ISSN: 0975-1556

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2022; 14(2); 257-264

Original Research Article

Comparison of Different RNA Extraction Methods for Detection of SARS-Cov-2by RT-PCR

Dinesh Kumar Jain[1], Manish Kumar[2], Shivra Batra[3], Parul Sinha[4], Sandeep Gupta[5], Nitya Vyas[6]

[1,2,3,4]Associate Professor, SMS Medical College, Jaipur

[5]Senior Demonstrator, SMS Medical College, Jaipur

[6]Senior Professor, SMS Medical College, Jaipur

Received: 03-12-2021 / Revised: 30-12-2021 / Accepted: 28-01-2022
Corresponding author: Dr Parul Sinha
Conflict of interest: Nil

Abstract

Background: Corona virus disease 2019 is a highly infectious disease which is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2. SARS-CoV-2 is transmitted from person to person mainly by respiratory droplets and aerosols as well as by direct or indirect contact.

Aims and objective: To compare different RNA extraction methods for detection of SARS-Cov-2 RNA from nasopharyngeal and oropharyngeal swabs using three different methods which are based on different techniques.

Material and methods: This analytical observational study was conducted in the department of Microbiology, Sawai Man Singh Medical College Jaipur, Rajasthan from December 2020 to January 2021. We selected 200 confirmed positive (extracted by Easy Mag automated system) (remnant) samples showing a wide range of different Ct values and 20 confirmed negative samples stored in Viral Transport Media VTM for this study.

In order to compare quality of three extractions methods, all samples were aliquoted separately for each extraction technique. (1) Extraction by manual method (spin column base): was done by as per manufacturer's instructions. (2) Extraction by QIA cube HT (vaccum column base): was done by as per manufacturer's instructions. (3) Extraction by Perkins Elmer chemagic 360: (magnetic beads based).

Result: A panel consisting of 200 Covid-19 positive and 20 Covid-19 negative samples were extracted by three methods (i.e. Manual column based, automated column-based and automated magnetic beads-based method). The extracted material/elutes were put for real-time RT-PCR assay for the detection of SARS CoV-2 RNA. There was no major difference seen in individual samples' ct values between three extraction system. CONCLUSION: In conclusion, we recommended all three RNA extraction methods (i.e. magnetic beads & silica column-based) are interchangeable in a diagnostic workflow for the SARS CoV-2 by RT-PCR and can be taken into account for SARS CoV-2 detection in possible future shortage of one kit or times of crisis in such pandemic time.

Keywords: RT-PCR, SARS CoV-2, RNA Extraction, Nasopharyngeal Swab, Oropharyngeal Swab.

Introduction

Corona virus disease 2019 (COVID-19) is a highly infectious disease which is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a member of the Coronavirus family. At the end of 2019, it was first reported in Wuhan, Hubei Province of China.SARS-CoV-2 is transmitted from person to person mainly by respiratory droplets and aerosols as well as by direct or indirect contact[1]. Symptomatic cases of COVID-19 are the main source of transmission, while asymptomatic and pre-symptomatic infected individuals might also be the potential sources of SARS-CoV-2 infection[2]. These characteristics may explain the rapid pan epidemic spread of this virus[1]. Thus in a short time SARS-CoV-2 spread from China to a dozen of countries and within a few months developed into a pandemic (outbreak)[2].

Social distancing and large-scale testing of the population are the essential measures required to control the current COVID-19 pandemic[3].

The World Health Organisation (WHO) recommends several public health

Measures[4].

I. Rapid diagnosis and immediate isolation of the cases
II. Rigorous tracking
III. Precautionary self-isolation

Though many efforts are being put forth on fast development of novel rapid and reliable diagnostic tests, to date, real time reverse transcriptase PCR or RT- PCR test for respiratory samples is still considered the gold standard to detect SARS-CoV-2 infection[5].

RT- PCR is highly sensitive and specific viral detection method as compare to other methods (e.g. viral antigen test, serology, electron microscopy) and is based on viral RNA extraction from respiratory specimens (i.e. Nasopharyngeal swabs or Oropharyngeal swabs) followed by highly sensitive reverse transcription and PCR[6].

RT- PCR result can be considerably affected by the efficiency of viral RNA extraction procedures and there are several methods that are used in molecular microbiology to separate RNA from clinical specimens[1].

The unprecedented global demand for commercial RNA extraction kits and ensuing shortage of these reagents [7] led to the establishment of several diagnostic workflows performed on patient samples with or without an intermediate RNA extraction step[7-11].

Viral RNA isolation from clinical samples depends on the rapid inactivation of virus particles, typically by detergent solubilization, and on the denaturation of ubiquitous RNases[12]. The latter may be accomplished by the use of chaotropic chemicals, such as guanidinum salts[13] or non-specific proteases that are active on both negative and denatured proteins such as proteinase K.

In either case, after virus particles lysis, RNA must be purified, since guanidinium salts, proteinase K and organic solvent inhibit the subsequent RT PCR steps.

RNA can be separated from proteins either by liquid phase separation using chloroform-aqueous emulsions after lysis with commercially available Trizol

(a mixture of guanidiniumthiocyanate and acid phenol) or by means of solid phase separation using silica[14]. Nucleic acid binding to negatively charged silica (SiO2) is facilitated by guanidinium salts and the basic pH of the lysis buffer[15].

To achieve a higher Nucleic acid binding capacity, silica-based Nucleic acid

extraction methods use either porous silica matrices that are embedded in column (spin column) G12, a tip (true tips)[16], or a suspension of micro-particles can be separated from the lysate either by centrifugation or by a magnetic field provided that the microparticle dense iron-containing cores are coated with porous silicon[17].

The efficiency of extraction method efficiency influences notably the yield of RNA and eventually RT- PCR result. This yield of RNA can be considerably affected by the efficiency of viral RNA extraction methods/kits.

Apart from this, several methods/kits are available in market & every commercial kit manufacturer claims their extraction methods/kits as rapid, reliable, cost-effective, sensitive and reproducible. In such pandemic times, when scarcity of diagnostic kits is predictable, the sharing of various protocols for RNA extraction among laboratories is required so-that laboratories doing RNA extraction for COVID-19 detection can choose reliable extraction methods.

Protocols and devices used for nucleic acid extraction have evolved from thiocynate-phenol-chlorofom manual techniques to user friendly column technology and automated platforms, but no general gold standard method has yet been established.

We have chosen Easy Mag Automated extraction system as a standard method of extraction, considering it as the most sensitive method present in our lab in view of the fact that the amount of sample taken in this system (i.e. 400 ml) is almost double in comparison to other extraction systems.

The objective of present study was to compare different RNA extraction methods for detection of SARS-Cov-2 RNA from nasopharyngeal and oropharyngeal swabs using three different methods which are based on different techniques (i.e. Manual spin column based, Automated vaccum column-based and automated magnetic beads based method).

Material and methods:

Sample collection

This analytical observational study was conducted in the department of Microbiology, Sawai Man Singh Medical College Jaipur, Rajasthan from December 2020 to January 2021.

We selected 200 confirmed positive (extracted by Easy Mag automated system) (remnant) samples showing a wide range of different Ct values and 20 confirmed negative samples stored in Viral Transport Media VTM (Vitromed healthcare, Biotech Park, Jaipur, Rajasthan) for this study.

In order to compare quality of three extractions methods, all samples were aliquoted separately for each extraction technique.

1. Extraction by manual method (spin column base): was done by as per manufacturer's instructions.
2. Extraction by QIA cube HT (vaccum column base): was done by as per manufacturer's instructions.
3. Extraction by Perkins Elmer chemagic 360: (magnetic beads based):

Was done after thorough vortexing , followed by brief centrifugation of the VTM, 300 µl of the sample was transferred to a 96 deep well processing plate to which 4 µl Poly (A) RNA , 10 µl of proteinase K, 300 µl lysis buffer along with 150µl magnetic beads and 900 µl of RNA binding buffer were previously dispensed.

The beads/ RNA mixture was washed with washing buffer and elutes were obtained in elution buffer in the automated system (PerkinsElmerchemagic 360).

Real time PCR (TruPCR master mix)

The primers used in TruPCR RT PCR kit are designed to target E gene, N/RdRp and RnasePgenes. For PCR, 10 ul RNA and 15 ul PCR master mix solution containing 10 ul master mix reagent, 0.35 ul Enzyme mix and 4.65 ul of primer probe mix. Cyclic conditions used as per the manufacturer's instructions were 50° C for 15 mins, 95°C for 5 mins, then 38 repeat cycles of 95°C for 5 secs, 60°C for 40 secs and 72°C for 15 secs, using BioradCFx platform.

Interpretation: The data was tabulated, and interpretation was done by using percentages.

Results:

Table 1: Analytical Sensitivity of three Extraction Methods

S. No	Extraction Method	RDRP or E and Rnase-P Gene Detected In Total 200 Covid-19 Positive Samples	
		Neat Sample	Sample at 1:10 Dilution
1.	Manual (Spin Column based)[@]	194(97%)/200	194(97%)/200
2.	Automated (Spin Column based)[#]	192(96%)/200	192(96%)/200
3.	Automated (Magnetic beads based)$	198(98%)/200	198(98%)/200

@ HI Media, # Qiacube HT from Qiagen, $perkins

Table 2:

Interpretation	Manual (Spin Column based)[@]	Automated (Spin Column based)[#]	Automated (Magnetic beads based)$
Positive*	193	188	198
Negative^	1	4	2
Invalid**	6	8	2
Extraction done[##]	194 (97%)	192 (96%)	198(99%)

@ HI Media, # Qiacube HT from Qiagen, $perkins

Table 3:

Interpretation	*RDRP*	*E*	*RNaseP*	Extraction[##]
*Positive**	+	+	+	Done
*Negative^*	_	_	+	Done
(Inconclusive result) IR[%]	+	+	_	Done
*Invalid***	_	_	_	Not Done
Result Interpretation		RDRP	E gene	RNaseP (Internal control)
Novel coronavirus SARS CoV-2 RNA detected		+	+	+/-
Novel coronavirus SARS CoV-2 RNA NOT detected		_	--	+
(Inconclusive result) IR		--	+	+/-
Invalid		_	_	_

Positive, Negative^, (Inconclusive result) IR[%], Invalid***

A panel consisting of 200 Covid-19 positive and 20 Covid-19 negative samples were extracted by three methods (i.e. Manual column based, automated column-based and automated magnetic beads-based method). The extracted material/elutes were put for real-time RT-

PCR assay for the detection of SARS CoV-2 RNA. All samples were also tested at a 1:10 dilution to detect the extraction efficiency and presence of PCR inhibitors if any.

Positive and negative controls were also put in every PCR run simultaneously.

Out of 200 Covid-19 positive samples, 194 (97%) samples showed E/RDRP and RNaseP genes with manual spin column-based method. (Hi-media) **Table 2** 6 (3%) samples showed invalid results where no gene were detected. **Table 2** By using Qia cube HT (Automatedvaccumcolumn based system from Qiagen) out of the 200 Covid-19 positive samples, 192 (96%) samples showed E/RDRP and RNase-P genes and 8(4%) samples showed invalid results. **Table 2**

With Automated Magnetic beads based extraction system, out of 200 Covid-19 positive samples, 198 (99%) samples showed E/RDRP and RNase-P genes. Only 2(1%) samples showed invalid results. **Table 2**

All 20 Covid-19 negative sample showed negative results by three methods without any invalid result.
There was no difference noted between neat sample and 1:10 diluted samples.

Positive control and negative control showed valid results.

There was no major difference seen in individual samples' ct values between three extraction system. (data not shown). (Difference of more than 3CT value between three extraction system for every sample were considered)

Table 4.: Time & Cost Comparison for three Extraction Methods

S. No	Extraction Method	No. of extracted specimens	Time Min/Run	Aprox. Cost In INR
1.	Manual (Spin Column based)	94 (24x4)	200 min	60
2.	Automated (Column based)	94	60-120 min	200
3.	Automated (Magnetic beads based)	94	40-45min	140

@ HI-Media, # Qiacube HT from Qiagen, $perkins

Discussion:

The Covid 19 pandemic is still ongoing globally, causing severe illness and deaths.

Although many antigen detecting rapid tests have been developed, detection of viral RNA by RT/PCR from oro nasopharyngeal swabs is still the most sensitive test to confirm SARS CoV-2 suspected cases.(Ad)

RNA extraction /isolation is the main step in diagnosis of Covid -19 and though many laboratories have extraction system available automated, many others mainly in developing countries do not have these options.

Besides this, shortage of products may lead to a situation that even automated laboratories may have to shift to manual extraction process to fulfill the diagnostic requirements.

Since the extraction efficiency influences notably the yield of RNA, laboratories doing RNA extraction for COVID-19 detection should choose reliable extraction methods.

In such pandemic time whenever a specifickit could be in short supply due to great demand of materials and reagents or dependencies on single suppliers may led to facing long delivery time when an increasing number of patients wait for diagnostic result, an alternative,

interchangeable, and reliable extraction method should be available.

In this study, performance of alternative methods of three nucleic acid extractions methods (i.e. automated magnetic beads, automated& manual silica column-based) were compared. We achieved diagnostic quality RT PCR results with all three methods, and there was no significant difference seen between the tested methods. The column-based silica methods (manual as well as automated) presented results almost as good as good as the magnetic beads method. *were comparable with both techniques.*

For the automated magnetic beads and automated silica column-based method, the Ct values reported for the SARS-CoV-2 genes were similarly comparable to those reported for the Manual method.

Automated magnetic beads-based technique is cheaper and took less processing time in comparison to automated column-based silica method. Table 4

Similar to our findings, other studies that have compared the magnetic beads with silica Column-based methods for human gut microbial community profiling obtained higher amounts of nucleic acids extracted with the magnetic beads methods and greater species diversity, it also showed that manual extraction presented similar results to the automated ones when taking into account the reproducibility of microbial profiles, although it might be different for nucleic acid yields[18].

However, most studies that have done comparison of different extraction methods have used stool samples for bacterial detection[19-20].

It is suggested by the present study that the manual method is a reliable method for RNA extraction and fully comparable to automated systems with respect to detection of SARS-COV-2 in oropharyngeal samples collected from patients with suspected cases of COVID-19. It is favored among scientists for small scale RNA purification setups, due to its low cost, robustness, versatility and ease of use. Table3 This method can be easily scalable to volumes usable for clinical diagnostics as a supplement to conventional automated systems[21].

While there are numerous advantages to the Manual method, there are also inherent limitations. In comparison to automated RNA extraction systems there is extensive hands-on time, technically demanding and inadvertently risks of human errors. However, well established workflows can minimize these risks to very low levels[21].

As technology and demand both trend upward, it’s becoming increasingly apparent that automation in labs is the next step in molecular microbiology research. Automated extraction systems tend to be specifically designed instruments that help simplify and increase output of nucleic acid extraction. Not only do automated machines decrease turnaround time and labor costs needed, it also increases safety, quality, and reliably high yield. Table3A major concern in the implementation of automated instrumentation to extract nucleic acid for use in amplification assay arethe potential for cross contamination of negative specimens as consequences of aerosolization, faulty robotics, or robotic error. However we did not find any contamination in all negative samples.Ref

In conclusion, we recommended all three RNA extraction methods (i.e. magnetic beads & silica column-based) are interchangeable in a diagnostic workflow for the SARS CoV-2 by RT-PCR and can be taken into account for SARS CoV-2 detection in possible future shortage of one kit or times of crisis in such pandemic time.

Reference:

1. Ambrosi C, Prezioso C, Checconi P, Scribano D, Sarshar M, Capannari M, Tomino C, Fini M, Garaci E, Palamara AT, De Chiara G, Limongi D. SARS-CoV-2: Comparative analysis of different RNA extraction methods. J Virol Methods. 2021 Jan; 287:114008.
2. Cucinotta D, Vanelli M. WHO Declares COVID-19 a Pandemic. Acta Biomed. 2020 Mar 19;91(1):157-160.
3. Comparison of Different Kits for SARS-CoV-2 RNA Extraction Marketed in Brazil Ana Karolina Antunes Eisen, Meriane Demoliner, Jul iana Schons Gularte, Alana Witt Hansen, Karoline Schallenberger, Larissa Mallmann, BrunaSaraiva Herm ann, Fágner Henrique Heldt, Paula Rodrigues de Almeida, Juliane Deise Fleck, Fernando Rosado Spilk.bioRxiv 2020.05.29.1223 58.
4. WHO, Strategies and plans.online under: https://www.who.int/emergencies/disea ses/novelcoronavirus-2019/strategies- and-plans, 2020. (WHO,2020a)
5. Klein S, Müller TG, Khalid D, Sonntag-Buck V, Heuser AM, Glass B, Meurer M, Morales I, Schillak A, Freistaedter A, Ambiel I, Winter SL, Zimmermann L, Naumoska T, Bubeck F, Kirrmaier D, Ullrich S, Barreto Miranda I, Anders S, Grimm D, Schnitzler P, Knop M, Kräusslich HG, Dao Thi VL, Börner K, Chlanda P. SARS-CoV-2 RNA Extraction Using Magnetic Beads for Rapid Large-Scale Testing by RT-qPCR and RT-LAMP. Viruses. 2020 Aug 7;12(8):863.
6. RNA Extraction Kits for COVID-19 Tests Are in Short Supply in US The Scientist. [(accessed on 6 August 2020)]; Available online: /www.the-scientist.com/news-opinion/rna-extraction-kits-for-covid-19-tests-are-in-short-supply-in-us-67250
7. Aitken J., The Crick COVID-19 Consortium. Ambrose K., Barrell S., Beale R., Bineva-Todd G., Biswas D., Byrne R., Caidan S., Cherepanov P., et al. Scalable and Robust SARS-CoV-2 Testing in an Academic Center. Nat. Biotechnol. 2020; 38:927–931.
8. Guruceaga X., Sierra A., Marino D., Santin I., Nieto-Garai J.A., Bilbao J.R., Lorizate M., Aspichueta P., coBIG (COVID19 Basque Inter-institutional Group) Mayor U. Fast SARS-CoV-2 Detection Protocol Based on RNA Precipitation and RT-qPCR in Nasopharyngeal Swab Samples. medRxiv. 2008
9. Zhao Z., Cui H., Song W., Ru X., Zhou W., Yu X. A Simple Magnetic Nanoparticles-Based Viral RNA Extraction Method for Efficient Detection of SARS-CoV-2. bioRxiv. 2020
10. Thi V.L.D., Herbst K., Boerner K., Meurer M., Kremer L.P., Kirrmaier D., Freistaedter A., Papagiannidis D., Galmozzi C., Stanifer M.L., et al. A Colorimetric RT-LAMP Assay and LAMP-Sequencing for Detecting SARS-CoV-2 RNA in Clinical Samples. Sci. Transl. Med. 2020:eabc7075
11. Lever M.A., Torti A., Eickenbusch P., Michaud A.B., Antl-Temkiv T.Å., Jã¸rgensen B.B., Šantl-Temkiv T., Jørgensen B.B. A Modular Method for the Extraction of DNA and RNA, and the Separation of DNA Pools from Diverse Environmental Sample Types. Front. Microbiol. 2015; 6:476.
12. Chomczynski P., Sacchi N. The Single-Step Method of RNA Isolation by Acid GuanidiniumThiocyanate–Phenol–Chloroform Extraction: Twenty-Something Years on. Nat. Protoc. 2006; 1:581–585.
13. Boom R., Sol C.J., Salimans M.M., Jansen C.L., Dillen P.M.W.-V., Van Der Noordaa J. Rapid and Simple Method for Purification of Nucleic Acids. J. Clin. Microbiol. 1990; 28:495–503.

14. Vandeventer P.E., Lin J.S., Zwang T., Nadim A., Johal M.S., Niemz A. Multiphasic DNA Adsorption to Silica Surfaces under Varying Buffer, pH, and Ionic Strength Conditions. J. Phys. Chem. B. 2012; 116:5661–5670.
15. Holmberg R., Gindlesperger A., Stokes T., Brady D., Thakore N., Belgrader P., Cooney C.G., Chandler D.P. High-throughput, Automated Extraction of DNA and RNA from Clinical Samples Using TruTip Technology on Common Liquid Handling Robots. J. Vis. Exp. 2013;76:e50356.
16. Lee A.H., Gessert S.F., Chen Y., Sergeev N.V., Haghiri B. Preparation of Iron Oxide Silica Particles for Zika Viral RNA Extraction. Heliyon. 2018;4:e00572.
17. Lim MY, Song EJ, Kim SH et al (2018) Comparison of DNA extraction methods for human gut microbial community profiling. SystApplMicrobiol 41.2 151-157
18. Mirsepase H, Persson S, Struve C et al (2014) Microbial diversity in fecal samples depends on DNA extraction method: easyMag DNA extraction compared to QIAamp DNA stool mini kit extraction. BMC Res. Notes 7.1 50
19. Persson S, de Boer RF, Kooistra-Smid AMD et al (2011) Five commercial DNA extraction systems tested and compared on a stool sample collection. Diagn Micr Infec Dis 69.3 240-244.
20. Dimke H, Larsen SL, Skov MN, Larsen H, Hartmeyer GN, Moeller JB. Phenol-chloroform-based RNA purification for detection of SARS-CoV-2 by RT-qPCR: Comparison with automated systems. PLoS One. 2021 Feb 24;16(2):e0247524.