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Original Article

Microbiology Section

Efficacy of Nucleic Acid Extraction by Manual versus Automated Magnetic Bead-based Method to Detect SARS-CoV-2

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ABSTRACT

Introduction: In December 2019, a rapid spread of highly infectious, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), was reported in Wuhan, China. The gold standard for diagnosis of SARS-CoV-2 infection is nucleic acid amplification technology by detecting its viral Ribonucleic Acid (RNA) from respiratory swabs (oropharyngeal and nasopharyngeal) by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) whose specificity is approximately 95%. Magnetic bead RNA extraction was benchmarked against the commercial QIAcube extraction platform.

Aim: To compare the efficacy of nucleic acid extraction by manual method and automated magnetic bead-based method to detect SARS-CoV-2.

Materials and Methods: The present cross-sectional observational study was conducted in the Department of Microbiology, Autonomous State Medical College, Firozabad, Uttar Pradesh, India. Duration of the study was from February 2022 to March 2022. A total of 470 oropharyngeal and nasopharyngeal samples were included in the present study to observe the efficacy of nucleic acid extraction by manual extraction and automated extraction for SARS-CoV-2. Data were entered in Microsoft Excel software and analysed using Statistical Package for the Social Sciences (SPSS) version 26.0.

Results: During the present study period, a total of 470 individual samples were tested in 94 pools. Out of these 470 individuals, 331 were males (70.5%) and 139 were females (29.5%). All 94 pools were found negative by both automatic and manual extraction methods. Envelope (*E*) gene was found in one pool (1.06%) by the manual RNA extraction method. The *E* gene was absent in 93 pools (98.94%) by manual method. Internal control was found highest in 88 pools (93.62%) by the automated extraction method.

Conclusion: Automated workflows avoid human error from the sample processing pipeline and also ensure as well as enhance the meaningful output, diagnostic precision, and testing capacity. Automated instruments are in wide usage because of their capability of processing thousands of samples per day with the support of minimal staff.

Keywords: Coronavirus disease 2019, Diagnostic techniques and procedures, Diagnostic tests, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

In December 2019, a rapid spread of highly infectious, SARS-CoV-2, was reported in Wuhan, China, which was declared a pandemic thereafter by the World Health Organisation (WHO) on 11th March 2020 [1]. Currently, the standard test for diagnosis of SARS-CoV-2 infection is the detection of its viral RNA from respiratory swabs (oropharyngeal and nasopharyngeal) by RT-PCR. This method involves the reverse transcription of the genetic material of the virus (RNA) to complementary DNA (cDNA), followed by amplification of some regions of the cDNA. In this two-step testing procedure, several primers and probes set by several COVID-19 detection kits for targeting one or more of the SARS-CoV-2 genes-nucleocapsid (*N*), an envelope protein (*E*), S glycoprotein (*S*), RNA-dependent RNA polymerase (*RdRp*) [2], or open reading frame 1ab (*ORF1ab*) region) is used in a single channel and utilises the detection of RNase P in a separate channel as the internal control [3]. The sensitivity and specificity of RT-PCR is not 100%. It is estimated to be 70-98% sensitivity and specificity is approximately 95%. In addition, the genetic diversity of the SARS-CoV-2 plays an important role and may affect the results of the RT-PCR test [4,5].

For measuring public health worldwide, the diagnosis of COVID-19 is implemented on a large scale. Various protocols are established for some preprocessing steps, like specimen lysis, along with the nucleic acid extraction of SARS-CoV-2 RNA, based on the magnetic bead-based nucleic acid extraction protocol [6]. These preprocessing steps can be processed through manual protocols with individual instruments or can be processed through automated protocols via automated instruments. Manual protocols for magnetic nucleic acid extraction consist of many steps which are lengthy, time taking, and prone to impurities [7]. These methods need heavy manpower, with a higher risk of cross-infections.

To overcome these drawbacks, automated protocols are convenient, simple, and companionable [8,9] by performing in 96-well plates in combination with a magnet plate optimised for 96 deep-well plates using automated robotic pipetting, which minimises the pipetting and handling errors [2]. Aim of the present study was to compare the process of manual and automated extraction of SARS-CoV-2 nucleic acid.

MATERIALS AND METHODS

The present cross-sectional observational study was conducted in the Department of Microbiology, Autonomous State Medical College (ASMC), Firozabad, Uttar Pradesh, India. An approval from the Head of Department, Department of Microbiology, In-charge, BSL-2 LAB, ASMC, Firozabad, Uttar Pradesh, India was obtained. Consent was not taken as no subject was involved, only a comparison of two different techniques done in the BSL-2 lab for the efficacy of better Coronavirus Disease 2019 (COVID-2019) results in the present study. Duration of this study from February 2022 to March 2022. A total of 470 oropharyngeal and nasopharyngeal samples were included in the present study. A total of 94 pools were made to cover these 470 samples. Each pool consisted of five samples.

Inclusion criteria: Samples of oropharyngeal and nasopharyngeal with proper identification and favourable temperature with triple-layer packing were included in the present study.

Exclusion criteria: Samples without proper marking, leakage and without maintaining temperature were excluded from the present study.

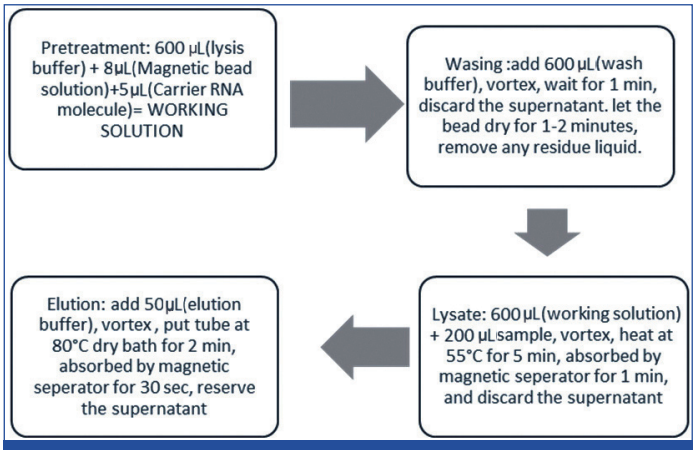
Study Procedure

In the present observational study, upper respiratory tract specimens (oropharyngeal and nasopharyngeal) of 470 individuals were collected. In the case of respiratory viral diseases, such as influenza or COVID-19, oropharyngeal and pharyngeal swabs were collected and tested for the presence of viral RNA. RNA isolation prior to detection is a pivotal step to ensure high specificity and sensitivity of detection in molecular assays [2]. This established protocol for extracting SARS-CoV-2 viral RNA from respiratory swabs is a magnetic bead-based nucleic acid extraction protocol which is done via two different methods i.e. manual extraction and automated extraction method to compare within.

Manual RNA extraction: In the manual process, magnetic bead RNA extraction was performed individually in eppendorf vials in combination with a magnetic stand and transferred to various wash buffers like triple distilled water or 70% ethanol via manual pipetting instruments.

Automated RNA extraction: In many automated instruments, magnetic microbeads coated with silica are used to capture nucleic acids and are sequentially transferred into various wash solutions by a robotic pipetting instrument with a magnetic head in 96-well plates [10].

For optimising the result in a 96-well RT-PCR plate, RNA of 94 pools, comprised of five samples in each, covering 470 random samples, one negative control and one positive control were extracted via manual protocol and automated protocol (KingFisher™ Flex for 96) by using Q-Line Molecular Viral Extraction Kit Magnetic Bead Method as per the kit manufacturing instructions [Table/Fig-1a,b]. The extracted RNA from both manual and automated processes are amplified and evaluated by Thermocycler (BIORAD CFX-96) using RT-PCR kit (DiAGSure nCoV-19 Assay), which contains primers and probes that are specific for SARS-CoV-2 as per kit [Table/Fig-2]. The process and results of the thermocycler obtained in the format of Ct value (cycle threshold) which were interpreted according to the kit manufacturing instructions [Table/Fig-3a-c].



[Table/Fig-1a]: Process of manual extraction method.

Step	Name	Plate position	Volume (µL)
1	-Load-	1	
2	Move	1	100
3	lysis	2	500
4	Wash buffer	3	600
5	Elution	4	50
6	-Unload-	1	

[Table/Fig-1b]: Process of automated extraction method (KingFisher™ Flex for 96).

STATISTICAL ANALYSIS

Data were entered in Microsoft Excel software and analysed using SPSS version 26.0. Distribution and differentiation parameters were mentioned as a percentage. To determine the p-value, Z test calculator for two population proportions was used. The p-value <0.05 was considered as statistically significant.

Kit name	Specific gene for SARS-CoV-2 detection	In-house gene
DiAGSure nCoV-19 assay (Multiplex, TaqMan based)	*ORF1ab* gene	RNase P
	E gene	

[Table/Fig-2]: Summary of RT-PCR kit used in this study.

Key components	Volume per reaction
WRTaqMan master mix	13 µL
Primer probe	2 µL
GRTScript enzyme	1 µL
Extracted RNA	9 µL
Total volume	25 µL

[Table/Fig-3a]: Summary of RT-PCR master mix composition used in the study.

Steps	Temperature (°C)	Time	No. of cycles
RT reaction	50	15 min.	1
Hold stage	95	5 min.	1
PCR stage	95	10 sec.	45
	60	40 sec.	
Approximate running time	Approx. 90 min.		
Threshold cut-off (Ct)	≤40		

[Table/Fig-3b]: Summary of RT-PCR amplification cycles.

Target	*ORF1ab* (confirmatory gene)	*E* gene	Internal control	Interpretation
Case-1	+	+/-	+/-	nCoV-19 positive
Case-2	-	+	+/-	nCoV-19 negative
Case-3	-	-	+	nCoV-19 negative
Case-4	-	-	-	Invalid

[Table/Fig-3c]: Interpretation of results.

RESULTS

A total of 470 individual samples were tested. Out of these 470 individuals, 331 were males (70.5%) and 139 were females (29.5%). All Ct value obtained in RT-PCR/thermocycler shown in 96-well RT-PCR plate format by both manual and automatic RNA methods is shown in [Table/Fig-4,5].

Manual Extraction

	1	2	3	4	5	6	7	8	9	10	11	12
A	ORF1ab- N/A Egene- N/A IC- 20.10	ORF1ab- N/A Egene- N/A IC- 21.34	ORF1ab-N/A Egene- N/A IC-19.56	ORF1ab- N/A Egene- N/A IC-18.10	ORF1ab- N/A Egene- N/A IC-8.31	ORF1ab- N/A Egene- N/A IC-20.99	ORF1ab- N/A Egene- N/A IC-14.27	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-16.93	ORF1ab- N/A Egene- N/A IC-17.39	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-15.18
B	ORF1ab- N/A Egene- N/A IC-16.35	ORF1ab- N/A Egene- N/A IC-20.70	ORF1ab- N/A Egene- N/A IC- N/A	ORF1ab- N/A Egene- N/A IC- N/A	ORF1ab- N/A Egene- N/A IC-33.63	ORF1ab- N/A Egene- N/A IC-25.85	ORF1ab- N/A Egene- N/A IC-28.09	ORF1ab- N/A Egene- N/A IC-25.79	ORF1ab- N/A Egene- N/A IC-30.28	ORF1ab- N/A Egene- N/A IC-35.60	ORF1ab- N/A Egene- N/A IC-28.52	ORF1ab- N/A Egene- N/A IC-34.34
C	ORF1ab- N/A Egene- N/A IC-34.76	ORF1ab- N/A Egene- N/A IC-34.58	ORF1ab- N/A Egene- N/A IC-33.78	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC- N/A	ORF1ab- N/A Egene- N/A IC-33.25	ORF1ab- N/A Egene- N/A IC- 32.84	ORF1ab- N/A Egene- N/A IC-32.42	ORF1ab- N/A Egene- N/A IC-29.96	ORF1ab- N/A Egene- N/A IC-29.27	ORF1ab- N/A Egene- N/A IC-28.00	ORF1ab N/A Egene- N/A IC- N/A
D	ORF1ab- N/A Egene- N/A IC-27.25	ORF1ab- N/A Egene- N/A IC-30.04	ORF1ab- N/A Egene- N/A IC-35.40	ORF1ab- N/A Egene- N/A IC-29.95	ORF1ab- N/A Egene- N/A IC-29.22	ORF1ab- N/A Egene- N/A IC-33.12	ORF1ab- N/A Egene- N/A IC- N/A	ORF1ab- N/A Egene- N/A IC-36.15	ORF1ab- N/A Egene- N/A IC-35.38	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-17.93	ORF1ab- N/A Egene- N/A IC-33.43
E	ORF1ab- N/A Egene- N/A IC-30.50	ORF1ab- N/A Egene- N/A IC-37.59	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-32.96	ORF1ab- N/A Egene- N/A IC-19.56	ORF1ab- N/A Egene- N/A IC-23.88	ORF1ab- N/A Egene- N/A IC-40.09	ORF1ab- N/A Egene- N/A IC-24.95	ORF1ab- N/A Egene- N/A IC-32.93	ORF1ab- N/A Egene- N/A IC-6.87	ORF1ab- N/A Egene- N/A IC-35.98	ORF1ab- N/A Egene- 37.66 IC-N/A
F	ORF1ab- N/A Egene- N/A IC-43.06	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-27.18	ORF1ab- N/A Egene- N/A IC-22.18	ORF1ab- N/A Egene- N/A IC-32.58	ORF1ab- N/A Egene- N/A IC-29.89	ORF1ab- N/A Egene- N/A IC-30.57	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-32.46	ORF1ab- N/A Egene- N/A IC-33.35	ORF1ab- N/A Egene- N/A IC-32.37	ORF1ab- N/A Egene- N/A IC-32.98
G	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-31.73	ORF1ab- N/A Egene- N/A IC-30.55	ORF1ab- N/A Egene- N/A IC-33.89	ORF1ab- N/A Egene- N/A IC-21.81	ORF1ab- N/A Egene- N/A IC-15.20	ORF1ab- N/A Egene- N/A IC-30.61	ORF1ab- N/A Egene- N/A IC-31.67	ORF1ab- N/A Egene- N/A IC-26.66	ORF1ab- N/A Egene- N/A IC-24.01	ORF1ab- N/A Egene- N/A IC-29.09	ORF1ab- N/A Egene- N/A IC-40.97
H	ORF1ab- N/A Egene- N/A IC-39.93	ORF1ab- N/A Egene- N/A IC-21.34	ORF1ab- N/A Egene- N/A IC-31.78	ORF1ab- N/A Egene- N/A IC-8.93	ORF1ab- N/A Egene- N/A IC- N/A	ORF1ab- N/A Egene- N/A IC-22.74	ORF1ab N/A Egene- N/A IC- 17.32	ORF1ab- N/A Egene- N/A IC-36.95	ORF1ab- N/A Egene- N/A IC- N/A	ORF1ab- N/A Egene- N/A IC- 32.66	NC	PC

[Table/Fig-4]: 96-well extraction plate depicting Ct values of manual RNA extraction method.

The test was considered invalid when there was no amplification of the internal control. In the automated extraction method, internal control was found highest in 88/94 pools (93.62%), while in the manual extraction method, internal control was found 78/94 pools (82.98%). The test was considered as failure 6/94 pools (6.38%) in automatic extraction method while 16/94 pools (17.02%) in manual extraction method. The peroration of test failure was significantly higher (p-value=0.0238) in the manual extraction method in comparison with the automatic extraction method.

Automatic extraction

	1	2	3	4	5	6	7	8	9	10	11	12
A	ORF1ab- N/A Egene- N/A IC- 27.21	ORF1ab- N/A Egene- N/A IC- 24.38	ORF1ab- N/A Egene- N/A IC-29.56	ORF1ab- N/A Egene- N/A IC-28.18	ORF1ab- N/A Egene- N/A IC-18.38	ORF1ab- N/A Egene- N/A IC-28.99	ORF1ab- N/A Egene- N/A IC-18.29	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-19.33	ORF1ab- N/A Egene- N/A IC-16.34	ORF1ab- N/A Egene- N/A IC-35.78	ORF1ab- N/A Egene- N/A IC-31.19
B	ORF1ab- N/A Egene- N/A IC-19.31	ORF1ab- N/A Egene- N/A IC-28.78	ORF1ab- N/A Egene- N/A IC-29.53	ORF1ab- N/A Egene- N/A IC- 35.90	ORF1ab- N/A Egene- N/A IC-32.69	ORF1ab- N/A Egene- N/A IC-26.85	ORF1ab- N/A Egene- N/A IC-20.09	ORF1ab- N/A Egene- N/A IC-22.99	ORF1ab- N/A Egene- N/A IC-36.25	ORF1ab- N/A Egene- N/A IC-38.60	ORF1ab- N/A Egene- N/A IC-22.58	ORF1ab- N/A Egene- N/A IC-22.39
C	ORF1ab- N/A Egene- N/A IC-31.77	ORF1ab- N/A Egene- N/A IC-16.58	ORF1ab- N/A Egene- N/A IC-19.79	ORF1ab- N/A Egene- N/A IC- N/A	ORF1ab- N/A Egene- N/A IC- 21.18	ORF1ab- N/A Egene- N/A IC-30.25	ORF1ab- N/A Egene- N/A IC- 30.84	ORF1ab- N/A Egene- N/A IC-29.42	ORF1ab- N/A Egene- N/A IC-27.44	ORF1ab- N/A Egene- N/A IC-30.22	ORF1ab- N/A Egene- N/A IC-26.02	ORF1ab- N/A Egene- N/A IC-26.13
D	ORF1ab- N/A Egene- N/A IC-11.25	ORF1ab- N/A Egene- N/A IC-20.09	ORF1ab- N/A Egene- N/A IC-18.86	ORF1ab- N/A Egene- N/A IC-29.12	ORF1ab- N/A Egene- N/A IC-11.22	ORF1ab- N/A Egene- N/A IC-32.97	ORF1ab- N/A Egene- N/A IC-25.57	ORF1ab- N/A Egene- N/A IC-11.15	ORF1ab- N/A Egene- N/A IC-31.86	ORF1ab- N/A Egene- N/A IC- N/A	ORF1ab- N/A Egene- N/A IC-19.32	ORF1ab- N/A Egene- N/A IC-35.82
E	ORF1ab- N/A Egene- N/A IC-36.50	ORF1ab- N/A Egene- N/A IC-32.89	ORF1ab- N/A Egene- N/A IC-30.68	ORF1ab- N/A Egene- N/A IC-31.91	ORF1ab- N/A Egene- N/A IC-11.56	ORF1ab- N/A Egene- N/A IC-29.82	ORF1ab- N/A Egene- N/A IC-31.09	ORF1ab- N/A Egene- N/A IC-21.93	ORF1ab- N/A Egene- N/A IC-39.33	ORF1ab- N/A Egene- N/A IC-18.82	ORF1ab- N/A Egene- N/A IC-32.83	ORF1ab- N/A Egene- N/A IC-25.40
F	ORF1ab- N/A Egene- N/A IC-28.43	ORF1ab- N/A Egene- N/A IC-23.74	ORF1ab- N/A Egene- N/A IC-29.88	ORF1ab- N/A Egene- N/A IC-16.42	ORF1ab- N/A Egene- N/A IC-12.78	ORF1ab- N/A Egene- N/A IC-31.73	ORF1ab- N/A Egene- N/A IC-40.87	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-37.44	ORF1ab- N/A Egene- N/A IC-30.76	ORF1ab- N/A Egene- N/A IC-32.05	ORF1ab- N/A Egene- N/A IC-32.47
G	ORF1ab- N/A Egene- N/A IC-N/A	ORF1ab- N/A Egene- N/A IC-38.84	ORF1ab- N/A Egene- N/A IC-28.59	ORF1ab- N/A Egene- N/A IC-41.78	ORF1ab- N/A Egene- N/A IC-22.80	ORF1ab- N/A Egene- N/A IC-13.70	ORF1ab- N/A Egene- N/A IC-12.61	ORF1ab- N/A Egene- N/A IC-27.49	ORF1ab- N/A Egene- N/A IC-20.64	ORF1ab- N/A Egene- N/A IC-14.71	ORF1ab- N/A Egene- N/A IC-10.09	ORF1ab- N/A Egene- N/A IC-36.91
H	ORF1ab- N/A Egene- N/A IC-31.43	ORF1ab- N/A Egene- N/A IC-26.98	ORF1ab- N/A Egene- N/A IC-32.77	ORF1ab- N/A Egene- N/A IC-16.42	ORF1ab- N/A Egene- N/A IC-34.64	ORF1ab- N/A Egene- N/A IC-19.75	ORF1ab- N/A Egene- N/A IC- 27.37	ORF1ab- N/A Egene- N/A IC- 24.97	ORF1ab- N/A Egene- N/A IC- N/A	ORF1ab- N/A Egene- N/A IC- 37.11	NC	PC

[Table/Fig-5]: 96-well extraction plate depicting Ct values of automatic RNA extraction method.

DISCUSSION

Within the last decades, the frequency of emerging virus outbreaks has increased globally [2]. Due to globalisation, many of the outbreaks have escalated the pandemic potential and produced a burden on society and health systems. The currently ongoing SARS-CoV-2 pandemic emphasises the urgency of appropriate response and preparedness. RT-PCR is adequately reliable and a fast technique for producing results in a few hours in a high output manner. The discovery of the RT-PCR method has paved the way for the detection of gene transcripts at trace levels, and the technique has been vastly utilised for contagious disease testing worldwide [11].

In the present observational study, the gold standard method for diagnosis of SARS-CoV-2 infection RT-PCR was used. The results of the thermocycler were interpreted according to the kit manufacturing instructions. The present study resulted in the peroration of test failure was significantly higher (p-value=0.0238) in the manual extraction method in comparison with the automatic extraction method.

Similarly, a review article based on automated SARS-COV-2 RNA extraction from patient nasopharyngeal samples using a modified DNA extraction kit found that automatic RNA extraction showed the efficient detection of RNA at low quantification cycle values (high nucleic acid/RNA extraction efficiency) [11]. A report on the use of a DNA extraction kit, after modifications, to extract viral RNA found that the fully automated liquid handling robotic RNA extraction systems is very likely suitable for isolation and downstream detection assays for any kind of viral RNA isolated from the pharyngeal swabs [12].

A similar study on validation of automated SARS-CoV-2 clinical diagnostics also stated that, automated workflows are more preferable than manual protocols to achieve a meaningful output, diagnostic accuracy, and also avoid human error [13] also, automated nucleic acid extraction has takes less hands-on time [14] with maximum purity of extracted RNA [5].

Limitation(s)

The limitation of the present study was the small sample size.

CONCLUSION(S)

A massive number of samples are being tested everyday, for which manual protocols are less preferable as the involvement of many lengthy steps which are time taking, and more prone to impurities. Manual methods also need heavy manpower, with a higher risk of cross-infection but do not affect the final costs of the test. Whereas, automated workflows avoid human error from the sample processing pipeline and also, ensure as well as enhance the meaningful output, diagnostic precision, and testing capacity. Automated instruments are in wide usage because of their capability of processing thousands of samples per day with the support of minimal staff. It also provides results in easy-to-use formats that are persuadable to point-of-care applications, without using complex instrumentation, but automatic protocols significantly increase the final costs, which hinders the massive testing in some areas, which is a drawback.

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