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COMPARISON OF A RAPID ANTIGEN DETECTION TEST WITH REAL TIME RT PCR FOR COVID 19 DIAGNOSIS: AN EXPERIENCE FROM A TERTIARY CARE HOSPITAL IN EASTERN INDIA.

Medical Microbiology

Apurva	Scientist C Medical, Department Of Microbiology, AIIMS Patna
Prathyusha Kokkayil*	Additional Professor, Department Of Microbiology, AIIMS Patna *Corresponding Author
Pramurtajyoti Debbarma	Ex, Senior Resident, Department Of Microbiology, AIIMS Patna
Asim Sarfaraz	Associate Professor, Department Of Microbiology, AIIMS Patna
Binod Kumar Pati	Additional Professor, Department Of Microbiology, AIIMS Patna
Bhaskar Thakuria	Professor And Head, Department Of Microbiology, AIIMS Patna

ABSTRACT

Introduction: Rapid antigen detection tests (RADTs) were added to the repertoire of COVID-19 diagnostics to expand testing capacities and obtain quicker results. Thus this study aimed to evaluate the performance of the Standard Q Covid-19 Antigen Test in comparison to Real-Time RT-PCR for the diagnosis of COVID-19 in a tertiary care hospital in Eastern India. **Methods:** A cross-sectional study was conducted on 554 subjects, from whom 1108 nasopharyngeal swabs and 554 oropharyngeal swabs were collected. The samples were tested using the Standard Q Covid-19 Antigen Test and Real-Time RT-PCR. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of the rapid antigen test were calculated, considering Real-Time RT-PCR as the reference standard. **Results:** The sensitivity and specificity of the rapid antigen test were 33.62% and 99.77%, respectively. The PPV and NPV were 97.44% and 85.44%, respectively. The diagnostic accuracy was 86.28%, with a moderate agreement (Cohen's Kappa value = 0.442) between the two tests. The median Ct values of PCR-positive but rapid antigen-negative samples were higher than those of PCR-positive and rapid antigen-positive samples, indicating that the rapid antigen test detects samples with high viral loads. **Conclusions:** The rapid antigen test demonstrated low sensitivity but high specificity. It cannot be used as a standalone test for detection in symptomatic patients and must be supplemented by PCR. However, it can be used to rule out further infection in convalescing patients and contact tracing strategies. The clinical usefulness of the kit among patients with Ct values > 32 is doubtful, as there is a higher probability of false-negative results.

KEYWORDS

Rapid antigen detection tests (RADTs), Real-Time RT-PCR, SARS CoV2, COVID 19.

INTRODUCTION -

SARS CoV2 virus causing COVID 19 has spread worldwide causing morbidity and mortality since December 2019. [1] While around 775 million COVID 19 cases have been reported worldwide, India has recorded 45 million cases and 534000 deaths as of May 4, 2024.[2] The common clinical features attributed to COVID 19 are fever, dry cough, shortness of breath, myalgia, headache, diarrhoea and vomiting. Nevertheless, the spectrum of severity of COVID 19 can range from asymptomatic infection to acute respiratory distress syndrome.[3] Current diagnostic modalities of COVID 19 include detection of viral RNA, viral antigen and antibodies against the virus. [3] Molecular diagnosis using Real Time RT PCR to detect viral RNA is presently the recommended gold standard test. [4] Real Time RT PCR for SARS CoV2 has a high specificity but the sensitivity of these commercial kits with emergency approval may range from 60 to 90%. [5] False negative results may also arise because of errors in sample collection and transport as well as varied genetic sequence of the virus due to mutations. [6] Moreover, the assay can pick up live as well as dead viral RNA thereby casting doubt on infectivity and recovery even 14 to 20 days after initial testing and symptomatic improvement. [5] On the technical side, Real Time RT PCR also requires dedicated infrastructure, trained staff and pre-analytical errors are various limiting factors in resource constrained settings. Rapid antigen detection tests (RADT) were added to the repertoire of COVID 19 diagnostics to expand testing capacities and to obtain quicker results. [3] Despite the global spread of COVID-19, limited research exists on the performance of rapid antigen tests, particularly in the Indian population. In spite of rampant use of the antigen detection test in Eastern India, no studies were undertaken so far to evaluate the performance from this region. Worldwide, there are some published studies which have compared the rapid antigen detection test to Real Time RT PCR. While Scohy A et al and Blaurion L et al have reported an overall sensitivity of around 30% in their evaluations, Porte L et al have reported an overall sensitivity of 93.9% and specificity of 100% for the rapid antigen detection test when compared to RT PCR. [7,8,9] A study by Mak GCK et al reported that the rapid antigen detection test detected between 28.6 % and 81.8 % of RT-PCR-positive high viral load samples from COVID-19 patients and 0–21.1 % for normal viral load samples. [10] The performance of the rapid antigen detection tests have also not been compared between different subsets of cases such as suspect cases, confirmed cases, and convalescent cases at the time of recovery and/or discharge from the hospital. Keeping these lacunae in view, it is crucial to evaluate diagnostic methods like the rapid antigen detection tests which are used for public health interventions. Thus the present study was undertaken to compare the effectiveness of rapid antigen and addresses important gaps in understanding the efficacy of rapid antigen detection test with RT PCR for COVID 19 diagnosis in a tertiary care hospital in Eastern India.

Methodology-

A cross-sectional study was conducted to compare the performance of the Standard Q Covid-19 Antigen Test (SD BIOSENSOR) with Real-Time RT-PCR using the kit from ICMR-NIV, Pune. The study included 554 subjects, from whom 1108 nasopharyngeal swabs and 554 oropharyngeal swabs were collected. Among the subjects, 353 were male, and 201 were female. The samples were collected from suspected cases, contacts of suspected cases, and convalescing patients in August 2020.

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The Standard Q Covid-19 Antigen Test, a rapid chromatographic immunoassay that qualitatively detected specific antigens of SARS-CoV-2 in human nasopharyngeal specimens. The test kit contained test devices individually packaged in foil pouches with a desiccant, extraction buffer tubes, nozzle caps, and sterile swabs. The test procedure involved collecting nasopharyngeal swab specimens, extracting the sample by mixing the swab in the extraction buffer tube, and applying three drops of the extracted sample to the specimen well

of the test device. Results were interpreted within 15-30 minutes, with the presence of both control (C) and test (T) lines indicating a positive result, and the presence of only the control (C) line indicating a negative result.

The Real-Time RT-PCR kit developed by ICMR-NIV, Pune, was used as the reference standard for detecting SARS-CoV-2 RNA in the collected clinical specimens. The kit contained primers and probes specific for the RdRp (RNA-dependent RNA polymerase) and ORF (Open Reading Frame) genes of SARS-CoV-2. The RdRp gene primers and probes used were RdRP_SARSr-F2, RdRP_SARSr-R1, and RdRP_SARSr-P2, while the ORF gene primers and probes were HKU-ORF1b-nsp14F, HKU-ORF1b-nsp14R, and HKU-ORF1b-nsp14P. The test procedure involved RNA extraction from clinical samples using QIAamp Viral RNA Mini Kit (QIAGEN) or an equivalent RNA extraction kit, followed by reverse transcription and real-time PCR amplification using Invitrogen III PlatinumÂ® One-Step Quantitative Kit or AgPath-ID One-Step RT-PCR Kit. The PCR reaction was carried out in a 25 µL volume containing 12.5 µL of 2x reaction mix, 1.5 µL of primer-probe mix, 1 µL of RT-PCR enzyme mix, 5 µL of nuclease-free water, and 5 µL of extracted RNA template. The cycling conditions included a reverse transcription step at 55°C for 30 minutes, followed by Taq inhibitor inactivation at 95°C for 3 minutes and 45 cycles of PCR amplification (denaturation at 95°C for 15 seconds and annealing/extension at 58°C for 30 seconds). The presence of SARS-CoV-2 was determined by detecting fluorescent signals from the FAM-labeled specific probes during the PCR cycles, with a Ct value less than 35 considered positive. The kit also included positive and negative controls to ensure the validity of the test results.

Secondary data, including relevant clinical information and corresponding laboratory data pertaining to the rapid antigen detection test and Real-Time RT-PCR test, were collected retrospectively after obtaining ethical clearance from the Institute Ethics Committee. The collected data were organized in Microsoft Excel and statistically analyzed using the Statistical Package for Social Sciences (SPSS) v.22. Quantitative variables were described using mean ± SD, while categorical variables were described using proportions. The performance of the Standard Q Covid-19 Antigen Test was assessed by calculating sensitivity, specificity, false-positive rate, false-negative rate, positive predictive value (PPV), and negative predictive value (NPV), considering Real-Time RT-PCR as the reference standard. Cohen's Kappa statistic was used to measure the agreement between the two testing methods.

This study design allowed for a comprehensive evaluation of the Standard Q Covid-19 Antigen Test's performance in comparison to the widely-used Real-Time RT-PCR method, providing valuable insights into its potential utility in the diagnosis and management of Covid-19 cases.

RESULT-

Among 554 subjects, the median age of the study population was 29 years (Range 6 to 89). Among the 554 subjects from whom nasopharyngeal samples were collected, 444 were symptomatic (80.1%), 110 were asymptomatic(19.9%). The predominant symptom was cough in 75 (13.5%) patients followed by sore throat and fever (55-9.9%, 47-8.5%). Detail of Baseline demographic features are depicted in Table 1.

Table 2 depicts Sensitivity – 33.62% (95%CI – 25.18% to 43.20%), Specificity – 99.77% (95% CI – 98.54% to 99.98%), PPV – 97.44% (95%CI – 84.065 to 99.64%), and NPV- 85.44% (95%CI – 83.72 to 86.99%) of antigen test with gold standard.

The diagnostic accuracy of the test was calculated as 86.28% with a positive likelihood ratio of 148. The agreement between the two tests was moderate with a Cohen Kappa value of 0.442.

Among 113 PCR positive samples, only 39 were detected in rapid antigen detection tests. The median Ct values of E gene and N gene were 21.395 and 21.410 respectively. The median Ct values of PCR positive but rapid antigen negative samples were 30.930 and 31.937 respectively.

Maximum positivity of rapid antigen detection was among symptomatic patients suspected of having COVID19. Among convalescents, though PCR picked up 41 samples with a median E gene Ct value of 30.805 and N gene CT value of 30.964, only one sample was detected by rapid antigen detection test. In contact cases, whereas PCR picked up 28 samples with a median E gene Ct value of 29.69 and N gene CT value of 30.15, only four samples were detected by rapid antigen detection test. Details of different patient groups are depicted in Table 3.

Table 1: Detail of Baseline demographic features

Baseline demographic features	No of participants (%)
Age	Median - 29 years
Male	353 (63.7%)
Female	201 (36.3%)
Symptomatic	444 (80.1%)
Asymptomatic	110 (19.9%)
Clinical features	
Cough	75 (13.5%)
Sore throat	55 (9.9%)
Fever	47 (8.5%)
Body ache/ Malaise	49 (8.8%)
Chest pain	10 (1.8%)
Breathlessness	3 (0.5%)
Diarrhoea	5 (0.9%)

Table 2: Antigen test verses Gold standard test.

Rapid antigen detection test	Real-Time RT PCR test		Total
	Positive	Negative	
Positive	38	1	39
Negative	75	440	515
Total	113	441	554

Table 3: Ct values of different patient groups.

Variables	Symptomatic Patients (110)	Convalescent (130)	Contacts (315)
RT PCR +	44	41	28
Ag +	34	1	4
Ct E Gene	23.49	30.80	29.69
Ct N Gene	24.50	30.96	30.15

DISCUSSION:

Rapid antigen detection tests (RADTs) were added to the repertoire of COVID-19 diagnostics to expand testing capacities and obtain quicker results. Following this, studies were conducted worldwide to compare real-time RT-PCR and antigen detection tests. The present study observed that the sensitivity of the rapid antigen detection test was only 33.62%, which is much lower than that claimed by the manufacturer. Similar findings were observed by Scohy A et al., with a sensitivity of 30.2%. [7] The specificity of the rapid antigen detection test was 99.77% in the present study, which was in accordance with Scohy A et al. at 100% .[7]

While the lower sensitivity of RADTs can give false-negative results, they can be used as a first-line diagnostic test in the convalescent phase to possibly reduce the burden of Real time RT-PCR testing in a pandemic situation. This is backed up by our observation in which the asymptomatic population was always RADT-negative and positive in RT-qPCR testing with higher CT values, as the viral load is less, rendering patients non-infectious. A much higher sensitivity of 75.16% was observed by Jyotsnamayee S et al. [11], stating that the test may be used in subjects with initial days of infection. It is also observed that false-negative RADT in symptomatic individuals having a Ct value greater than or equal to 27 may be less infectious.[12, 13]

The positive predictive value (PPV) and negative predictive value (NPV) are quite promising when considering the kit for screening among symptomatic and asymptomatic individuals. Various studies have established that a mere positive RT-PCR does not conclude the infectivity of the patients. Viral load and infectivity can be partially analyzed by the cycle threshold (Ct) value. There are studies where the antigen came positive for a Ct value of 33, considering it as a cut-off for infectivity, and had good clinical correlation [14] in comparison to our kit, which has detected up to 30.96 Ct as the higher cut-off value. Comparison of Ct values of samples that were PCR-positive and antigen-positive with PCR-positive antigen-negative samples suggest that antigen is detected in samples with high viral load. This may also reflect the phase of viral replication when antigen is produced. Among convalescent patients, the rapid antigen test detected only one sample since the Ct values indicate a low viral load in these patients. A

comparison of antigen detection with viral culture or sub genomic PCR is required to rule out dead viral genome, which may be picked up by PCR in convalescent patients. [15,16]

CONCLUSIONS:

Since the rapid antigen test has a low sensitivity, it cannot be used as a stand-alone test for detection in symptomatic patients and has to be supplemented by PCR. However, it has a high specificity and can be used as a test to rule out further infection in convalescing patients and contact tracing strategies that contribute to the control of the outbreak. As per WHO recommendations, the sensitivity of the RAT kit needs to be > 80% to be used at the point of care testing. The kit studied here has shown less sensitivity. It can be used with certain precautions, like in patients having the onset of illness within 5 days, which is the time when human-to-human transmission is more. Also, this study suggests that there is no specific relation between Ct value and antigen positivity/negativity. The clinical usefulness of using this kit among patients having a Ct value > 32 is doubtful, as there is a higher probability of getting a false-negative result.

In conclusion, the rapid antigen detection test has demonstrated its value as both a diagnostic and prognostic tool in the management of COVID-19. Its ability to predict infectivity and aid in patient cohorting makes it a valuable asset, especially in resource-limited settings. As we continue to learn from the COVID-19 pandemic, the lessons learned from the use of RADT can serve as a template for managing future outbreaks and optimizing patient care.

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