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Original Research Paper

Microbiology

COMPARATIVE ANALYSIS OF COBAS 6800, RT-PCR, AND TRUENAT SARS-COV-2 IN ROUTINE DIAGNOSIS OF COVID19.

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ABSTRACT **Introduction -** In December 2019, a group of patients of unknown aetiology with atypical pneumonia emerged in Wuhan, China [1]. Severe Acute Respiratory Syndrome Corona Virus 2 (SARS CoV-2), a novel beta-coronavirus was identified as the cause of these cases [2]. RTPCR testing leads to delay in diagnosis in cases where early and rapid diagnosis of COVID-19 is warranted which leads to tension and anxiety both among patient and treating health care workers. **Material And Methods-** This retrospective observational study was designed and conducted at the Department of Microbiology; Moti Lal Nehru Medical College (MLNMC), Prayagraj Uttar Pradesh. This study was approved by the Institutional Ethics Committee (IEC). All study data was retrieved from Hospital Information System of medical college. **Result -** Among 200 patients, sample for Truenat, RT-PCR and Cobas 6800 testing were collected during study period. Out of 200 samples 57(28.5%) samples were positive by Cobas 6800 with 15(7.5%) invalid results and 128(64%) negative results. In RT-PCR 71(35.5%) samples were positive and 129(64.5%) samples were negative with no invalid results. 200 positive samples were then selected from the RTPCR and COBAS 6800 positive samples for Truenat testing. Out of these 200 positive samples results of 60(30%) samples were presumptive positive and 128(64%) were negative invalid results 12(6%). **Conclusion-** we have concluded that RT-PCR is the best diagnostic method for the detection of COVID-19 in a country like India, where there is shortage of trained man power, well equipped labs and poor infrastructure. It also reduces the financial burden in the era of pandemics, where whole population needs to be catered.

KEYWORDS : SARS-CoV-2, RT-PCR COBAS 6800, TRUENAT, COVID-19

INTRODUCTION

In December 2019, a group of patients of unknown aetiology with atypical pneumonia emerged in Wuhan, China [1]. Severe Acute Respiratory Syndrome Corona Virus 2 (SARS CoV-2), a novel beta-coronavirus was identified as the cause of these cases [2]. As of May 29th 2020, according to the Johns Hopkins University COVID-19 Dashboard, there were over 5.8 million confirmed cases of COVID-19 and more than 360,000 deaths worldwide [3]. Diagnosis of the cases became utmost important and thus the testing capabilities were absolutely essential for managing a pandemic; as before admission of a patient in the hospitals, invasive procedures, major and minor surgeries etc, COVID-19 testing became mandatory. Currently Real Time Polymerase Chain Reaction (RTPCR) is the gold standard test for the diagnosis of COVID -19; however, it requires turnaround time of 6–8 hours, well equipped biosafety laboratory level-II and/or III along with trained manpower. RTPCR testing leads to delay in diagnosis in cases where early and rapid diagnosis of COVID-19 is warranted which leads to tension and anxiety both among patient and treating health care workers.

So, a cheap and rapid, molecular diagnostic test with high specificity and sensitivity is urgently required for SARS CoV-2 detection, especially in rural areas and developing countries where there is lack of well-equipped labs and poor infrastructure [4]. The Cobas 6800 system is a high throughput fully automated solution, that in a number of ways can drive laboratory efficiency. It can run up to 1200 samples in a day. True Nat assay is a chip based rapid molecular diagnostic test for detecting SARS CoV-2. This technology is based on portable, light weight, battery powered TaqMan probe based Real Time Polymerase Chain Reaction technology developed and manufactured by Molbio Diagnostics Private Limited, Goa, India. The Truenat device can also be used for detection of more than 25 pathogens like malaria, tuberculosis, hepatitis B, HIV, dengue, H1N1 influenza, chikungunya, Rabies, Influenza, SARS Cov-2 etc [5,6].

MATERIAL AND METHODS

Sample collection

Nasopharyngeal and oropharyngeal swabs from the known SARS-Cov-2 patients were collected in Viral Transport Medium (VTM) as per the guideline provided by the Indian Council of Medical Research (ICMR).[7]

Processing of samples

All the samples were processed in BSL3 labs with standard precaution, and proper cold chains were maintained for the storage (80°C) of the sample.[8]

Study site and population

This retrospective observational study was designed and conducted at the Department of Microbiology; Moti Lal Nehru Medical College (MLNMC), Prayagraj Uttar Pradesh. This study was approved by the Institutional Ethics Committee (IEC). All study data was retrieved from Hospital Information System of medical college. The samples for RT-PCR testing were collected at triage of a dedicated COVID-19 sample collection centre and received for processing by maintaining proper cold chain at COVID-19 molecular lab, For Truenat testing single oropharyngeal swab was collected in Viral Lysis Media (VLM) provided by Molbio diagnostics Ltd, whereas for RT-PCR and Cobas 6800 both oropharyngeal and nasopharyngeal swabs were collected in VTM (HImedia Labs, India) and transported to the COVID-19 laboratory in a cold chain. At time of COVID-19 pandemic peak (April–May 2021) any patient requiring treatment at MLNMC, PRAYAGRAJ had to undergo mandatory COVID-19 testing and all cases in which both samples for RTPCR and Truenat were collected within time gap of less than 24 hours by treating physician were included in this study.

COVID-19 detection by Real Time PCR

Ribonucleic acid extraction

Viral Ribonucleic acid (RNA) for RT-PCR was extracted in an automated 96 well Thermo- Fisher extractor with the use of ThermoFisher reagents and plasticware which includes (1) Tip comb plate, (2) Elution plate containing 100 μl of elution buffer/ well, (3) Wash II plate containing 1000 μl 80% ethanol/well, (4) Wash I plate containing 500 μl wash buffer/well and (5) sample plate containing 200 μl sample + master mix (265 binding solutions + 10 μl magnetic beads + 5 microliter proteins k).[8]

Qualitative Real Time PCR

A 20 μL reaction was prepared for detection of SARS CoV-2 by RT-PCR utilizing 5 μL of extracted RNA, 6.0 μL of Covisure master mix, 3.0μL of prime probe and sequences targeting N genes, Orf1 and RnaseP as per Covisure kit protocol[7]. The Thermal Cycling was performed at 50°C for15 min for reverse transcription, followed by 95°Cfor 3 min and then 40 cycles of 95°C for 10 s, 60°C for 30s using Quant Studio 5 Real Time PCR system (Thermo Fisher Scientific, Massachusetts USA). All samples were screened for N gene and positive samples were confirmed by detection of specific Orf1 gene. Cut off threshold (Ct value) <40 was considered as positive.

Truenat workstation

The True Nat workstation consist of a nucleic acid extraction device (True prep AUTO V2) and a real time polymerase chain reaction analyser (True lab Uno Dx/Uno/Quattro), along with accessories such as a RNA cartridge, True Nat Chips, micro tip holding stand etc. Both devices are portable, powered by a rechargeable battery and can run continuously for approx., 6 hours on single charge. True prep AUTO V2 is fully automated RNA extractor and uses a disposable fluidic cartridge to extract RNA from VLM within 15 minutes. 6 μL of extracted RNA is added to a PCR tube consisting of room temperature stabilized real time PCR reagents and the mixture is added onto a disposable microchip. The chip is loaded in Truelab analyser and programme is selected for appropriate assay. COVID -19 testing was done using two step strategy; all samples are initially tested by E gene assay and all positive samples with cut off threshold of <32 is confirmed by RNA dependent RNA polymerase (RdRp) gene assay[9]. All samples that are positive by RdRP assay with Ct value < 32 are considered as true positives. We used Truelab Quattro model in this study. Four samples can be processed at a time taking about 1 hour, including sample preparation. One device can process 64 samples per day in a 24-hour working laboratory.

Cobas 6800 Automated System

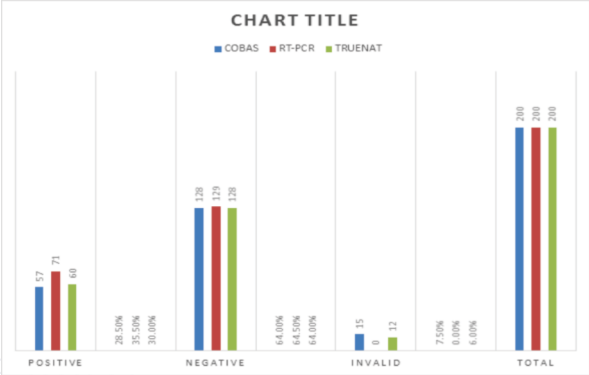
Due to safety considerations, for the automated system, all of the samples were heat-inactivated (56 C for 10 min) and handled inside a class BSC II biosafety cabinet before being loaded onto the Cobas 6800 instrument (Roche Molecular Systems, Rotkreuz, Switzerland). The Cobas SARS-CoV-2 kit was used. The Cycle Threshold (Ct) values reported by the Cobas SARS-CoV2 test were either "detected" (ORF1 gene and E gene detected), "presumptive positive" (ORF1 gene not detected; E gene detected), or "not detected" (ORF1 gene and E gene not detected). The limit of detection for this assay was determined to be 4.4 copies per reaction (package insert, AccuPlex™ SARS-CoV-2 reference material kit). All protocols were performed according to the manufacturer's instructions.

RESULT

Among 200 patients, sample for Truenat, RT-PCR and Cobas 6800 testing were collected during study period. Out of 200 samples 57(28.5%) samples were positive by Cobas 6800 with 15(7.5%) invalid results and 128(64%) negative results. In RT-PCR 71(35.5%) samples were positive and 129(64.5%) samples were negative with no invalid results. 200 positive samples were then selected from the RTPCR and COBAS 6800 positive samples for Truenat testing. Out of these 200 positive samples results of 60(30%) samples were presumptive positive and 128(64%) were negative invalid results 12(6%) The detailed analysis of the 19 Truenat negative and RT PCR positive samples showed that of these 13 samples were Truenat positive for E gene with CT value more than 32, but were considered negative as per Truenat interpretation guidelines.

	POSITIVE	NEGATIVE	INVALID	TOTAL
COBAS	57	128	15	200
RT-PCR	71	129	0	200
TRUENAT	60	128	12	200

Category	Positive		Negative		Invalid		Total
	#	%	#	%	#	%	
COBAS 6800	57	28.5%	128	64.0%	15	7.5%	200
RT-PCR	71	35.5%	129	64.5%	00	00%	200
Truenat	60	30.0%	128	64.0%	12	6.0%	200



Conventional molecular diagnostic methods include several manual steps, such as nucleic acid extraction, master mix preparation and RT-PCR setup, as well as the interpretation of results. These steps are labour intensive and time consuming. A fully automated system allows for the handling/testing of large numbers of samples and also significantly reduces the hands-on time required for sample processing and RNA extraction. In addition, it is easier and quicker to train personnel who are not familiar with molecular diagnostic assays to work with automated tests. However conventional molecular diagnostic methods include several manual steps, such as nucleic acid extraction, master mix preparation and RT-PCR setup, as well as the interpretation of results. These steps are labour intensive and time consuming.

Detection of SARS CoV-2 by RTPCR is the current gold standard for diagnosis of COVID-19. World health organization has repeatedly stressed the importance of the molecular diagnosis of COVID-19 for prompt management of patients, isolation and contact tracing and limit its spread. However, for performing RTPCR testing a fully functional air-conditioned biosafety level-II microbiology laboratory is required; equipped with specialized instruments like biosafety cabinets class II, automated RNA extractors, Real time PCR machine and trained manpower to process the samples while ensuring biosafety and bio security. But according to our results the sensitivity and specificity of RT-PCR is higher and it gives more reliable results. Also RT-PCR being an open system for testing, it allows to do the pooling of samples in high throughput labs which helps to gives timely results in restricted and high patient sample load environment.

CONCLUSION

On comparing the three diagnostic methods we assessed that the turnaround time (TAT) for Truenat was the shortest being around 1 hour, COBAS 6800 turnaround time was 3 hours and RT-PCR turnaround time was the shortest at an average of 2 hours and 30 minutes which can vary with different Covid-19 RT-PCR Kit manufacturers.

Truenat is simple, easy to use, good and rapid molecular diagnostic tool for COVID-19. It is capable of producing faster results than standard RT-PCR tests. It has a contamination/ evaporation resistant design as it is a closed system and it enables point of care testing and reporting of COVID-19 in areas with poor infrastructure but testing capacity remains a challenge for Truenaat. Cobas is intended for fully automated sample-to-result qualitative detection of SARS-CoV-2 in nasopharyngeal and oropharyngeal swab samples collected from patients. Full automation reduces the potential for human error and free-up technicians for other tasks. But the high cost of the instrument and reagents and requirement of advanced infrastructure prove to be a hurdle in its path. On the other hand, laboratory testing with RT-PCR has an advantage over the other two methods that it has high sample throughput processing ability because it allows pooling of samples that cannot be achieved by Truenat or Cobas 6800. Further the results of the study show the high specificity of the RT–PCR with no invalid results and its accuracy and precision level is higher than other methods. Therefore, after comparing all the aspects of our study, we have concluded that RT-PCR is the best diagnostic method for the detection of COVID-19 in a country like India, where there is shortage of trained man power, well equipped labs and poor infrastructure. It also reduces the financial burden in the era of pandemics, where whole population needs to be catered.

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