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ORIGINAL RESEARCH

Laboratory detection of Sars Cov 2-Comparative Evaluation of a Lateral Flow Assay And Truenat™ RT- PCR

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Abstract

Introduction: The world witnessed a pandemic in the form of Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2) beginning in late 2019 initiating from the focus of wet markets in China. The infection got rapidly transmitted via droplets/aerosols & contact with respiratory secretions, leading to the development of mild/moderate/severe pneumonia. Although only 15-20 % of infections were of a moderate/ severe nature, they were sufficient to overwhelm the healthcare system of any country. Early detection of COVID-19 infection was of utmost importance to ensure timely isolation of positive cases to contain transmission in community settings. Therefore development and continued evaluation of the performance of the point-of-care tests were essential. for the purpose.

Aim: To compare the diagnostic performance& usefulness of Standard™ Q COVID-19 Ag kit (SD Biosensor®, Republic of Korea) (index test) against TrueNat RTPCR (Molbio Diagnostics, India) which is a modified RTPCR (reference test) and approved by ICMR, in early detection of COVID cases so that early treatment can be instituted and prompt isolation of Covid positive patients can be done.

Material & Methods: This study was carried out in the molecular biology laboratory of the Department of Microbiology, Noida International Institute of Medical Sciences, Greater Noida between April 2021 and September 2021. Sixty-two nasopharyngeal specimens from patients clinically suspected of having COVID-19attending OPD and admitted to wards of NIIMS hospital were tested both by COVID-19 lateral flow assay and TrueNat RTPCR assay simultaneously. Results of specimens tested with either COVID lateral flow assay or TrueNat RTPCR alone were excluded. The data were analyzed statistically.

Result: SARS CoV2 was detected in 8 specimens by RT PCR assay and in 5 specimens by lateral flow assay, all 5 being positive by RT PCR assay.

The sensitivity of rapid antigen detection lateral flow assay was 62.5% while the specificity was 100% at a 95% confidence interval. The positive predictive value was 100% and the negative predictive value was 94.7%.

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Conclusion: Our study indicated that STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) is a useful test having high specificity and moderate sensitivity which can be used as a screening test for rapidly identifying positive patients in community settings. However, for the detection of all positive Covid patients, RTPCR should be used wherever feasible.

Keywords: COVID-19, Laboratory Diagnosis, Rapid Antigen Test, TrueNat RTPCR.

Introduction

The world witnessed the Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2) pandemic in the last 2 years which started in December 2019, initiating from Huanan wet market in Wuhan, the capital city of Hubei province, China. The scale of the pandemic was unprecedented. Despite all the advances in science and medicine, emerging & re-emerging infections pose a threat to human lives, the global economy, and the healthcare system, which keep challenging us to devise newer methods of diagnosis& control.

The Coronaviruses belong to the subfamily of Orthocoronavirinae of the family of Coronaviridae.[1,2] They are enveloped viruses having a positive-stranded RNA and a helical nucleocapsid.[3] Coronaviruses are large, spherical particles around 125 nm in diameter. It consists of a lipid bilayer envelope, with the membrane (M), envelope(E), and spike (S)structural proteins attached to it.[4] The S protein is composed of an S1 and S2 subunit which helps in binding the virus to the receptors present on the host cell. The E and M protein, on the other hand, are required by the virus to maintain its structure.[5] Inside the envelope is the nucleocapsid (N)protein, which is bound to a single-stranded RNA genome.[6]The virus attaches itself to the host cell, introducing its genome in the cytoplasm of the host cell where the replication starts. RNA-dependent RNA polymerase (RdRp)protein is the main replicase enzyme.

The detection of the nucleic acid sequence of the virus by either real-time reverse-transcription polymerase chain reaction (RT-PCR) analysis, nucleic acid next-generation sequencing (NGS), or other molecular tools is currently the gold standard for diagnosing SARS-CoV-2 infection. The targets of the SARS-CoV-2 sequence used for genomic detection currently include three conserved gene sequences in the viral genome viz. the open reading frame (ORF), nucleocapsid protein (N) gene, and envelope protein (E) gene.[7]The specimens for testing can be nasopharyngeal swabs, sputum, other lower respiratory tract secretions, blood, and feces.

Despite the high sensitivity of the RT-PCR assay, the quality of the specimens, collection methods, sample storage method, the time interval between transportation to viral RNA extraction, and the reagents used for extraction can all contribute to the variability of the detection sensitivity. On the other hand, specimens inadvertently contaminated by amplicons generated from other samples could create false positivity given the molecular amplification involved in the diagnostic system.[8]

Early and reasonably accurate detection of SARS-CoV 2 is necessary for isolating and treating the infected individuals. WHO has recommended molecular methods as the method of choice for the detection of SARS CoV 2 viral infection. As setting up a molecular laboratory incurs high costs & a high level of expertise is involved in performing RNA extraction and analyzing PCR assays, early detection of COVID-19becomes challenging, especially in resource-poor settings of developing countries. The main burden of diagnosis becomes restricted to centralized reference laboratories with skilled manpower and elaborate infrastructure. Therefore the results are available to the clinicians with a longer turnaround time. Even then due to the multiple steps involved in the process, there are chances of errors.[9]

Therefore, a reliable one-step/point of care assay taking less time to perform and less expertise to interpret, has low cost & not requiring elaborate infrastructure, and providing

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satisfactory test results for COVID-19 was urgently sought after. The Indian Council of Medical Research (ICMR) on April 10, 2020, validated the usage of the TrueNat assay (Molbio Diagnostics, India), a diagnostic platform for Tuberculosis, for COVID-19 tests. This chip-based real-time RT-PCR assayis a modified real-time RT-PCR that is easy to perform, has a rapid turnaround time, and is cost-effective when only a few samples have to be tested per da. This does not require a biosafety cabinet and staff with minimal training can perform the test.

The rapid antigen Test (RAT) tests are immunochromatographic tests, commercially available as lateral flow assays, which detect viral antigens by the immobilized SARS-CoV-2 antibody coated on nitrocellulose paper. These lateral flow assays which use monoclonal antibodies specific to SARS-CoV-2 antigens extracted from the nasopharyngeal and oropharyngeal swabs can be utilized as screening tests. The RAT test takes less time giving the results within 30 min without the need for any specialized instrument. Hence, RAT tests can drastically improve turnaround time and reduce the workload in already strained diagnostic hospitals and laboratories if their performance is found satisfactory. In India, an advisory was issued by the Indian Council of Medical Research (ICMR) (on 14th June 2020) regarding the usage of RAT for quick detection of COVID-19 positive patients.[10]

We conducted this study in our molecular biology laboratory where we used lateral flow assays and TrueNat RTPCR assay to detect and diagnose COVID-19 during the second covid wave in India. We intended to do a comparative evaluation of a rapid SARS-CoV-2 antigen detection test using Standard™ Q COVID-19 Ag kit (SD Biosensor®, Republic of Korea)(index test)against TrueNat RTPCR(reference test), to assess the usefulness of lateral flow assay test in early detection of COVID cases so that early treatment can be instituted and prompt isolation of positive patients can be done.

Material & Methods

This study was disbursed within the molecular biology laboratory of the Department of Microbiology, Noida International Institute of Medical Sciences, Greater Noida in between April 2021 and September 2021. Test results of 62 specimens from OPD & IPD patients who were suspected of having COVID-19wereused for comparison which was tested both by COVID-19 lateral flow assay and TrueNat RTPCR assay simultaneously. Results of specimens tested with either COVID lateral flow assay or TrueNat RTPCR alone were excluded. The results of the lateral flow assay (index test) were compared against TrueNat RTPCR (reference test).

Sample collection

From each patient, two sets of both nasal and oropharyngeal specimens were collected using the specimen collection kits for respective tests. Sterile nylon flocked nasopharyngeal specimen collection swab was introduced in one nostril of the patient until resistance was felt at the nasopharynx, rotated 180°, then withdrawn. A sterile oropharyngeal specimen collection swab was introduced within the mouth, and until reaching the oropharynx, then the swab was rubbed on the posterior wall for 10–15 seconds.[11]Both swab applicators were placed into a vial of viral transport media. (Molbio for TrueNat assay and SD BIOSENSOR for lateral flow assay)

Two sets of samples from each patient were collected, one for TrueNat RTPCR and another for lateral flow assay. The samples for RT-PCR were immediately transported to the laboratory for SARS-CoV-2 RNA determination in cold box and stored at 4^0C till testing, while SARS-CoV-2 antigen analysis was carried out immediately.

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SARS-CoV-2 RNA detection using real-time RT-PCR[12]

RNA Extraction TrueNat™

RNA from the patient sample was extracted using Trueprep® AUTO/AUTO v2 Universal Cartridge based Sample Prep Device and Trueprep® AUTO/AUTO v2 Universal Cartridge based Sample Prep Kit as per manufacturer's instructions. The process took 20 minutes.

Real Time RT-PCR Assay

Chip-based Real Time Duplex PCR Test for COVID-19

The RT PCR assay was done using TrueNat™ Chip-based Real Time Duplex PCR Test for COVID-19. TrueNat COVID-19 is a disposable, room temperature stable, chip-based Real Time duplex PCR test with dried $MgCl_2$ in the reaction well and freeze-dried RT PCR reagents in a microtube for performing Real Time RT-PCR test and runs on the Truelab® Real Time micro PCR Analyzer (Truelab® Quattro). It required only six (6) µL of purified RNA to be added to the reaction well for the analysis. The target sequence for this kit was E and Orf1a genes of the SARS Cov-2 virus and internal positive control was the human RNase P gene. Detection of the human RNase P gene serves as a full process internal positive control (IPC) for proper swab collection, nucleic acid extraction and PCR. The process took 40 minutes. The RTPCR test result was displayed in real-time in the inbuilt monitor of the machine. Additionally, a printout was taken from the attached printer.

Rapid SARS-CoV-2 antigen detection assay[13]

The nasopharyngeal swab specimen collected with the STANDARD™ Q COVID-19 Ag Test kit's material (SD BIOSENSOR)was used for this test. The swab was placed within the extraction buffer provided in the kit and gently rotated several times to resuspend the sample. Three drops from this medium were deposited in the cassette well of STANDARD™ Q COVID-19 Ag Test card (SD BIOSENSOR), which detects the SARS-CoV-2 C-terminal-nucleocapsid (N) antigen in respiratory specimens. Results were visible in 15-30 minutes. The test was considered positive when bands were observed in the control and test positions. Negative results were interpreted when only a band in the control position was observed. The test was invalidated when no bands were detected.

Statistical analysis

The test results were processed using Microsoft Excel. Descriptive statistics were expressed as mean±standard deviation (SD). p values <0.05 were considered statistically significant.

Results

Out of 62 samples,8 (12.9%) were positive by Truenat RTPCR while54 (87.09%) were negative. STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) detected 5 (8.06%) samples as positive while 57 (91.94%) were negative (Table 1). All 5 specimens positive by lateral flow assay were positive in Truenat RTPCR assay. The antigen test showed a performance sensitivity of 62.5% and a performance specificity of 100%; this test exhibited a positive predictive value of 100% and a negative predictive value of 94.7%(Table 2).Ct-values from those patients positive to the antigen test presented a Ct-mean of 21.85±3.74&20.67±3.52for E gene and ORF1a gene respectively. The three specimens with negative results in the antigen test but positive in RTPCR assay exhibited Ct-mean of 26.99±2.60 & 28.06±3.87 in E gene and ORF1a gene respectively. (p value$\leq$0.05)

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Table 1: Comparison of COVID-19 test results by STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) lateral flow assay & Truenat RTPCR

Test	Positive	Negative	Total
TrueNat™ RTPCR	8 (12.90%)	54 (87.09%)	62
STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR)	5 (8.06%)	57 (91.94%)	62

Table 2: Test results of STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) (index test) against TrueNat™ RTPCR (reference test)

	TrueNat™ RTPCR (REFERENCE TEST)		
	POSITIVE	**NEGATIVE**	**TOTAL**
STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) (INDEX TEST)			
POSITIVE	TP=5	FP=0	5
NEGATIVE	FN=3	TN=54	57
TOTAL	8	54	62

Abbreviations: FN: False-negative; FP: False-positive; TN: True-negative; TP: True-positive.

Table 3: Performance of STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) against TrueNat™ RTPCR

Statistical Parameter	Estimated Value (%)
Sensitivity	62.5%
Specificity	100%
Positive Predictive Value	100%
Negative Predictive Value	94.7%

Discussion

Early detection of COVID 19 infection helps in early isolation & quarantining of the patient so that the spread can be controlled and early treatment interventions can be taken. The gold standard method of detection by RTPCR involves expertise and takes a longer time in giving results. Also running samples on a conventional RTPCR system is not cost-effective when only a few samples have to be tested per run. Therefore, soon after the emergence of COVID 19, there was a search for methods of detection which could give results in less time, and cost and involve less expertise in analysis. ICMR validated the use of TrueNat RTPCR for COVID testing in April 2020. TrueNat RTPCR is a modified chip based RTPCR which takes around 60 minutes to give results of COVID-19 test and is cost-effective when only a few samples have to be tested per day. ICMR later in June 2020 introduced Rapid Antigen Test for COVID 19 which was approved for screening purposes. RAT results are available in 20-30 minutes. These tests improved the result timeline, nonetheless, their validity and reliability must be evaluated thoroughly in practice.

STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) is a novel diagnostic tool based on immunochromatographic principle that detects specific viral antigens from SARS-CoV-2 recovered from nasopharynx samples. Its use and interpretation are simple, the use of a safetycabinetor highly trained personnel are not needed, thus improving the procedure costs and helping in the resolution of the public health problem.

In our study, we observed sensitivity of 62.5% and a specificity of 100 % for STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) in comparison to TrueNat RTPCR assay. Sensitivity in our study is slightly less than and specificity is similar to results obtained by Krüttgen A et al.[14] who reported sensitivity of 70.6% and specificity of 100% and Cerutti F et al.[15] who reported sensitivity of 70.7%, and specificity of 96%. The manufacturer reported a

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sensitivity of (62.8-86.4%)[13]and a specificity of (98.6-99.6%) in the kit literature which is similar to our study.

Chutikarn C et al.[16] in their study showed a higher sensitivity using antigen-based detection test (98.33%); although this study used clinical specimens collected in viral transport media, a pre-treatment was performed on the sample previous to the antigen analysis.This pre-treatment probably increased the efficiency of the procedure, but also increased the cost and the need for highly trained personnel.

The antigen detection assay's sensitivity and specificity was found to be highly dependent on viral load, decreasing with the decrease of viral load which has been demonstrated by other authors .[14,15]Therefore, the test may underdiagnose patients during the early or late phase of the infection when viral load is less, and clinical correlation must be done and a second test for confirmation must be done keeping a high index of suspicion. Some researchers have also demonstrated that samples with high Ct value/ low viral load rarely become culture positive for the virus.[15,17,18]

The results from our study indicate that STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) rapid antigen test is a useful test having high specificity and moderate sensitivity which can be used as a screening test for rapidly identifying positive patients ensuring timely treatment and isolation. A diagnostic test with high specificity has few false positives; those who test positive are indeed mostly positive as was found in this study. A test with high specificity is therefore particularly suitable for confirming disease in the event of a positive result. But the test results must be interpreted in conjunction with clinical symptoms, adding a confirmatory RTPCR test as the need arises.

Conclusion

Early detection of COVID-19 with a point of care test, having reasonable performance characteristics, which is also cost-effective, requires less time and expertise to perform and interpret is the game changer in the control of spread and management of the disease. Rapid antigen tests were developed with the same purpose. Several manufacturers have come up with their own RAT claiming specific performance characteristics. These should be evaluated time and again to assess their usefulness for the purpose for which they are used. The results from our study indicate that STANDARD™ Q COVID-19 Ag Test (SD BIOSENSOR) rapid antigen test is a useful test having high specificity and moderate sensitivity which can be used as a screening test for rapidly identifying positive patients ensuring timely treatment and isolation.

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