Original Research Article

Evaluation of antigen based rapid diagnostic test in comparison to RT PCR in diagnosis of Sars CoV2 with respect to duration of illness and Ct value of corresponding RT PCR

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Abstract

Background: SARS-CoV-2 is the third highly pathogenic corona virus introduced into mankind after Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS-CoV) in twenty-first century for which the development and validation of rapid and easy-to-perform diagnostic methods are of high priority. **Objective:** In this study we evaluated performance characteristics of RAT, the STANDARD Q COVID19 Ag by SD-Biosensor for rapid detection of SARS CoV 2. **Material and methods:** Samples were collected from 1168 patients and we performed both RAT and RT PCR and the results of RAT were compared with that of RT PCR as gold standard. **Result:** Detection rates of SARS CoV-2 by RAT and RT-PCR were 19.17% and 29.53%, respectively; false positivity rate was 2.67%.False positive and false negative rate was 2.6% and 13.45% respectively.RAT sensitivity, specificity, negative and positive predictive values were 63.18%, 99.27%, 97.32% and 86.54% respectively. Statistical analysis considered the calculation of sensitivity, specificity, positive predictive value, negative predictive value using standard formulae. **Conclusion:** A high sensitivity, specificity, positive predictive value and fairly high negative predictive value of RAT might prove to be promising in situations where pre-test probability of having infection is high.

Key words: RAT, RT PCR, SARS CoV-2

Introduction

On 11 Mar 2020, World Health Organization (WHO) declared Coronavirus Disease 2019 (COVID-19) as a pandemic[1]. COVID-19 is not the first severe respiratory disease outbreak caused by the coronavirus. Just in the past two decades, coronaviruses have caused three epidemic diseases, the other two beingSevere Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)[2]. In context of COVID-19 pandemic, development of rapid and easy-to-perform diagnostic methods is of high priority, to shorten the turn-around time and is a situation that demands cost-efficient approach. Gold standard for detection of SARS-CoV-2 relies on viral RNA amplification by real-time RT-PCR (RT-q PCR) which requires hours to release reports[3].

Current pandemic highlights the limits of production and trade of molecular based tests as we are facing a worldwide shortage of reagents. Point-of-care diagnostic tests (POCTs) for detecting viral antigens in clinical samples, thus, would be very helpful for the diagnosis of COVID-19 as mass-screening and for rapid isolation of patients[4].

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Rapid antigen tests (RAT)detect viral antigen by immobilized coated SARS-CoV-2 antibody on the nitrocellulose membrane. According to WHO, the role of RAT for antigen detection of SARS−CoV-2 needs to be evaluated and is not recommended for clinical diagnosis[5]. However, there is scanty data on the performance and diagnostic accuracy of these RATs[6].

In view of above, this study was undertaken in the Department of Microbiology, Unit of Virology, Calcutta School of Tropical Medicine, Kolkata for a period of three months from July 2020 to September 2020 to determine the performance characteristics of RAT kits in comparison to molecular based test viz. RT PCR.

Objective

To evaluate performance characteristics ofrapidantigen test (STANDARD Q COVID19 AgSD-Biosensor) for detection of SARS CoV-2 nucleoprotein in comparison to gold standard RT-PCR.

Material and methods

A study of performance characteristics of rapid antigen detection test (RAT) for detection of SARS CoV-2 compared to RT-PCR was conducted. Samples were derived from patients with influenza like illness (ILI), patients who are asymptomaticbut having an epidemiological risk factor for COVID 19 infection (close contactwith laboratory confirmed case) and patients having other

features suggestive of COVID 19 like anosmia, loss of taste sensation, cough, diarrhoea, malaise, sore throat, headache, etc were included.
One thousandthree hundred and two (1302) patients attending Fever Clinic of Calcutta School of Tropical Medicine during the study period out of which one thousand one hundred and sixty-eight(1168) patients fulfilling inclusion criteriawere selected in the study.
Samples were collected by trained personnel in separate "sample collection room". Nasopharyngeal (NP) and oropharyngeal(OP) swabs were placed together in a 10-ml tube of viral transport medium(VTM, Hi Media Laboratories) and processed for SARS CoV-2 by RT-PCR using Viral Detect II(Genes2me). Remaining part of the suspension was stored at −80 °C until use in this study.
The STANDARD Q COVID-19 Ag (SD BIOSENSOR) test was performed on all patients following manufacturer's instructions.A sterile flocked swab inserted and rubbed into the nasopharynx of the patient was taken out and dipped into extraction buffer tube, swab was then stirred 5 times both clock wise and anti-clock wise while squeezing sides of the tube. Nozzle cap then pressed tightly and 3 drops of extracted specimen was added to well of test device and result was read in 15-30 minutes. Samples showing both positive and negative result in RAT further underwent RT PCR test for confirmation.
RT PCR assay included a negative control and an RNaseP internal PCR control. Its target genes wereenvelope (E) gene, nucleoprotein (N) gene and RNAdependent RNA polymerase (RdRP) gene of SARS CoV 2 to meet WHO requirements. Samples showing an exponential growth curve and cycle threshold (Ct) value of 37 was taken as cut off for differentiating between positive and negative samples.
Technician performing RAT was blinded to the RT-PCR result.
Results of RAT were compared with RT-PCR. For samples with discordant result, tests were repeated. Demographic and clinical data were obtained from the mandatory ICMR forms filled up for each patient and were analysed in anonymized manner.
Statistical analysis considered the calculation of sensitivity, specificity, positive predictive value, negative predictive valueusing standard formulae.
Result of RAT was also compared to the corresponding Ct value of RT PCR and relation to duration of illness of the patient.

Standard formulae
True positives (TP)= Samples showing positive result by both RAT and RT- PCR.
True negatives (TN)= Samples showing negative result by both RAT and RT-PCR.
False positive (FP)= Samples showing positive result by RAT but are negative by RT-PCR.
False negative (FN)= Samples showing negative result by RAT but are positive by RT-PCR.
Sensitivity and specificity were calculated as per following formulae:
SENSITIVITY= true positives/ (true positives+ false negatives)
SPECIFICITY= true negatives/ (true negatives+ false positives)
POSITIVE PREDICTIVE VALUE (PPV) = TP/ TP+FP
NEGATIVE PREDICTIVE VALUE (NPV) = TN/TN+FN

Results
Number of samples where RAT and RT PCR both done = 1168
Total RAT positives = 224
Total RAT negatives = 944
Total RT PCR positives = 345
Total RT PCR negatives = 823
RAT negative / RT PCR positive (False negative) = 127
RAT positive / RT PCR negative (False positive)= 06
Detection rates of SARS CoV-2 by RAT and RT-PCR were 19.17% (224/1168) and 29.53%(345/1168), respectively; false positivity rate was 2.67% (06/224) (Table I).
RAT sensitivity, specificity, negative and positive predictive values were 63.18 %, 99.27 %, 97.32% and 86.54% respectively, calculated by standard formulae.

Table no I: Correlation of RAT and RT PCR enabling calculation of the important variables

	RT PCR POSITIVE	RT PCR NEGATIVE	TOTAL
RAT POSITIVE	224- 06 = 218 TRUE POSITIVE (TP)	06 FALSE POSITIVE (FP)	224 (TOTAL POSITIVES)
RAT NEGATIVE	127 FALSE NEGATIVE (FN)	944-127= 817 TRUE NEGATIVE (TN)	944 (TOTAL NEGATIVES)
TOTAL	345	823	1168
Sensitivity of RAT = 218/345*100% = 63.18% Specificity of RAT = 817/823*100% = 99.27% POSITIVE PREDICTIVE VALUE (PPV) = TP/ TP+FP = 97.32% (= 218/218+06 = 218/224) NEGATIVE PREDICTIVE VALUE (NPV) = TN/TN+FN = 86.54% (817/ 817+127)			

Table no. II: Correlation of RAT positivity with corresponding Ct values of RT PCR

RT PCR Ct values	RAT positivity (%)
<24	163 (72.7%)
24-30	53 (23.6%)
>30	08 (3.5%)
Total	224 (100%)

Table no. III: Correlation of RAT positivity with duration of illness

Duration of illness	RAT positivity	
< 5 days	151 (67.3%)	Total = 224 (100%)
>5 days	73 (32.58%)	

Discussion
The STANDARD Q COVID19 Ag (SD-Biosensor) is among growing number of diagnostic assays available for COVID-19. Itis an immuno-chromatoghaphic test(ICT) formatand is approved to be used with nasopharyngeal swab. In our experience, the system was easy to use and gave a qualitative result for an individual sample in approximately 15-30 minutes. This significant throughput is encouraging given the large number of samples processed in many COVID-19 testing points and the potential use of RATs as a large-scale decentralized screening tool, especially in resourcepoor settings. Present study sheds light onperformance ofthis RATfor detection of SARS CoV-2 nucleoprotein in comparison to gold standard RT-PCR.

RAT identified 224 out of 345 total RT PCR positive samples during the study period (64.92%). False positive rate detected was2.6%. False negative rate was 13.45%, which is why RAT negative patients, especially when symptomatic or having history of close contact with lab confirmed COVID positive patient, need to undergo RT PCR as confirmatory test.

A high sensitivity (63.18%), specificity (99.27%), positive predictive value (97.32%) and fairly high negative predictive value (86.54%) of RAT might prove to be promisingin situations where pre-test probability of having infection is higher and as a preliminary sieve of COVID positive patients in emergency situations eg. SARI wards, Triage area, before emergency surgery,etc while awaiting RT PCR result.

When compared with corresponding Ct values of RT PCR, RAT positive samples had lower Ct value than that of RAT negative/RT PCR positive samples. Majority i.e 163/ 224 (72.7%) RAT positive samples had corresponding Ct valueless than 24 followed by 23.6% samples with Ct value between 24-30 and 3.5%samples with Ct value more than 30.

Majority of patients (67.3%) showing RAT positive result had been symptomatic for less than 5 days duration which supports the idea that antigen detection test is effective during acute phase of illness within a few days after onset of symptoms when viral load in upper respiratory tract is at its peak.

Clinical performance of RATlargely depends on circumstances in which they are used and the appropriate setting should be identified.

The COVID-19 pandemic poses a major global challenge, with a massive yet possibly underestimated burden and several unknowns. With a subtle clinical presentation and asymptomatic carriage, and in the absence of specific treatment or vaccine, it is clear that an early and accurate diagnosis is crucial for control of the disease. So for that, improvement of SARS CoV-2 diagnosis with easy, rapid and cost-efficient approach is urgently required. Rapid antigen detection tests (RAT) for detection of SARS-CoV-2 viral antigens are quite promising, advantages of RATs are rapidity, ease of interpretation, limited technical skill and infrastructure required, and this continues to make them worth pursuing. However, principal concerns are false-negative rate, probably due to low viral load.

In view of above, our study shows that adopting RAT for detection of SARS CoV-2 is more promising in centers for mass screening where prevalence of COVID-19 is lower. But at the same time our study shows fairly high positive predictive value of RAT, which means it might also be used in emergency situations e.gin patients admitted to the emergency room where pre-test probability of having COVID-19 is significantly higher and false negative results are relevant for correct management of patients. As safety of health care professionals, laboratory staff and scavengersis of utmost importance, adopting RAT as a preliminary test in emergency situations, especially in triage area, might be helpful for cautious management of COVID positive patients keeping in mind the shortage of personal protective equipments (PPE) faced globally, PPE can be meticulously used as and when required.

Also, in situations where only RT PCR is used and chance of infection spread from patient to patient admitted in various wards is high, RAT offers a quicker method in isolating these patients awaiting RT PCR result.

Last but not the least, RATs in mass screening testing could decrease the burden on virology laboratories that have been overwhelmed during this pandemic and the curve does not seem to flatten in near future!

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Conflict of interest

None to declare

References

1. P. Zhou, X.L. Yang, X.G. Wang, et al., A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature (2020), https://doi.org/10.1038/s41586-020-2012-7.
2. E. de Wit, N. van Doremalen, D. Falzarano, et al., SARS and MERS: recent insights into emerging coronaviruses, Nat. Rev. Microbiol. 14 (2016) 523e534, https://doi.org/10.1038/nrmicro.2016.81.
3. V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Eurosurveillance [Internet] 25 (3) (2020). Jan 23 [cited 2020 Feb 11]. Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020. 25.3.2000045.
4. G.C. Mak, P.K. Cheng, S.S. Lau, K.K. Wong, C. Lau, E.T. Lam, et al., Evaluation of rapid antigen test for detection of SARS-CoV-2 virus, J. Clin. Virol. 129 (August) (2020), 104500.
5. WHO, Laboratory Testing Strategy Recommendations for COVID-19: Interim Guidance, 21 March. Available from: (2020) https://apps.who.int/iris/handle/ 10665/331509.
6. A. Scohy, A. Anantharajah, M. Bodéus, B. Kabamba-Mukadi, A. Verroken, H. Rodriguez-Villalobos, Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis, J. Clin. Virol. (May) (2020) 21 2020 published online ahead of print.