoV specific RNA detected	

RESULTS

Table 2 represents the final SARS-CoV-2 results in 96 NP/OP samples using three different RNA extraction kits. These results were interpreted as per the manufacturer's criteria.

The CT values for gene E were similar in most samples, without significant variations. For the analysis of gene N, Himedia showed higher CT values in comparison with other kits. As for the IC, for all the samples, Ct came within the range of 25-30 and there was no significant difference among the mean Ct values of HiMedia, Perkin and Zybio Extraction Kits.

The results presented in Table 2 show that the analyses with Himedia, Perkin and Zybio RNA Extraction kits were the same regarding the final outcome (detected or not detected) for individual samples; however, results from Perkin, Zybio presented 100% sensitivity while Himedia presented 97.9% sensitivity to the original results after first analysis for diagnosis. The original Ct values used for diagnosis were obtained using Quantiplus Multiplex COVID-19 Detection Kit. No false positive SARS-CoV-2 results were detected using the three RNA extraction kits (specificity of 100.0%).

Zybio was compared with Himedia, taken as the gold standard. The performance metrics including accuracy; the area under the receiver operating characteristics (AU-ROC) curve, sensitivity, and specificity were 99%, 99%, 100%, and 98%, respectively (Table 3).

Similarly, Perkin was compared with Himedia, taken as the golden standard. The performance metrics including accuracy; the area under the receiver operating characteristics (AU-ROC) curve, sensitivity, and specificity were 99%, 99%, 100%, and 98%, respectively (Table 4).

Table 2: Comparison of different kits used in their positivity and compatibility.

Medium	RNA extraction procedure	Kit/ protocol	Original positive results	Positive	Negative	Total	Positivity percentage	Concordance with original results for diagnosis
VTM	Perkin	Quantiplus Multiplex COVID-19 detection kit.	48	48	48	96	100	Yes
VTM	Himedia		48	47	49	96	97.9	Yes
VTM	Zybio		48	48	48	96	100	Yes

Table 3: Comparison of HiMedia and Zybio.

Confusion matrix			
	Zybio		
HiMedia	**Negative**	**Positive**	**% Correct**
Negative	49	1	98
Positive	0	47	100
Overall % correct			98.969
Performance metrics		**P value**	
Accuracy		0.99	
AUC		0.99	
Sensitivity		1	
Specificity		0.98	

Table 4: Comparison of HiMedia and Perkin.

Confusion matrix			
	Perkin		
HiMedia	**Negative**	**Positive**	**% Correct**
Negative	48	1	98
Positive	0	47	100
Overall % correct			98.969
Performance metrics		**P value**	
Accuracy		0.99	
AUC		0.99	
Sensitivity		1	
Specificity		0.98	

Table 5: Comparison of Perkin and Zybio.

Confusion matrix			
	Perkin		
HiMedia	**Negative**	**Positive**	**% Correct**
Negative	49	0	100
Positive	0	48	100
Overall % correct			100
Performance metrics		**P value**	
Accuracy		1	
AUC		1	
Sensitivity		1	
Specificity		1	

Perkin was also compared with Zybio, taken as the golden standard. The performance metrics including accuracy; the area under the receiver operating characteristics (AU-ROC) curve, sensitivity, and specificity were 100%, 100%, 100%, and 100%, respectively (Table 5).

Table 6: Correlation of CT values.

Pearson's correlations		Pearson's r	P value
HiMedia	Perkin	0.601	<0.001
HiMedia	Zybio	0.636	<0.001
Perkin	Zybio	0.66	<0.001

The significant correlation between the CT values of these tests is shown in table 6.

Ultimately, the obtained results showed that all the three kits were effective in providing nucleic acid from samples in the different media tested. The detection of genes N, E and IC were found viable in all the biological samples.

DISCUSSION

The COVID-19 pandemic is still ongoing worldwide, causing severe illness and death. Although rRT-PCRs were proven to be efficient and sensitive approaches for COVID-19 diagnosis, the extraction efficiency influences significantly the yield of RNA. Thus, the method used for RNA extraction is the most important variable to determine the positivity of sample for SARS-CoV-2 genome.[16]

We tested samples from a biorepository with three RT-qPCR kit/protocols. The results obtained by the COBAS SARS-CoV-2 test (Roche diagnostics) were considered as truly positive and negative samples, and the sensitivity and specificity were compared accordingly. All the three RNA Extraction procedure proved to be efficient for identification of specific genes of SARS-CoV-2 genome. For rRTPCR assays, among many, the N gene was chosen because it is highly abundant during viral replication and conserved among coronaviruses.[17]

In this study some samples subjected to the RNA extraction procedure via HiMedia were reported with low sensitivity and increased Ct values. The reason can be attributed to the presence of residues (a multitude of nucleic acids and proteins) in the samples or by RNA fragmentation during heating.[18] In addition, results through Himedia might show errors due to magnetic beads in the elusion plates as they inhibit PCR detection and amplification methods.[19] Hence this should be duly noted during reporting if high Ct values of the samples are obtained as was in the case of HiMedia.

According to our comparative analysis, Perkin Elmer® Coronavirus Nucleic Acid Detected Kit was found to have the lowest limit of detection among the used extraction kits making it the most sensitive commercially available molecular kit, as also supported by FDA Reference Panel.[20]

Because of the reason mentioned above (Ct value) and due to cost-effectiveness, sensitivity, expensive buffer, time consumption of RNA Extraction etc. several recent studies now have been conducted to bypass RNA extraction using only hot shock procedures without buffer addition.

We compared the three Extraction procedures based on parameters like no. of steps, turnaround time, ease of performance, sensitivity and limitation as follows:

After analysing the parameters, Perkin Extraction Kit was found to be the best choice during outbreaks.

This study has few limitations. A dataset consisting of 96 samples might lack the statistical power needed for robust analysis. Ct values, between 20 and 32 were observed in samples but do not capture low viral loads; this may result in overlooking cases with weak positivity potential. Conducting the research at an institution could restrict its relevance, to clinical environments that follow different protocols. Relying on COBAS as the only reference platform may overlook variability from different assays

CONCLUSION

Many laboratories around the world are already using rapid nucleic acid extraction reagents to promptly obtain nucleic acids for RT-PCR assay of SARS-CoV-2. Fast RNA extraction reduces costs with reagent and plastics, reduces the overall time to diagnose and reduce the enormous pressure that the technicians suffer during routine assays.

Concluding the comparison of the three procedures in our study, Himedia has high equipment cost and showed high CT in some samples, it has moderate performance with less handling issues and turnaround time. Zybio has high sensitivity, lowest equipment cost, and min. turnaround time. Perkin, though having more turnaround time, has highest sensitivity. Hence, during increased demands in outbreaks, Perkin followed by Zybio should be preferred for the timely results and in cases of limited resources and increased healthcare expenditure.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Eisen AK, Demoliner M, Gularte JS, Hansen AW, Schallenberger K, Mallmann L, et al. Comparison of different kits for SARS-CoV-2 RNA extraction marketed in Brazil. BioRxiv. 2020:2020-05.
2. Worldometer. Reported cases and deaths by country or territory. Available at: https://www.worldometers.info/coronavirus/#countries. Accessed 10th May 2022.
3. World Health Organisation. Naming the coronavirus disease (COVID-19) and the virus that causes it. Available at: https://www.who.int/emergencies/diseases/novelcoronavirus2019/technical-guidance/naming-the-

coronavirus-disease-(covid-2019)-and-thevirus-thatcauses-it. Accessed 10th May 2022.
4. Cucinotta D, Vanelli M. WHO Declares COVID-19 a Pandemic. Acta Biomed. 2020;91(1):157-60.
5. Helmy YA, Fawzy M, Elaswad A, Sobieh A, Kenney SP, Shehata AA. The COVID-19 pandemic: a comprehensive review of taxonomy, genetics, epidemiology, diagnosis, treatment, and control. J Clin Med. 2020;9(4):1225.
6. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3.
7. Zhang T, Wu Q, Zhang Z. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 Outbreak. Curr Biol. 2020;30(7):1346-1351.e2.
8. Lotfi M, Hamblin MR, Rezaei N. COVID-19: Transmission, prevention, and potential therapeutic opportunities. Clin Chim Acta. 2020;508:254-66.
9. Ghaderzadeh M, Asadi F. Deep learning in the detection and diagnosis of COVID-19 using radiology modalities: a systematic review. J Healthc Eng. 2021;2021(1):6677314.
10. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2021;19(3):141-54.
11. Mayo Clinic. Coronavirus disease 2019 (COVID-19). Available at: https://www.mayoclinic.org/diseases-conditions/coronavirus/symptoms-causes/syc-20479963. Accessed 12th May 2022.
12. Güner R, Hasanoğlu I, Aktaş F. COVID-19: Prevention and control measures in community. Turk J Med Sci. 2020;50(SI-1):571-7.
13. Hozhabri H, Piceci Sparascio F, Sohrabi H, Mousavifar L, Roy R, Scribano D, et al. The Global Emergency of Novel Coronavirus (SARS-CoV-2): an update of the current status and forecasting. Int J Environ Res Public Health. 2020;17(16):5648.
14. Liu R, Han H, Liu F, Lv Z, Wu K, Liu Y, Feng Y, Zhu C. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. Clin Chim Acta. 2020;505:172-5.
15. Rong G, Zheng Y, Chen Y, Zhang Y, Zhu P, Sawan M. COVID-19 diagnostic methods and detection techniques. Encycloped Sensor Biosens. 2022:17.
16. Ambrosi C, Prezioso C, Checconi P, Scribano D, Sarshar M, Capannari M, et al. SARS-CoV-2: Comparative analysis of different RNA extraction methods. J Virol Meth. 2021;287:114008.
17. Li D, Zhang J, Li J. Primer design for quantitative real-time PCR for the emerging Coronavirus SARS-CoV-2. Theranostics. 2020;10(16):7150.
18. Smyrlaki I, Ekman M, Lentini A, Rufino de Sousa N, Papanicolaou N, Vondracek M, et al. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. Nature communicat. 2020;11(1):1-2.
19. Berensmeier S. Magnetic particles for the separation and purification of nucleic acids. Appl Microbiol Biotechnol. 2006;73(3):495-504.
20. Food and Drug Administration. SARS-CoV-2 Reference Panel Comparative Data. Available at: https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2reference-panel-comparative-data. Accessed 14th May, 2022.

Cite this article as: Jhajharia A, Bhargava R, Malhotra B, Tak A, Gupta P. Comparative analysis of different RNA extraction kits used in detection of Covid-19 VIRUS. Int J Res Med Sci 2024;12:4574-9.