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Original Research Article.

A Comparative Study of Silica Spin Column Based and Magnetic Bead Based RNA Extraction and Purification Kits on Covid -19 Samples

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ABSTRACT

Introduction: The purpose of this study to find out better Extraction and Purification of RNA kits between silica spin column based and magnetic bead based to detect Corona virus 2 (SARS-CoV-2). Isolating high-quality RNA is the most critical step for successfully performing a broad range of assays, from RT-qPCR or microarray analysis to cDNA library preparation, as well as Northern blot studies.

Materials and Methods: Nasopharyneal and oropharingeal swab poured in Viral Transport medium sample were extracted and purified by magnetic beads method MAG Pure Corona Virus (SARS-CoV) Nucleic Acid Purification Kit, which was adapted for a manual procedure using magnetic racks, Silica spin column-based method MAGSPIN-73 PLUS for total nucleic acid extraction and detected through qRT-PCR.

Results: These tests were performed on 100 known samples in which 25 samples were positive and rest 75 samples were negative. Average relative fluorescence units (RFU) of E gene, RdRp gene and Human RNase P gene were higher in Spin Column based kit. Average CUT off Threshold Value (Ct Value) of E gene, RdRp gene and Human RNase P gene were lower in Spin Column based kit.

Conclusion: The yield of extracted and purified RNA in Spin Column based kit was more than Magnetic bead-based kit. Spin Column based kit is better compare to Magnetic bead-based kit, because, only 01 sample Extraction and purification of RNA was failed against 10 samples of Magnetic bead based kit. Concordant results for positive and negative samples were more in Spin Column based kit comparison of Magnetic bead-based kit.

Key Words: Silica Spin Column, Magnetic Bead, Extraction Kits, Corona Virus.

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INTRODUCTION

Corona virus disease 2019 (COVID-19) is an infectious acute respiratory disease caused by a novel Corona virus. The World Health Organization (WHO) was informed of cases of pneumonia of unknown microbial etiology associated with Wuhan City, Hubei Province, China on 31 December 2019. The WHO later announced that a novel Corona virus had been detected in samples taken from these patients. Since then, the epidemic has escalated and rapidly spread around the world, with the WHO first declaring a public health emergency of international concern on 30 January 2020, and then formally declaring it a pandemic on 11 March 2020.

A potentially severe acute respiratory infection caused by the novel Corona virus severe acute respiratory syndrome Corona virus 2 (SARS-CoV-2). The clinical presentation is generally that of a respiratory infection with a symptom severity ranging from a mild common cold-like illness to a severe viral pneumonia leading to acute respiratory distress syndrome that is potentially fatal. Characteristic symptoms include fever, cough, and dyspnea, although some patients may be asymptomatic. Complications of severe disease include, but are not limited to, multi-organ failure, septic shock, and venous thromboembolism.

Etiological agent of the disease was identified as being a new Corona virus originating from bats with a possible relation to a Corona virus identified in pangolins of Manis javanica species.[2,3,6] The new Corona virus was classified in the same species of the first SARS of 2003 as Severe acute respiratory syndrome-related Corona virus (SARS-CoV-2), within the subgenre Sarbecovirus, genre Betacoronavirus, subfamily Orthocoronavirinae and Coronaviridae family. Members of this viral Family are mainly spherical and have lipid envelopes with prominent proteins named Spikes, which ones give the crown appearance that inspired family name. Genome is composed by a positive single stranded RNA that measures about 26 - 31 kb and is read through 6 ORFs. Besides SARS-CoV-2, six other Corona virus are already known

by cause infection in humans, among them the SARS and MERS-CoV pandemic viruses that may cause severe disease and pneumonia and the other four human Corona virus (HCoV) that are causal agents of common colds OC43, 229E, HKU1 and NL63.[8,9] Covid-19 is a mainly respiratory disease that have as most common symptoms the fewer, cough, shortness of breath, fatigue and difficulty breathing, other symptoms as chills, sore throat, myalgia, loss of smell and taste may also occur with minor frequency, besides gastrointestinal manifestations as diarrhea, nausea and vomiting. In most severe cases the disease course with severe pneumonia that usually led to hospitalization and the need to use mechanical respirators.[10-12]

Two essential measures are required to control of the pandemic while no vaccines are available: social distancing and large scale testing of the population. Due to high infected number, even testing only symptomatic patients who looked for medical care there is still a huge sample volume to do the diagnostic. Thereby, alternative methods may be required when lack of kits and reagents would occur due to high demand, mainly in nucleic acids extraction prior to detection by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Here we compared four different manual methods for SARS-CoV-2 RNA isolation and purification aiming detection by qRT-PCR marketed as commercial kits in India. Isolating high-quality RNA is the most critical step for successfully performing a broad range of assays, from RT-qPCR or microarray analysis to cDNA library preparation, as well as Northern blot studies. It is even critical for high-throughput transcriptome analysis using next-generation sequencing techniques. High-quality experiments require high-quality samples and maximizing yield of non-degraded RNA isolation is key. Two of the most common RNA extraction techniques for Extraction and purification of RNA kits were included in study. These are Manual nucleic acid extraction by magnetic beads and Spin Column based method. The spin column extraction method is a solid phase extraction technique to bind and isolate RNA within filter-based spin columns. These spin columns utilize membranes that contain silica or glass fiber to bind nucleic acids. Samples are lysed in a buffered solution containing RNase inhibitors and a high concentration of chaotropic salt. The lysates are passed through the silica membrane using centrifugal force, with the RNA binding to the silica gel at the appropriate pH. The membrane containing residual proteins and salt is then washed to remove impurities, with flow-through discarded. RNA is subsequently eluted with RNase-free water, as RNA is stable at a slightly acidic environment. Magnetic particle extraction method strategy for bioseparation utilizes beads with a paramagnetic core (in other words, they have properties of magnetism only when in proximity to an external magnetic field) coated with, most commonly, a matrix of silica for binding nucleic acids. In this method, cells are lysed in a buffer with RNase inhibitors and then incubated with the magnetic beads, allowing the particles to bind RNA molecules. The magnetic beads can then be quickly collected by being placed in proximity to an external magnetic field. The supernatant is removed and then subsequently washed and resuspended with removal of the magnetic field. This process can be easily repeated for multiple washes. The RNA is eluted from the magnetic beads with RNase-free water into solution, and the supernatant (containing the pure RNA) can then be transferred.

MATERIALS AND METHODS

Methods compared in this study include magnetic beads method MAG Pure Corona Virus (SARS-CoV) Nucleic Acid Purification Kit (APS LAB), which was adapted for a manual procedure using magnetic racks, Silica spin column-based method MAGSPIN-73 PLUS (APS LABS) for total nucleic acid extraction. The same amount of cells and the manufacturer protocols were followed for each kit.

Sample and Control

100 Oropharyngeal and nasopharyngeal swab samples of suspected patients are collected as per the ICMR guidelines and transfer to Laboratory into Viral Transport Medium are used as sample for the comparison.

For control, a set of SARS-CoV-2 positive nine clinical samples already tested in our lab was chosen based on cycle threshold value (CT), being three of each CT range in qRT-PCR for SARS-CoV-2 (allotted as low, medium and high).

In order to properly compare the different methods, all final samples were aliquoted separately for each technique and freezed (ultra freezer -80ºC) only one time before total nucleic acids/RNA extractions were done, the same attention was taken with the nucleic acids extracted before analysis by qRT-PCR.

Manual Nucleic Acid Extraction by Magnetic Beads

Magnetic beads method MAG Pure Corona Virus (SARS-CoV) Nucleic Acid Purification Kit (APS LAB) is designed for the isolation of RNA from nasal/throat swabs collected in viral Transfer medium (VTM). The procedure is based on the absorption of nucleic acid to paramagnetic beads under buffer condition. Sample lysis is achieved by incubation with a lysis buffer containing chaotropic ions digestion. After precipitating with isopropanol, MAG Pure beads are added to the lysate. After magnetic separation, paramagnetic beads are washed to remove containing and salts using wash buffers. Residual ethanol from previous wash steps is removed by air drying. Finally, highly pure RNA is eluted with low salt elution buffer or nuclease free water. Purified RNA used for further application. This kits contents Mag Pure beads, lysis buffer, wash buffer 1 & 2 elution buffer, carrier RNA (on aliquot store at -20^{0}C), Magnetic separator.

Protocol For Preparation of Buffers

Wash Buffer: add the following required volume of 100 % ethanol to the concentrated wash buffer and mix well. Store at room temperature with tightly closed cap.

Protocol For RNA Isolation

Prepare pre mixture of lysis buffer working solution by adding the following components per reaction lysis buffer, carrier RNA and MAG bead 102 µl, 8 µl and 40 µl respectively. Add 200 µl of VTM containing nasal / throat swabs samples to a 2ml microcentrifuge tube and add 150 µl lysis buffers with carrier RNA. Mix well by vortexing for 30 seconds and keep on shaker/ rocker for 10 minutes at 56^{0}C on thermo mixer. Add 300 µl of ethanol mix well by vortexing for 30 seconds and keep on shaker/ rocker for 5 minutes at 37^{0}C or room temperature at 900 RPM. Separate the magnetic beads by placing the tubes in a magnetic stand. Wait for 1 minute till all beads are collected. While the tubes are still on the magnetic stand, remove entire supernatant by gentle pipetting without disturbing the magnetic beads. Remove the tubes from the magnetic separator and add 500 µl wash buffer-1, resuspend the beads completely by vortexing and keep on thermo mixer for 3 minutes at 37^{0}C at 900 RPM. Separate the magnetic beads by

placing the tubes in a magnetic stand. Wait for 1 minute all beads are collected. While the tubes are still on the magnetic stand, remove entire supernatant by gentle pipetting without disturbing the magnetic beads. Remove the tubes from the magnetic separator and add 500 µl wash buffer -2, resuspend the beads completely by vortexing and keep on thermo mixer for 3 minutes at 37^{0}C at 900 RPM. After second wash, completely remove supernatant and fully dry the beads at 65^{0}C for 5 minutes. Add 60 µl of elution buffer, resuspend the beads completely by vortexing and incubate for 5 minutes at 56^{0}C on thermo mixer at 900 RPM speed. Perform magnetic separation by keeping the tubes at magnetic separator for 1 minute. Transfer the supernatant containing viral RNA to a new, nuclease free micro centrifuge tube and use the purified viral nucleic acid in downstream application immediately or keep the samples in the ice or 4^{0}C till perform assay.

Manual Nucleic Acid Extraction by Spin Column

Spin Column based method MAGSPIN-73 PLUS Corona Virus (SARS-CoV) Nucleic Acid extraction Kit (APS LAB) is designed for the isolation of RNA from nasal/throat swabs collected in viral Transfer medium (VTM). Spin column-based (silica matrix) nucleic acid purification is a solid phase extraction method to quickly purify nucleic acids. This method relies on the fact that nucleic acid will bind to the solid phase of silica under certain conditions. If the pH and salt concentration of the binding solution are optimal, the RNA will bind to the silica gel membrane, than elute RNA from column. Purified RNA used for further application. This kit contents VNE buffer, wash buffer -1 (concentrate) W1, wash buffer -2 (concentrate) W2, elution buffer, carrier RNA, spin column and collection tube.

Protocol For Manual Nucleic Acid Extraction by Spin Column

Prepare pre mixture of VNE buffer and carrier RNA by adding 16 µl carrier RNA per 100 µl VNE buffer. Add 100 µl of VNE buffer into micro centrifuge tube. Add 200 µLVTM / sample to the VNE buffer in the micro centrifuge tube. Mix by vortexing for 15-30 sec. To ensure efficient VNE, it is essential that the sample is mixed through with VNE buffer to yield a homogenous solution. Frozen samples that only thawed once can also be used. Incubate at room temperature (15 -25^{0}C) for 10 minutes. Add 270 µl ethanol (96- 100 %) to the sample, and mix by vortexing for 15 sec. after mixing briefly centrifuge the tube to remove drops from inside the lid carefully apply entire lysed sample solution from step 5 to spin column (in a 2 ml collection tube). Close the cap and centrifuge at 8000 RPM for one minute and discard the flow through. Add 500 µl wash buffer-1 and centrifuge at 8000 RPM for one minute. Discard the flow through. Add 500 µl wash buffer-2 and centrifuge at 12000 RPM for one minute. Discard the flow through. Remove the spin column from the tube and place it in a centrifuge at 12000 RPM for one minute as a dry wash to remove the residual from the spin column. Place the spin column in a clean 1.5 ml micro centrifuge tube and add 50 µl pre warmed at 56^{0}C elution buffer equilibrated to room temperature. Incubate at room temperature for one minute. Spin at 12000 RPM for one minute and collect the residue. The collected residue is ready to use RNA for further application or store at -80^{0}C.

Detection by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Real –Q 2019 nCoV detection Kit V2 kit is a real time reverse transcription polymerase chain reaction (rRT-PCR) test. The 2019 nCov primer and probe sets is designed to detect genes Envelop (E) gene, RNA dependent RNA polymerase (RdRP) gene and human Rnase P gene (HRP) in clinical specimens of nasopharyngeal swab, oropharyngeal swab, sputum, endotracheal aspirates, bronchoalveolar lavage (BAL) with sign and symptoms infection who are suspected of COVID-19. The target gene for detection and fluorescent dye of probe are E gene, RdRp gene and Human RNase P gene FAM, Cy5 and HEX respectively. It is designed to detect TaqMan Probe flurescence signals in three wavelength signals in a single tube. This kit contents 2X PCR reaction mixture (PCR MIX), nCov probe & primer mixture -1 (PROBE-1), primer mixture -2 (PROBE-2), RT-PCR enzyme (ENZYME), and Positive Control. All components are taken out immediately before use, thawed and used for centrifugation. Immediately, after use store below -20^{0}C.

Protocol for using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) kits

Prepare a real time PCR master mixture with 2X PCR reaction mixture (PCR MIX) 6µl, nCov probe & primer mixture -1 (PROBE-1)3µl, primer mixture -2 (PROBE-2) 3µl, RT-PCR enzyme (ENZYME) 3µl for each reaction. After mixing master mixture well (without vortexing), dispense 15 µl in strip tube or plates. Dispense 5 µl of the sample RNA and positive control into each well. Add 5 µl of water to the negative control well to confirm contamination of the PCR reaction. After dispensing, close the tube well with a cap and centrifuge lightly. After attaching strip tube or plate to CFX 96 equipment, inspect it under following conditions. The florescence is designated by selecting FAM, HEX and Cy5 at 60^{0}C, the last step of the cycling step.

Real Time PCR conditions

Step	Temperature	Time	Cycle	Acquisition mode
1	**50^{0}C**	15 minutes	1	
2	**95^{0}C**	5 minutes	1	
	95^{0}C	10	40	None
3		Seconds		
	60^{0}C	30 Seconds		Acquiring on FEM, HEX, Cy5

Other materials also required to perform the reaction are CFX-96 touch real time PCR detection system, variable volume pipettes, magnetic separator, bio safety cabinet, laminar flow, PCR hood, -80^{0}C and -20^{0}C deep freeze, power free gloves, tabletop centrifuge with different rotors, optical 96 well reaction plate or tube, optical cap or optical adhesive cover, aerosol barrier, RNases, DNase free tips.

RESULTS

A total 100 samples of Covid 19 (SARS-CoV-2) were analyzed which tested in laboratory. Out of which 25 (25%) were positive and rest 75 (75%) negative.

These samples were extracted and purified by two different principal based RNA Extraction kits and amplified on the Real –Q 2019 nCoV detection Kit V2 kit. Out of 100 samples, 65 samples were negative and 21 samples were positive by both the kits. 10 samples were not amplified, which were extracted by magnetic bead kit but 09 negative and 01 positive amplified, which were

extracted by spin Column based kits. Only 01 sample was not amplified in spin column based out of 100 samples. 03 samples were amplified but shown variable result.

Concordant result for positive (25) and negative (75) in Magnetic bead based RNA extraction kit were 22 (88%) and 68 (90.67%) respectively. Concordant result for positive (25) and negative (75) in Spin column based RNA extraction kit were 24 (96%) and 75 (100%) respectively. Disconcordant result for positive (25) and negative (75) in Magnetic bead based RNA extraction kit were 03 (12 %) and 07 (09.33 %) respectively. Disconcordant result for positive (25) and negative (75) in Spin column based RNA extraction kit were 01 (04 %) and 00 (00%) respectively. Amplification of Human RNaseP concordant result in Magnetic bead based RNA extraction kit and Spin column based RNA extraction kit were 90 (90%) and 99(99%) respectively and disconcordant result were 10(10%) and 01 (01%) respectively.

In Magnetic bead based RNA extraction kit, 36 samples shown more than average and 29 samples shown less than average RFU Value of RNA extraction and purification for Human RNAaseP in COVID 19 Negative (65) in both kits.

In Spin column based RNA extraction kit, 41 samples shown more than average and 24 samples shown less than average RFU Value of RNA extraction and purification for Human RNAaseP in COVID 19 Negative (65) in both kits.

In Magnetic bead based RNA extraction kit, 35 samples shown more than average and 30 samples shown less than average Ct Value of RNA extraction and purification for Human RNAaseP in COVID 19 Negative (65) in both kits.

In Spin column based RNA extraction kit, 36 samples shown more than average and 29 samples shown less than average Ct Value of RNA extraction and purification for Human RNAaseP in COVID 19 Negative (65) in both kits.

Table 1: Known samples of Covid 19

Known sample	No of Known sample
Positive	25
Negative	75
Total sample	100

Table 2: Segregation of results in both magnetic bead and spin column based

Negative in magnetic bead and spin column based both	65
Positive in magnetic bead and spin column based both	21
Not Amplification in magnetic bead and Negative spin column based	09
Not Amplification in magnetic bead and positive spin column based	01
Negative in magnetic bead and Not Amplification spin column based	01
Negative in magnetic bead and Positive spin column based	02
Positive in magnetic bead and Negative spin column based	01
Total	100

Table 3: Amplification of RNA in two different extraction kits

	Magnetic bead based RNA extraction kit				**Spin column based RNA extraction kit**			
	Number of concordant	**Percentage of concordant**	**Number of disconcordant**	**Percentage of disconcordant**	**Number of concordant**	**Percentage of concordant**	**Number of disconcordant**	**Percentage of disconcordant**
Positive (out of 25)	22	88%	03	12%	24	96%	01	04%
Negative (Out of 75)	68	90.67%	07	09.33%	75	100%	00	00
Amplification in Human RNaseP gene (Out of 100)	90	90%	10	10%	99	99%	01	01%

Table 4: RFU and Ct Value of RNA extraction and purification for Human RNAaseP in COVID 19 Negative in both kits (Number of Negative are 65)

	Magnetic bead based RNA extraction kit		Spin column based RNA extraction kit	
	RFU (Average value= 199.6)	Ct Value (Average value= 32.12494)	RFU (Average value= 211.78)	Ct value (Average value= 32.12194)
Number of Negatives More than Average (out of 65)	36	35	41	36
Number of Negatives less than Average (out of 65)	29	30	24	29

Table 5: RFU and Ct Value of RNA extraction and purification for Human RNAaseP in COVID 19 Positive in both kits (Number of Positive are 21)

	Magnetic bead based RNA extraction kit		Spin column based RNA extraction kit	
	RFU (Average value= 200.5263)	Ct Value (Average value= 32.08728)	RFU (Average value= 214.0625)	Ct value (Average value= 32.07548)
Number of positives More than Average (out of 21)	10	06	10	08
Number of positives less than Average (out of 21)	11	15	11	13

Table 6: RFU and Ct Value of RNA extraction and purification for E gene in COVID 19 Positive in both kits (Number of Positive are 21)

	Magnetic bead based RNA extraction kit		Spin column based RNA extraction kit	
	RFU (Average value= 708.5714)	Ct Value (Average value= 29.26762)	RFU (Average value= 853.3333)	Ct value (Average value= 27.4081)
Number of E gene positives More than Average (out of 21)	10	10	09	09
Number of E gene positives less than Average (out of 21)	11	11	12	12

Table 7: RFU and Ct Value of RNA extraction and purification for RdRp gene in COVID 19 Positive in both kits (Number of Positive are 21)

	Magnetic bead based RNA extraction kit		Spin column based RNA extraction kit	
	RFU (Average value= 539.0476)	Ct Value (Average value= 32.42095)	RFU (Average value= 811.4286)	Ct value (Average value= 30.2045)
Number of RdRp gene positives More than Average (out of 21)	13	11	7	11
Number of RdRp gene positives less than Average (out of 21)	8	10	14	10

Table 8: Comparison of average values RFU and Ct Value of different gene in Magnetic bead based RNA extraction kit and Spin column based RNA extraction kit.

Target	Magnetic bead based RNA extraction kit		Spin column based RNA extraction kit	
	Average RFU	Average Ct Value	Average RFU	Average Ct Value
Human RNase P in Negative sample	199.6	32.12494	211.78	32.12194
Human RNase P in Positive sample	200.5263	32.08728	214.0625	32.07548
E gene	708.5714	29.26762	853.3333	27.4081
RdRp gene	539.0476	32.42095	811.4286	30.2045

In Magnetic bead based RNA extraction kit, 10 samples shown more than average and 11 samples shown less than average RFU Value of RNA extraction and purification for Human RNAaseP in COVID 19 positive (21) in both kit.

In Spin column based RNA extraction kit, 10 samples shown more than average and 11 samples shown less than average RFU Value of RNA extraction and purification for Human RNAaseP in COVID 19 positive (21) in both kits.

In Magnetic bead based RNA extraction kit, 10 samples shown more than average and 11 samples shown less than average Ct Value of RNA extraction and purification for Human RNAaseP in COVID 19 positive (21) in both kit.

In Spin column based RNA extraction kit, 08 samples shown more than average and 13 samples shown less than average Ct Value of RNA extraction and purification for Human RNAaseP in COVID 19 positive (21) in both kits.

In Magnetic bead based RNA extraction kit, 10 samples shown more than average and 11 samples shown less than average RFU Value of RNA extraction and purification for E gene in COVID 19 positive (21) in both kit.

In Spin column based RNA extraction kit, 09 samples shown more than average and 12 samples shown less than average RFU Value of RNA extraction and purification for E gene in COVID 19 positive (21) in both kits.

In Magnetic bead based RNA extraction kit, 10 samples shown more than average and 11 samples shown less than average Ct Value of RNA extraction and purification for E gene in COVID 19 positive (21) in both kit.

In Spin column based RNA extraction kit, 09 samples shown more than average and 12 samples shown less than average Ct Value of RNA extraction and purification for E gene in COVID 19 positive (21) in both kits.

In Magnetic bead based RNA extraction kit, 13 samples shown more than average and 08 samples shown less than average RFU Value of RNA extraction and purification for RdRp Gene in COVID 19 positive (21) in both kit.

In Spin column based RNA extraction kit, 07 samples shown more than average and 14 samples shown less than average RFU Value of RNA extraction and purification for RdRp Gene in COVID 19 positive (21) in both kits.

In Magnetic bead based RNA extraction kit, 11 samples shown more than average and 10 samples shown less than average Ct Value of RNA extraction and purification for RdRp Gene in COVID 19 positive (21) in both kit.

In Spin column based RNA extraction kit, 11 samples shown more than average and 10 samples shown less than average Ct Value of RNA extraction and purification for RdRp Gene in COVID 19 positive (21) in both kits.

Average values RFU Value of Human RNase P Negative, Human RNase P Positive, E gene and RdRp gene in Magnetic bead based RNA extraction kit were 199.6, 200.5263, 708.5714 and 539.0476 respectively and in Spin column based RNA extraction kit were 211.78, 214.0625, 853.3333 and 811.4286 respectively.

Average values Ct Value of Human RNase P Negative, Human RNase P Positive, E gene and RdRp gene in Magnetic bead based RNA extraction kit were 32.12494, 32.08728, 29.26762 and 32.42095 respectively and in Spin column based RNA extraction kit were 32.12194, 32.07548, 27.4081 and 30.2045 respectively.

DISCUSSION

Sudden exposure of pandemic of SARS –Cov-2 all over the world draw all attention to development of detection kit and Extraction and purification RNA kits of Covid-19. Hundreds of kits various principal and methods were developed worldwide. An effort was undertaken using to know the comparison of two RNA Extraction and purification kits to detect RNA of Covid-19 (Sars-Cov-2). This study highlights two facts First, the Extraction and purification RNA of COVID 19 and second, to know the yield of Extracted and purified RNA. These tests were performed on 100 known samples in which 25 samples were positive and rest 75 samples were negative. Extraction and purification RNA is a decisive step in diagnosis of COVID 19, because amplification depends upon product obtained from the Extraction and purification. Therefore, a sensitive, specific and reliable Extraction and purification RNA kit require finding out real number of COVID cases. Automated as well manual methods are available, but due to uninterrupted supply system in Indian health scenario, a manual method is effective and sustainable. In order to evaluate performance of two manual Extraction and purification RNA kits, in this study, we have compared two different principal based manual nucleic acid Extraction and purification kits for detection of E gene, RdRp gene and Human RNase P gene. In this pandemic scenario, due to load of testing of samples and rapidness of reporting, magnetic bead based method did not stand in front of manual spin Column based kits. Magnetic bead based kit is more time consuming, lengthy and complicated procedure compare to manual Spin Column based kits. Magnetic stand require for magnetic bead based and Centrifuge machine require for Spin Column based kits. Of

course, Centrifugation machine is costly compare to magnetic stand, but placing of tubes in magnetic stand is very tedious job in limited magnetic stand. On the contrary, centrifugation in centrifugation machine is time saving and convenient process in pandemic condition. Spin Column based kit is more better compare to Magnetic bead based kit, because, only 01 sample Extraction and purification of RNA was failed against 10 samples of Magnetic bead based kit. Concordant result for positive and negative samples were more in Spin Column based kit comparison of Magnetic bead based kit because of purification was high in Spin Column based kit.

Average relative fluorescence units (RFU) of E gene, RdRp gene and Human RNase P gene were higher in Spin Column based kit. Average CUT off Threshold Value (Ct Value) of E gene, RdRp gene and Human RNase P gene were lower in Spin Column based kit. That's indicate, the yield of extracted and purified RNA in Spin Column based kit was more than Magnetic bead based kit.

Higher Number of samples were more than average RFU value and less than average Ct Value for E gene, RdRp gene and Human RNase P gene in Spin Column based kit comparison to Magnetic bead based kit. This finding again shows that extraction and purification and yield of RNA were higher in Spin Column based kit.

The spin column extraction method is simple, procedure to perform. It is a ready to use kit format, which adds convenience. Amenable to large-scale and high-throughput processing, flexible for use with both centrifugation or vacuum based systems. But starting with too much sample or incomplete homogenization can clog the membrane and/or result in contamination with proteins or genomic DNA and incomplete cellular lysis can lead to low yields.

Magnetic particle extraction method is non-filter method reduces concern for clogging and no organic solvent hazardous waste. But viscous samples can impede migration of magnetic beads. This technique can be laborious when performed manually with large numbers of samples and risk of contamination of RNA samples with residual magnetic beads.

CONCLUSION

Two different manual RNA extraction and Purification kits were used for SARS-CoV-2 detection by qRT-PCR with clinical samples and control dilutions. We aimed to evaluate and identify most reliable and accurate method is for diagnostic purposes. In this regard, silica spin column-based methods are convenient and reliable choices.

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