Simplified Magnetic Bead Based Viral RNA Extraction for Rapid Detection of COVID-19

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Abstract

Rapid and large-scale diagnosis has helped in mitigation the recent ongoing pandemic of corona virus disease of 2019 (COVID-19). The pandemic had a devastating effect on global economy. The molecular detection system has evolved over last two decades and is rapidly replacing the conventional confirmatory techniques in diagnostic virology. However the major limitation in implementation of available molecular detection assays is the non availability of field deployable nucleic acid isolation platform. The standard laboratory diagnosis rely on confirmation of presence of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in respiratory specimens of suspected patients. Preparation of viral nucleic acid is a critical step involved followed by downstream molecular diagnostic platforms. For good quality of viral RNA extraction many commercial extraction kits, are available. These are developed in a surge of pandemic scenario keeping in view the large demand for testing. The commercial RNA extraction kits available on either column based or magnetic extraction are limited and, alternative, non-commercial protocols are rapidly required. Here, we have standardized an in-house magnetic bead RNA extraction method which utilises simple in-house reagents and manual extraction method that doesn't require any high-end equipments. The in-house assay was evaluated against the commercial available silica column and magnetic extraction kits using a panel of 100 throat /nasal swab samples. A high correlation in viral RNA detection with TaqMan qRT-PCR was observed with excellent sensitivity and specificity. Interestingly, the developed method is very simple, cost effective, rapid and can be quickly add up any downstream amplification platform for SARS-CoV-2 detection.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported from Wuhan in China in December 2019. Subsequently, there is a rapid spread of this virus across the globe. India also witnessed the emergence and rapid spread across the country. Currently, India occupies the 2nd position in the numbers of SARS CoV-2 positive cases. To slow its spread, large-scale diagnostics and the enforcement of strict public health measures are required to be continuously implemented. The current standard test for SARS-CoV-2 detection and diagnosis is based on viral RNA extraction from a naso-pharyngeal swab followed by highly sensitive reverse transcription and quantitative PCR (RT-qPCR). Several primer sets targeting one or more of the SARS-CoV-2 genes—nucleocapsid (N), envelope protein (E), Spike glycoprotein (S), or RNA-dependent RNA polymerase (RdRp), Open Reading Frame (ORF) 1ab have been used in both singleplex and multiplex formats^{1–5}. At a global scale, there is a shortage in availability of commercial RNA extraction kits was observed in this current pandemic scenario and this led to shortage of the RNA extraction reagents in the establishment of molecular diagnostic platforms for patient samples^{6–7}. The isolation of viral RNA from a clinical sample depends on the rapid inactivation of viral particles, like by add of solubilisation buffers, and the denaturation of RNases that are omnipresent⁸. The later may be accomplished by the use of chaotropic chemicals, such as guanidinium salts⁹ or proteases that are active on proteins, like proteinase K. After virus particle lysis, RNA must be purified, since guanidinium salts, proteinase K and organic solvents inhibit the subsequent RT-qPCR step. RNA

purification from proteins either liquid phase separation applying chloroform-aqueous phase with the add of Trizol or by applying silica as a solid phase¹⁰. Negatively charged silica (SiO₂) bind to nucleic acid which is facilitated by guanidinium salts and the pH of lysis buffer¹¹. Silica-column based nucleic acid extraction methods use either porous silica matrices embedded in spin column¹² or a tip like TruTips¹³, or a suspension of microparticles to achieve a high nucleic acid binding capacity. These microparticles than separated from the lysate by applying centrifugation or magnetic field iron cores coated with porous silica.

The protocol established in this study aimed at extracting SARS-CoV-2 RNA from respiratory patients swabs (oropharyngeal and nasopharyngeal) and is based on in-house synthesized magnetic bead and buffers based nucleic acid extraction protocol. Magnetic bead RNA extraction was performed manually with the use of magnetic stand. The manual pipetting system, which is less expensive than automated pipetting robots, was used to minimize the pipetting and handling errors. Here, we showed that the yield of in-house magnetic bead-based RNA extraction protocol is comparable to the commercially available Qiagen and magnetic bead based viral RNA extraction kit, as determined by the commonly applied amplification methods RT-qPCR.

Methods

Clinical samples and sample lysis

A selection of upper respiratory tract specimens (Nasopharyngeal/Throat swab) sent to the Defence Research & Development Establishment, Gwalior Madhya Pradesh India through different medical health authorities for laboratory diagnosis of SARS-CoV-2 were used for the study. A total of 100 residual anonymized and de-identified swab samples including 40 SARS-CoV-2 positive and 60 negative by RT-qPCR were included in the study. The following steps were performed in a biosafety level 3 (BSL-3) laboratory according to standard microbiological and diagnostic practices. The experimental protocols of this study were approved by Defence Research and Development Establishment- Institutional Biosafety Committee, (DRDE-IBSC) vide no IBSC/VIRO-01/2021/PKD. Ethical approval and informed consent waiver for the study has been granted vide no. VCH/VEC/June-2021/04 of Vidya Ethics Committee, Gwalior, India. All methods were performed in accordance with the relevant national biosafety guidelines and regulations.

Magnetic bead particles were prepared using modified Stober's process¹⁴. Briefly, five hundred milligram of Fe₂O₃ nanoparticles (Aldrich, USA, Cat. No. 544884) were sonicated (30 min) in a solution having 320 ml of ethanol and 80 ml of water followed by addition of 10 ml of 30% NH₄OH solution. To this solution was added 1 gm of tetraethyl orthosilicate (Aldrich, USA, Cat No. 86578) in 45 min and the contents were allowed to stir at room temperature for additional 24 h. Thus formed silica coated nanoparticles were then separated under magnetic field, washed repeatedly with ethanol and finally dried under vacuum (70°C, 24 h). The average size of particles was found 2.3 µm and BET surface area was 17 m²/g. Finally these particles were used as suspension of 50 mg of particles/ml nuclease free water.

Buffers

Three buffer solutions were employed for extraction of viral RNA *viz*, lysis/binding buffer (3.96% Ammonium sulphate, 0.8% NP-40 in 0.2M Tris acetate, pH – 4), washing buffer (0.5% NP-40 in 0.01 M Tris-HCl, (pH - 6.8)) and elution buffer (10 mM Tris-HCl, (pH- 8.5)).

Extraction of viral RNA employing silica coated magnetic bead Thirty micro liter of magnetic bead suspension were added to lysis buffer (760 µl) and sample (nasal/throat swab (140 µl), further the tubes were incubated at room temperature for 8 min. Subsequently, proteinase K (25µl) was added, to the tubes and further incubated for additional 2 min. The magnetic bead particles were separated and supernatant was discarded. Subsequently, washing buffer (500 µl) containing proteinase K solution (2.5 ml) (20mg/ml) was added, and the beads were mixed vigorously. Further the mix was incubated for 10 min with washing buffer, the magnetic beads particles were separated by applying magnetic field and the remaining supernatant was pipetted out. The washing step was repeated again following removal of washing buffer and elution buffer (50 µl) was applied to the bead and mixed for uniform suspension. This was further kept at room temperature for 10 min. Finally the beads were separated and supernatant was collected in RNase-free tube by applying magnetic field. The purity and concentration of eluted RNA was carried out in Qubit 4.0 Fluor meter (Thermo, USA). The suitability of extracted viral RNA for molecular assay was confirmed through SARS-CoV-2 specific TaqMan RT-qPCR²¹. The eluted RNA was stored at -80°C.

Extraction of viral RNA employing Qiagen kit

The extraction of viral RNA was carried out from infected material using the Qiagen viral RNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. In brief, AVL buffer (560 µl) was added to nasopharyngeal/throat swab sample (140 µl) and thoroughly vortexed (15 sec), then the mixture was further kept at room temperature (10 min). Subsequently, ethanol (560 µl) was applied to this mixture and the mixture was passed through QIAamp spin column, by centrifugation (6000rpm, 1 min), the spin column residuals were discarded. Spin column were further sequentially washed using wash buffer AW1(500 µl) and AW2 (500 µl). Finally viral RNA was extracted in elution buffer (50 µl) added to the centre of the column followed by centrifugation at 8000 rpm for 2 min. The purity and concentration of viral RNA was analyzed with Qubit 4.0 Fluormeter (Thermo, USA). The suitability of extracted viral RNA for molecular assay was confirmed through SARS-CoV-2 specific TaqMan RT-qPCR². Finally the eluted RNA (50 µl) was stored at –80 °C.

Extraction of viral RNA employing commercial magnetic beads extraction kit

Viral RNA from clinical samples was extracted using MGI Kit, Wuhan, China using manufacture's protocol. Briefly 200µl of sample was added to 481µl lysis buffer (containing 200 µl MLB, 250µl 100% ethanol, 15µl Proteinase K, 15µl Magnetic Beads and 1µl Enhancer buffer). It was mixed well and centrifuged at 1050 RPM for 2 min. The lysate was then allowed to incubate at 65°C for 5 minutes. Later it was mixed well and placed on magnetic stand for 1 minutes to achieve a magnetic bead pellet and the supernatant

was discarded. Subsequently, 500µl of MW1 buffer followed by 500µl MW2 and 600µl of Absolute ethanol with the similar magnetic bead pelleting procedure as mentioned above was applied and supernatant was discarded. After this, ethanol was dried by subjecting the tube at 56°C for 1 minute. At the end, the viral RNA was eluted in 60µl of RNase free water at 65°C for 10 min and further stored at -80°C until use.

SARS-CoV-2 RNA detection by RT-qPCR

RT-qPCR detection of the SARS-CoV-2 was carried out using both by both screening and confirmatory assay by targeting (E/RNaseP) and (RdRP/ORF1ab gene) respectively ². For the mastermix, 0.5 µl of Primer/Probe mix SARS and Wuhan CoV E-gene (Eurofins, India) and 0.5 µl of Primer/Probe mix Human RNaseP gene (Eurofins, India) or 0.5 µl of Primer/Probe mix SARS and Wuhan CoV RdRP-gene (Eurofins, India) and 0.5 µl of Primer/Probe mix Human ORF1ab gene (Eurofins, India) was mixed with 5.5 µl RNase-free water, 12.5 µl Invitrogen master mix (Thermo, USA), and 0.5 µl reverse transcriptase enzyme (Thermo, USA) per sample. RT-qPCR was performed in ABI 7500Dx Instrument (Thermo, USA) with 30 min of reverse transcription at 56 °C, initial denaturation at 95 °C for 5 min, and subsequent 45 amplification cycles with 95 °C for 5 sec, 60 °C for 30 sec. Cycle threshold (CT) was determined, where the fluorescence signal of the amplification reaction was above the background fluorescence using the ABI software (Thermo). Data analysis on raw CTs was performed in Excel and Correlation between three methods were calculated.

Estimation of the detection limit of magnetic bead RNA extraction using RNaseP

Five µl in vitro transcribed RNA targeting RNaseP gene (6.1×10^{10} copies) was ten fold serially diluted and used in RT-qPCR reaction. 5µl RNA template was added covering a range from 10^1 to 10^6 RNA molecules per reaction. RT-qPCR was performed for human RNaseP gene using similar primer/probe as described earlier for all the three methods i.e. in-house magnetic bead, Qiagen, commercial magnetic bead. RT-qPCR cycling program for RNaseP gene amplification was performed as described in earlier in method section.

Evaluation with clinical samples

One SARS-CoV-2 positive Naso-pharyngeal swab sample, was 10 fold serially diluted in RNase-free water prior to RNA isolation a 10-fold dilution series up to 10^5 fold, in order to compare the magnetic bead RNA extraction protocol with commercial Qiagen and magnetic bead extraction method. The extracted RNA from all three protocols were subjected to RT-qPCR using RNaseP gene primers that act as QC of sample. A panel of 40 SARS-CoV-2 positive and 60 SARS-CoV-2 negative were used for comparative evaluation of all three viral RNA extraction protocols in conjunction to RT-qPCR.

Results

The magnetic bead RNA extraction protocol was established in a simple manual format as part of the detection workflow (Fig. 1). The complete workflow of RNA extraction can be conducted in less than 30 min without use of any electrical equipment.

Initial experiments were performed with silica coated magnetic bead based nucleic acid extraction protocol along with commercially available QIAamp viral RNA kit/ and commercial magnetic bead kit in order to study the relative quality and quantity of RNA. The comparative evaluation revealed the concentration of RNA to be 50ng/μl for in house magnetic bead protocol with an integrity value of 6.0, whereas it was 48ng/μl in Qiagen protocol with an integrity value of 7.0 and concentration of RNA to be 45ng/μl for in commercial magnetic bead protocol with an integrity value of 6.0. The suitability of extracted RNA for both screening & confirmatory molecular assay (TaqMan RT-qPCR) was also confirmed, as demonstrated by positive reaction through amplification curve (Fig. 2). The RNA extracted from a panel of samples through all three methods revealed 100% concordance, which was confirmed though TaqMan RT-qPCR. The CT value obtained is summarized in Table 1.

Table 1

Comparison of viral RNA extraction protocol using in-house silica coated magnetic bead and Qiagen/commercial magnetic bead based viral RNA kit from COVID-19 positive clinical samples.

S No	Details	Commercial Magnetic bead extraction + RT-qPCR (Ct Value E/RNaseP)	Qiagen Extraction + RT-qPCR (Ct Value E/RNaseP)	In-house Magnetic bead extraction + RT-qPCR (Ct Value E/RNaseP)
1	RNA extracted from COVID-19 Positive sample (Low Ct)	20/22	21/23	21/24
2	RNA extracted from COVID-19 Positive sample (High ct)	30/33	32/33	33/34
3	RNA extracted from COVID-19 negative sample	No Ct	No Ct	No Ct
4	PTC	22/23	20/22	23/24
5	NTC	No Ct	No Ct	No Ct

RNA extraction using magnetic beads yields RT-qPCR results that comparable to commercial extraction Kit

Viral RNA was detected in samples diluted up to 10^5 fold after either Qiagen or magnetic bead RNA extraction. RT-qPCR of the extracted RNA by all of the three methods showed approximately equidistant amplification curves with an interval of 2 CT values for each 10-fold dilution step (Fig. 3A-C). The CT value obtained is summarized in Table 1.

A dilution series of human RNaseP RNA from in-vitro transcribed RNA was subjected to RT-qPCR using RNaseP primers to further evaluate linearity of the magnetic bead RNA extraction method across a broad range of defined RNA inputs (Fig. 3A-C). CT values were linear over three different methods that resulted with a goodness of fit of $R^2 = 0.997$ was less than 10 RNA copies/reaction as depicted in (Fig. 3A-D).

RT-qPCR performed on magnetic bead extracted RNA shows high detection sensitivity and specificity

In order to evaluate the magnetic bead RNA extraction protocol on larger sample sets, 40 confirmed SARS-CoV-2 positive and 60 negative samples were included. The samples were subjected to RNA extraction through three independent protocols (Qiagen, Germany; Magnetic bead,Wuhan; In-house magnetic bead). All these RNA were subjected to RT-qPCR using primers for the E gene and RNaseP gene. The sensitivity and specificity of the three independent methods were calculated. The samples were distributed in small groups of positive and negative samples (n = 12). The sensitivity and specificity was calculated as 100% as none of the positive sample was found missed and none of the negative sample was picked up by RT-qPCR (Table 2).

Table 2

Comparative evaluation of Magnetic bead RNA extraction conjunction to RT-qPCR with Commercial Qiagen and magnetic RNA extraction conjunction to RT-qPCR.

Magnetic bead based RNA extraction coupled RT-qPCR	Qiagen and magnetic RNA extraction conjunction to RT-qPCR		
	Positive	Negative	Total
Positive	40	0	40
Negative	0	60	60
Total	40	60	100
Concordance = 100%, Sensitivity = 100%, Specificity = 100%			

Discussion

The emerging virus outbreaks occurring frequency has increased globally in last few decades ¹⁵⁻¹⁶, possibly as a result of anthropogenic environmental changes that has increased the risk of further the zoonotic transmission¹⁷. The large scale of globalization, have increased pandemic potential and pose a high burden on society and health supporting systems. The current and ongoing COVID-19 pandemic surged the urgency to preparedness and responses. Despite of availability of therapeutic or vaccine, the early detection and identification of patients remains the most effective way to stop further human-to-human spread in mitigation strategies. In the case of a respiratory viral disease, such as influenza/COVID-19, naso-pharyngeal swabs are used to extract viral RNA and further test by downstream molecular assays. To achieve high sensitivity and specificity in RNA isolation for downstream applications as well as detection this becomes a critical step. Therefore a simplified field compatible RNA purification protocols that can be easily coupled to downstream molecular diagnostic

assays. However, the majority of commercial available RNA isolation kits are costly and based on multiple components in kits with add of buffers in vary compositions generally not provided owing to safeguard commercial interest. Therefore, these, commercial available kits do not offer flexibility and rapid availability when any large epidemic or pandemic scenario occurs.

Here, we report a magnetic bead-based RNA extraction protocol that is, to a large extent, producer independent, does not rely on unique components that are difficult to replace, and is scalable for mass testing. Because of the large surface binding capacity and rapid separation in solution by magnetic field, silica coated magnetic beads are compatible with a variety of plastic wares and they can be used in a user friendly format as well as in small scale testing. The assay was also reported for other viruses like Chikungunya in conjunction to field applicable isothermal formats fulfilling the ASSURED criteria of diagnostics¹⁸. In addition, magnetic beads were prepared in-house in this study and all reagents i.e. lysis, washing and elution buffers were also prepared manually without the need of centrifugation equipments. Various magnetic bead based RNA purification protocol has been established for automated, high throughput COVID-19 diagnostics⁴. However, a sophisticated infrastructure is required to carry out the protocol, and the protocol is partially dependent on commercial buffers. Our aim was to provide a simplified protocol that can be rapidly implemented and carried out in most of the peripheral laboratories in emergency crisis. We established a magnetic bead RNA isolation protocol that takes approximately 30 min using in-house reagents obviating the need of high end equipments as part of workflow for SARS-CoV-2 diagnostic. The assay was found equally sensitive to commercial platforms using either silica column or magnetic bead and can be integrated with multiple downstream molecular diagnostic platforms. The quality and integrity of the RNA extract was found suitable for RT-qPCR detection.

In conclusion, we report a detailed step-by-step RNA extraction protocol from naso-pharyngeal swabs, which is based on in-house magnetic beads and buffers and does not need any hi-end equipments. Our protocol was validated with a panel of 100 clinical samples and compared with commercially available silica column /magnetic bead based protocols that revealed similar sensitivity and specificity for detection of SARS-CoV-2 RNA by RT-qPCR. This provides a reliable RNA extraction for a diagnostic pipeline that can be rapidly deployed during similar pandemic and is also accessible to regions with insufficient laboratory capacities. This might be of importance in case of possible future shortages of commercial RNA extraction kits and enables diagnostic laboratories to be well prepared in case of a new rise of SARS-CoV-2 variant cases.

Declarations

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Author's Contributions: SS, DP, PKD- designed experiments, SS-Optimization of magnetic bead viral RNA extraction DP, DKD- Synthesis of magnetic beads SS,DP,PKD-wrote the manuscript; SS,PY,SD,AS,SKS- conducted viral RNA extraction, SS,PY,SD,JSK-Conducted RT-PCR, PKD, DKD-Supervised and critically reviewed results and manuscript. Finally all authors have reviewed and approved the final version of manuscript.

References

1. Vogels, C.B.F. *et al.*, Analytical Sensitivity and Efficiency Comparisons of SARS-CoV-2 RT–qPCR Primer–Probe Sets. *Nat. Microbiol.* **5**, 1299–1305 (2020) doi: 10.1038/s41564-020-0761-6.
2. Corman V.M. *et al.* Detection of 2019 Novel Coronavirus (2019-nCoV) by Real-Time RT-PCR. *Eurosurveillance.* **25**:2000045 (2020). doi: 10.2807/1560-7917.ES.2020.25.3.2000045.
3. 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.
4. Aitken J. *et al.*, Scalable and Robust SARS-CoV-2 Testing in an Academic Center. *Nat. Biotechnol.* **38**:927–931 (2020). doi: 10.1038/s41587-020-0588-y.
5. Guruceaga X. *et al.*, 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) doi: 10.1101/2020.04.26.20081307.
6. Zhao Z.*et al.*, A Simple Magnetic Nanoparticles-Based Viral RNA Extraction Method for Efficient Detection of SARS-CoV-2. *bioRxiv.* (2020) doi: 10.1101/2020.02.22.961268.
7. Thi V.L.D. *et al.*, A Colorimetric RT-LAMP Assay and LAMP-Sequencing for Detecting SARS-CoV-2 RNA in Clinical Samples. *Sci. Transl. Med.* :eabc7075(2020). doi: 10.1126/scitranslmed.abc7075.
8. Lever M.A. *et al.*, A Modular Method for the Extraction of DNA and RNA, and the Separation of DNA Pools from Diverse Environmental Sample Types. *Front. Microbiol.* **6**:476 (2015). doi: 10.3389/fmicb.2015.00476.
9. Chomczynski P. *et al.*, The Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate–Phenol–Chloroform Extraction: Twenty-Something Years on. *Nat. Protoc.* **1**:581–585 (2006). doi: 10.1038/nprot.2006.83.

10. Boom R., *et al.*, Rapid and Simple Method for Purification of Nucleic Acids. *J. Clin. Microbiol.* **28**:495–503 (1990). doi: 10.1128/JCM.28.3.495-503.1990
11. Vandeventer P.E., *et al.*, Multiphasic DNA Adsorption to Silica Surfaces under Varying Buffer, pH, and Ionic Strength Conditions. *J. Phys. Chem. B.* **116**:5661–5670 (2012). doi: 10.1021/jp3017776.
12. Borodina T.A., *et al.*, DNA Purification on Homemade Silica Spin-Columns. *Anal. Biochem.* **321**:135–137 (2003). doi: 10.1016/S0003-2697(03)00403-2.
13. Holmberg R., *et al.*, High-throughput, Automated Extraction of DNA and RNA from Clinical Samples Using TruTip Technology on Common Liquid Handling Robots. *J. Vis. Exp.* **76**:e50356 (2013). doi: 10.3791/50356.
14. Lee A.H. *et al.*, Preparation of Iron Oxide Silica Particles for Zika Viral RNA Extraction. *Heliyon.* **4**:e00572(2018). doi: 10.1016/j.heliyon.2018.e00572.
15. Jones G., *et al.*, Global Trends in Emerging Infectious Diseases. *Nature.* **451**:990–993 (2018). doi: 10.1038/nature06536.
16. Smith K.F., *et al.*, Global Rise in Human Infectious Disease Outbreaks. *J. R. Soc. Interf.* **11**:20140950 (2014). doi: 10.1098/rsif.2014.0950.
17. Lindahl J., *et al.*, The Consequences of Human Actions on Risks for Infectious Diseases: A Review. *Infect. Ecol. Epidemiol.* **5**:30048(2015). doi: 10.3402/iee.v5.30048.
- 18 Sharma S, *et al.*, Development of magnetic bead based sample extraction coupled polymerase spiral reaction for rapid on-site detection of Chikungunya virus. *Sci. Rep.* **10**:11651 (2020).doi.org/10.1038

Figures

Lysis of Swab sample in Lysis Buffer

Magnetic Bead extraction

RT-qPCR

Figure 1

Overview of sample processing using in-house magnetic bead RNA extraction protocol for COVID-19

Figure 2

SARS-CoV-2 Gene amplification by TaqMan RT-qPCR

Figure 3

RT-qPCR of diluted invitro transcribed human RNaseP RNA for determination of LOD by three assays (A- Qiagen, B- commercial magnetic bead, C-in-house magnetic bead,) SARS-CoV-2 gene amplification by TaqMan RT-qPCR D.- Comparative analysis of ct values versus RNA dilution by plotting standard curve by all three methods.