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Coronavirus Pandemic

"David *vs.* Goliath": A simple antigen detection test with potential to change diagnostic strategy for SARS-CoV-2

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Abstract

Introduction: As regard to all pandemics, the current COVID-19 pandemic, could also have been better managed with prudent use of preventive measures coupled with rapid diagnostic tools such as rapid antigen tests, but their efficacy is under question because of projected lower sensitivity as compared to Real Time Reverse Transcriptase Polymerase Chain Reaction, which although considered gold standard has its own limitations.

Methodology: A prospective, single centre study was carried out to evaluate the performance of Standard Q COVID-19 Ag, a rapid immunochromatographic assay for antigen detection, against TrueNat, a chip-based, point-of-care, portable, Real-Time PCR analyzer for diagnosis of COVID-19; on 467 nasal swab samples from suspected subjects at a fever clinic in North India in month of July 2020.

Results: Of the 467 specimens tested, TrueNat showed positive result in 29 (6.2%), majority of whom were asymptomatic (72.4%) while 4/29 (13.9%) had influenza like illness and 2/29 (6.8%) presented with severe acute respiratory illness. Compared to TrueNat, Rapid antigen test gave concordance for 26 samples, while for 2 samples the result was false positive; giving an overall sensitivity of 89.7% (95% CI = 72.6-97.8) and a specificity of 99.5%, indicating strong agreement between two methods.

Conclusion: Community prevalence plays an important role is choosing the laboratory test and result interpretation. Rapid antigen detection tests definitely have a big role to play, especially in resource limited setting, for early diagnosis as well as for source control to halt the spread.

Key words: COVID-19; TrueNat; rapid antigen test; RAT; RT-PCR; immunochromatographic assay.

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Introduction

Indeed a Goliath of this time, SARS-CoV-2, the causative agent of COVID-19 pandemic, has devastated the world in a span of few months since its outbreak which began in Hubei province of China in the end of 2019 [1-3]. To counteract and contain the pandemic one of the key measure is large scale testing and early identification of cases followed by preventive measures to curtail the spread [3]. But the attempt at early diagnosis of COVID-19 cases is easier said than done. Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) and viral culture being the gold standards in the diagnosis of SARS-CoV-2 infection, take many hours to detect the nucleic acid and days to isolate the virus [4]. Besides, due to its limited availability and technicality, virus isolation is mainly useful for research rather than diagnostic purpose.

The requirement of expertise, infrastructure and long turn over time is not only taking a toll on health care system, but is also jeopardizing the complete process of containing the COVID-19 pandemic. Antigen detection tests based on Immunochromatography / Rapid antigen tests (RAT) have the potential for besides being rapid; simple, inexpensive, point of care (POC) test for diagnosis of COVID-19 [5] in other words, the young "David".

Indian Council of Medical Research (ICMR), New Delhi, has recently approved Standard Q COVID-19 Ag (SD Biosensor Inc, Republic of Korea), a rapid immunochromatographic assay. It earlier approved TrueNat (MolBio Inc, India) a chip-based, point-of-care, portable, Real-Time micro PCR Analyzer for the detection of SARS-Cov-2. Though the RAT have potential for widespread use in resource limited settings, their efficacy is often doubted because of their reported low sensitivity of as low as 50% in few studies [11,12].

As compared to rapid antigen tests, RT-PCR based assays are limited by the need for investment in the equipment and infrastructure, trained manpower, maintenance issues besides longer turnaround time and cost involved, thereby limiting their utility in pandemic situation.

The aim of the study is to assess the performance of COVID-19 Rapid antigen test, Standard Q COVID-19

Ag as compared against TrueNat, Real-Time micro PCR Analyzer.

Methodology

This prospective single center study was carried out on 467 nasal swab samples taken in duplicate, which were subjected to both TrueNat procedure and RAT. Study subjects included patients with influenza like illness (ILI), presenting to the fever clinic as well as, asymptomatic contacts of known COVID-19 patients and asymptomatic patients requiring aerosol generating surgical / non-surgical interventions at a tertiary care hospital in Lucknow, Uttar Pradesh in the month of July 2020; as per the guidelines for Rapid antigen testing, issued by the ICMR, New Delhi [11].

The Standard Q COVID-19 Ag assay is a rapid chromatographic immunoassay/rapid antigen test for the qualitative detection of Nucleocapsid (N) antigen of SARS-CoV-2 present in human nasopharynx. TrueNat™ SARS CoV-2 works on the principle of chip based RT PCR, using Taqman chemistry detecting Envelope (E) gene in screening test and RNA dependent RNA polymerase (RdRp) in confirmatory assay. The result is displayed as "high detected", "medium detected", "low detected" and "very low detected" corresponding to Cycle threshold (Ct) value range of 15-20, 21-25, 26-30 and >30 respectively as per the software of the TrueNat machine. The Ct value is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run.

For both the RAT and TrueNat, sample collections as well as the tests were performed as per the manufacturer's instructions. Two separate nasal swabs were collected from each patient for both the procedures. In the laboratory, separate sets of technicians performed the two tests on all 467 samples in single blinded manner.

Rapid antigen testing procedure

In brief, the swab was collected by inserting sterile swab into patient's nostrils reaching the surface of posterior nasopharynx followed by swabbing over the posterior nasopharynx. The swab was withdrawn from nasal cavity and inserted into the extraction buffer tube. While squeezing the buffer tube, swab was stirred for more than 5 times. The swab was removed while squeezing the sides of tube to extract the liquid from swab and discarded in 0.5% hypochlorite solution. Nozzle cap was tightly pressed onto the tube. Three drops of extracted specimen were applied to the specimen well of the test device. Entire procedure from collection to this point was carried out within 60 minutes. The results were read after 15-30 minutes and read visually as positive if the control lines and test lines both showed band. The test was read as negative if only the control line showed band and invalid if only test line showed band.

TrueNat procedure

TrueNat was performed on another set of nasal swab collected from the same patients as mentioned above. The swab was transported in the viral lysis media as provided in the kit. The viral RNA from the swab was extracted using Trueprep AUTO/AUTO v2 Universal® Cartridge based Sample Prep Device and Trueprep AUTO/AUTO v2 Universal Cartridge based Sample Prep Kit and assayed using TrueNat Beta CoV chip. If the sample tested positive for Beta CoV, six (6) µL of the same extracted RNA from the Beta CoV positive sample was dispensed into the reaction well of the TrueNat™ SARS CoV-2 chip. The chip was inserted in the Truelab Real Time Quantitative micro PCR Analyzer where the RNA is first converted into complementary DNA (cDNA) by the Reverese transcriptase enzyme and further thermal cycling takes place. A positive amplification causes the dual labelled fluorescent probe in the chip-based Real Time PCR test to release the fluorophores in an exponential manner which is then captured by the built-in optoelectronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run.

Statistical analysis

Data was described using descriptive analysis. For categorical variables, frequencies and percentages were reported. For continuous variables, mean and standard deviation were used to summarize the data. Distribution of Ct values was represented using Box and Whisker plot and both tests were compared using Weighted Cohen Kappa index. The analysis was done using MedCalc Software.

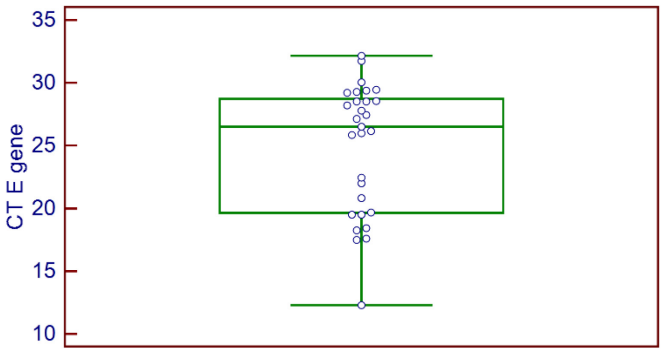
Results

Nasal swabs collected from 467 subjects were independently tested by TrueNat and RAT. Among study subjects, male to female ratio was 1.04: 1 with median age of 32 years (range 2 to 85 years) with majority (87.8%) belonging to age group of 18 to 59

years. TrueNat testing showed positive result for 29 (6.2%) patients. The demography of patients has been described in Table 1. The overall median Ct value for E gene among positive patients was 26.5 (IQR = 12.3 - 32.14; mean = 24.8) and median Ct value for RdRp gene was 26.3 (IQR = 12.3-33.09; mean = 24.9) (Table 1 and Figure 1). Among those detected by TrueNat 'high detected' was seen in 7 (24.1%) patients, 'medium detected' in 4 (13.8%) patients while 'low detected' was seen among 18 (62%) patients (Table 2). Amongst those flagged 'high detected', symptoms were present in 2/7 (28.5%) patients in 'medium detected' symptoms were present in 1/4 (25%) patient while 5/18 (27.7%) patients were symptomatic among 'low detected'. Majority of those detected positive by TrueNat, 21/29 (72.4%) were asymptomatic. Of 8/29 symptomatic TrueNat positive patients, 4 (13.9%) had ILI symptoms and 2 (6.8%) presented with severe acute respiratory illness (SARI) as defined by World Health Organization (WHO) [13].

Results of RAT were concordant with TrueNat results in 26 patients, for remaining three the RAT was not able to detect the presence of SARS CoV-2. Whereas for 2 subjects, while RAT showed positive result, the TrueNat remained negative even on repeat testing. RAT when compared with TrueNat test showed sensitivity of 89.7% (95% CI = 72.6- 97.8) and specificity of 99.5%. Agreement kappa index between two methods was 0.9 (95% CI = 0.82 to 0.98) indicating strong agreement between two methods (Table 3).

Figure 1. Graph depicting interquartile range of Ct for E gene in patients positive by TrueNat test.



Discussion

In the present study both TrueNat and Rapid antigen test demonstrated good concordance. All but two RAT negative samples were also TrueNat negative (two were false positive detected only by RAT). Besides RAT picked up 26/29 samples as positive which were detected by TrueNat, it however missed three TrueNat positive samples thus bringing its sensitivity to 89.5%. This is much more than what is mentioned in ICMR advisory (50.6% to 84%) and as low as 30.2% in the study carried out by Anais Scohy *et al* [14]. The specificity of RAT (99.5%) falls well within the range claimed by ICMR advisory of 99.3% to 100%.

The possible explanation for two false positive results of RAT samples could be due to cross reaction of antigen between common cold causing Coronavirus and SARS-CoV-2 to antibody present on test strip [15].

Table 1. Demography of cases included in the study.

		All	TrueNat Positive	TrueNat Negative
Total		467	29	438
Gender	Male	239 (51.2)	19 (65.5)	220 (50.2)
	Female	228 (48.8)	10 (34.5)	218 (49.8)
Age	Median	32	44	31
	Range	2-85	2-80	7-85
	0 to 17 years	15 (3.2)	2 (6.9)	13 (3.0)
	18 to 59 years	410 (87.8)	22 (75.9)	388 (88.6)
	≥ 60 years	42 (8.9)	5 (7.2)	37 (8.4)
Clinical feature	ILI	12 (2.6)	4 (13.9)	8 (1.8)
	SARI	2 (0.4)	2 (6.8)	0
	Breathlessness	4 (0.9)	2 (6.9)	2 (0.5)
	Asymptomatic	449 (96.1)	21 (72.4)	428 (97.7)
Ct Value E gene (overall)	Median		26.5	-
	IQR		12.3- 32.14	-
	Mean		24.82	-
Ct value RdRp gene (overall)	Median		26.3	-
	IQR		12.3- 33.09	-
	Mean		24.9	-

ILI: Influenza Like Illness: SARI; Severe Acute Respiratory Illness; IQR: Inter Quartile Range; Ct: cycle threshold of TrueNat; E gene: Envelope; RdRp: RNA dependent RNA polymerase.

Table 2. Details of Ct of E gene and RdRp gene among COVID-19 positive cases.

		Median	Mean	Range
E gene	High detected	18.25	17.6	12.3 - 19.7
	Medium detected	21.4	21.2	19.5 - 22.45
	Low Detected	28.5	28.4	25.8 - 32.14
RdRp Gene	High detected	18.0	17.3	12.3 - 19.2
	Medium detected	21.2	21.3	20.1 - 22.5
	Low Detected	28.7	28.4	24.3 - 33.09

E gene: Envelope; RdRp: RNA dependent RNA polymerase.

Both the patients showing false positive result with antigen testing were asymptomatic at the time of testing with no history of contact with a COVID-19 positive patient. Antigen tests are cost effective, requiring minimal or almost nil infrastructural /technical expertise requirement to perform as compared to RT-PCR and allow for earlier diagnosis (within 30 minutes), fulfilling the criteria for rapid diagnostic/POC tests. Well studied antigen assays for influenza and RSV have a high specificity [9,10]. They have also been developed for SARS-CoVand MERS-CoV [6,8]. The major limitation of the available antigen assay is its lower sensitivity compared to RT-PCR [9,10].

On May 9, 2020 the United States Food and Drug Administration (U.S. FDA) granted the first emergency use authorization (EUA) for COVID-19 antigen test (Quidel Corporation) for use as POC, which is only 80% sensitive compared to RT-PCR and 100% specific for detection of Nucleocapsid (N) protein of SARS CoV-2 from naso-pharyngeal and nasal swabs [6].

The low sensitivity detected in the both the studies carried out by Anais Scohy *et al* [14] and Mak *et al* [4] on evaluation of rapid antigen test for COVID-19 could be due to multiple reasons including kit variability as well as the time to performance of the test after sample collection. Upon sample storage there is a possibility that the virus antigen may degrade leading to lower detection rates [11].

There were initial suggestions that rapid antigen tests may miss very low level of virus [14]. But in our study we found that RAT picked up many samples with "low level" of virus as detected in TrueNat assay. RAT detected 18 samples which were flagged 'low detected' in TrueNat with Ct values ranging from 25.83 to 32.14.

Antigen based immuno-assays definitely have a role in the resource limited settings. Rapid Antigen test is almost 100% specific and have much less turnaround time in comparison to TrueNat test and RT-PCR. It can easily be used in rural health care set up especially in a developing country like ours since it does not require much technical expertise, any expensive equipment/biosafety cabinets, electricity or any specific temperature conditions. Kits can be easily transported from one place to another at room temperature.

Rapid antigen tests can also be part of an algorithm for diagnosis of SARS-CoV-2 in health care set up. Therefore, if the existing set up has TrueNat or RT-PCR testing facility, it can be supplemented by addition of RAT. The sample collection being similar, no additional effort or risk is involved to the collecting personnel. One of the sample pairs can easily be tested initially by rapid antigen test to be followed by TrueNat or RT-PCR if the first sample of the pair comes out negative and the history is suggestive.

There are very few research articles that are available to support the high sensitivity of antigen based immuno-assays for diagnosis of SARS-CoV-2. Positivity rate is one the epidemiological variables to assess the impact and spread of COVID-19, which is defined as the percentage of all coronavirus tests performed that are actually positive, or: positive tests/total tests × 100% [18]. The number of tests carried out in India has increased substantially with the start of RAT but due to the projected low sensitivity rate

Table 3. Specificity and sensitivity of antigen detection in total and in different subgroups.

				Antigen detection test				
				Positive	Negative	Sensitivity		Specificity
Cases		**TrueNat**	**n**	**n**	n	**%**	**95% CI**	**%**
All		Positive	29	26	3	89.7	72.6 - 97.8	99.5
		Negative	438	2	436			
Gender	Male	Positive	19	17	2	89.4	66.8 - 98.7	99.5
		Negative	220	1	219			
	Female	Positive	10	9	1	90.0	55.5 - 99.7	99.4
		Negative	218	1	217			

of this test the "Positivity rate amongst the total number of tests performed" has lost importance, which we feel is a wrong projection of this useful test in view of high sensitivity found in our study. We must also not forget that the sensitivity of RT-PCR varies with different samples and accuracy of collection. As per one study the sensitivity was highest with broncho-alveolar lavage (93%) followed by sputum (72%), nasal swabs (63%) and least for throat swabs (32%)[16]. Not only does sensitivity varies with type of sample, it also depends on the day of sample collection before onset of symptoms. As per study conducted by Kucirka *et al* the probability of a obtaining a false-negative result in an infected person decreases from 100% on day 1 to 67% on day 4. The false negativity reduces further to 38% on the day of symptom onset and further to 20% on day 8; post which it starts to rise from 21% on day 9 to almost 66% on day 21 [17]. According to New York times although PCR test seemed best options for detecting coronavirus when this pandemic started but as for now there is a dire need for tests which are rapid and cheap enough to test everyone in need [19]. The cost ofperforming rapid antigen testing is 3 times cheaper compared to PCR based assays which, with sensitivity and specificity equivalent to PCR based assay, could be a boon for developing countries.The major limitations of our study is that we were not able to compare the RAT against a more open platform like RT-PCR, which is considered gold standard for COVID-19 diagnosis.

Conclusions

A combination of clinical, epidemiological and laboratory considerations are essential for accurate diagnosis of emerging infections such as COVID-19. Community prevalence or disease activity plays an important role in choosing the laboratory test and in interpretation of results. Rapid antigen assays may contribute in a more meaningful manner in containment of this pandemic.More studies performed under optimal conditions to evaluate its true sensitivity are needed to bring out it's true potential.RAT for COVID-19 definitely has a chance in diagnosis like a 'Young David' against the 'Goliath' that the SARS CoV-2 is.

Authors' contributions

Study conception and design: Prof. Jyotsna Agarwal, Dr Anupam Das and Dr Jaya Garg. Data acquisition: Dr Pranshu Pandey. Data analysis and interpretation: Dr Anupam Das and Dr Pranshu Pandey. Draft revision: Dr Jaya Garg and Dr Manodeep Sen, Jyotsna Agarwal. All the authors approved the final version for submission.

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