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Accuracy of Rapid Antigen Detection Test Device (COVID-19 Ag Rapid Test Device) in Diagnosis of Acute COVID-19 Infections

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ABSTRACT

Objective: To evaluate the accuracy of the COVID-19 Ag Rapid test device for detection of SARS-CoV-2 with Reverse Transcriptase Polymerase Chain Reaction as the gold standard.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Virology, Armed Forces Institute of Pathology, Rawalpindi, in the month of Sept 2020.

Methodology: A total of 106 patients suspected of COVID-19 were tested, including 63 patients admitted to the COVID-19 ward of Pak Emirates Military Hospital and 43 from the emergency department of Combined Military Hospital, Rawalpindi Pakistan respectively. The samples were transported to the Virology department and subjected to Polymerase Chain Reaction and Antigen testing. In addition, the diagnostic accuracy of the COVID-19 Ag Rapid test device was compared to Reverse Transcriptase Polymerase Chain Reaction for detection of SARS-CoV-2 infection.

Results: Out of 106 nasopharyngeal swab samples tested, 48 (45.2%) samples were positive by Rapid test device and Reversed Transcriptase Polymerase Chain Reaction, and 52 (49.0%) samples tested negative by both methods. Inconsistent results (False Negative) were obtained in 6 (5.6%) samples. COVID-19 Ag Rapid test device has detected the maximum number of cases, 41 (85.4%), during the first week of illness. Its sensitivity decreases as the duration of infection progress.

Conclusion: The overall sensitivity of the Rapid test device is much less than the Polymerase chain reaction due to potential false negative results. However, it can be helpful in the early isolation of cases in an outbreak in a closed community and for case management in peripheral setups, where Polymerase chain reaction facilities are unavailable.

Keywords: Rapid Antigen Detection test, RT-PCR, SARS-CoV-2 diagnosis.

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INTRODUCTION

In December 2019, a novel coronavirus was identified in Wuhan, a city in Hubei Province of China, as the cause of pneumonia in a cluster of patients.[1] The virus was highly contagious, and it rapidly spread to all the continents and finally declared as a global pandemic by World Health Organization (WHO) on 11th Mar 2020.[2] The manifestation of SARS-CoV-2 infection is characterized by fever, cough, myalgia, dyspnea and anosmia.[3] Most infections are not severe. However, some patients may progress to critical illness, especially in advanced age and having underlying medical comorbidities.[4] Accurate and timely detection leading to effective management are very helpful in stopping the spread of the virus. Traditionally, SARS-CoV-2 detection relied on RT-PCR, a technique widely used in molecular biology to amplify DNA samples. PCR test has played an important role in the early detection and isolation of cases.[5] As the number of new COVID-19 cases rises and deaths continue to occur around the world, the common goal of scientists and healthcare workers is to develop assays that can rapidly detect SARS-CoV-2 infection.[6,7]

In the past, Point-of-care testing (POCT) for respiratory viruses has shown relatively low sensitivity, and its use for reliable screening is limited. Studies conducted in the 2009/10 flu pandemic period reported sensitivities ranging from 20% to 70% for influenza RDT.[8] In one systematic review and meta-analysis conducted in Canada, the sensitivity of RDT for Respiratory Syncytial Virus (RSV) was 29% to 81% for adults and children, respectively. The most important cause of disagreement regarding implementing POCT as a screening test for respiratory viruses was the lack of sensitivity and variable in test results under different conditions.[9]

During a current pandemic, a need was felt to evaluate a rapid screening test for detecting SARS-CoV-2 infection with high sensitivity and specificity. Such rapid tests have the obvious benefits; a fast turn-around time, ease of use, high throughput and

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particularly their utility in outbreak situations in the resource-limited setting.[10] Therefore, we carried out this study to find the accurateness of a Rapid Antigen Test Device in detecting SARS-CoV-2 infection and its utility as Point-of-care testing (POCT) so that it can be implemented.

METHODOLOGY

This study was conducted in the month of Sept 2020 at the Department of Virology, Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan. The Institutional Review Board (VIR-1/READ-IRB/20 /1054) of the institute approved the study format.

Inclusion Criteria: Suspected patients of COVID-19 infection having cough, fever, shortness of breath, body aches or anosmia were included in the study.

Exclusion Criteria: Patients with a history of major maxillofacial surgery and patients with debilitating illnesses were excluded from the study.

Participants were explained the purpose of the study and informed written consent was obtained. A total of 106 patients, 63 admitted to the COVID-19 ward at Pak Emirates Military Hospital (PEMH), Rawalpindi Pakistan and 43 individuals who had attended the emergency department at Combined Military Hospital (CMH), Rawalpindi were enrolled in the study. Sample collection was done by trained medical staff wearing personal protective equipment (PPE), N95 mask, face shield/goggles, gown and gloves. Two nasopharyngeal Swab (NPS) samples were collected from each patient; one swab was placed in VTM for RT-PCR testing, and the second swab was placed in an extraction tube filled with extraction buffer with COVID-19 Ag rapid test device. The samples were transported by maintaining a cold chain (2°C to 8°C) to Virology department AFIP, Rawalpindi and simultaneously subjected to PCR and RDT. Initial processing of all specimens for SARS-CoV-2 was performed in a Biosafety Cabinet (Class II), wearing recommended PPE. The nucleic acid extraction was done by an automated extraction method using magnetic beads technology, and amplification was performed using the SARS-CoV-2 amplification kit on Thermal Cycler. Significant data was obtained from the medical history sheet of the patients.

All available medical records were assembled into a data collection sheet, which comprised of two sections; the first portion was for the demographic characteristics of the patients like age, gender and occupation, while the second portion contained clinical complaints like fever, cough, shortness of breath, myalgia, history of any significant history of exposure and High-Resolution Computed Tomography (HRCT) Chest finding. Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Mean ± SD were calculated for quantitative variables like age, while all qualitative Variables, e.g., genders, age groups, symptoms and HRCT-Chest findings, were expressed as frequency and percentages. The sensitivity and specificity of the RDT test were calculated against PCR (Gold Standard). The chi-square test was used for comparisons.

RESULTS

Both RT-PCR and RDT tested one hundred six nasopharyngeal swab samples. The mean age of participants was 39 +13.4 years. 72 (68%) patients were males, and 34 (32%) were females. Age group distribution showed that a maximum number of patients (67.9%) were in age group-2 (21-40 years). Out of the total of 106 patients, 66 (62.3%) of patients were symptomatic; the most frequent symptoms were fever (54.7%), myalgia (43.4%), cough (32.1%), and only 8 (7.5%) had HRCT-Chest finding suggestive of COVID-19 (Table-I).

Table-I: Demographic and Clinical Characteristics.

Characteristics	n (%)
Gender	
Male	72 (67.9%)
Female	34 (32.1%)
Age Groups	
Group - 1 (1-20 years)	4 (3.8%)
Group - 2 (21-40 years)	72 (67.9%)
Group - 3 (41-60)	22 (20.8%)
Group - 4 (61-80 years)	8 (7.5%)
Symptoms	
Symptomatic	66 (62.2%)
Asymptomatic	40 (37.7%)
HRCT-Chest finding	
Suggestive for COVID-19	8 (7.5%)
Unremarkable study	38 (35.8%)
Not Done	60 (56.6%)

According to RT-PCR results, 52 (49.0%) samples were negative, and 54 (50.9%) samples were positive, with a wide-ranged cycle threshold (Ct) value (range: Ct12 to Ct 30). Among these 54 (50.9%) positive samples, RDT detected 48 (45.2%) positive samples. 52 (49.0%) samples were negative for both RT-PCR and RDT. However, six positive samples (5.6%) on RT-PCR were found negative by RDT, with resultant sensitivity of 88.8% and specificity of 100% for RDT (Table-II).

Table-II: Comparison of rapid test device (RDT) and RT-PCR results.

Diagnostic Parameters	Values	
PCR Result	Positive	Negative
	54 (50.9%)	52 (49.0%)
RDT Result	48 (45.2%)	58 (54.7%)

The comparison of clinical manifestations with RT-PCR and rapid test device (RDT) were shown in the Table-III.

Table-III : Comparison of clinical manifestations with RT-PCR and rapid test device (RDT).

	PCR Results		Rapid Test Device (RDT)		*p*-value PCR	*p*-value RDT
	Yes	No	Yes	No		
Present						
Fever	46 (79.3%)	8 (16.7%)	41 (70.7%)	7 (14.6%)	0.001	0.001
Absent	12 (20.7%)	40 (83.3%)	17 (29.3%)	41 (85.4%)		
Present						
Cough	23 (67.6%)	31 (43.1%)	21 (61.8%)	27 (37.5%)	0.018	0.019
Absent	11 (32.4%)	41 (56.9%)	13 (38.2%)	45 (62.5%)		
Present						
Shortness of breath	8 (100.0%)	46 (46.9%)	7 (87.5%)	41 (41.8%)	0.004	0.013
Absent	-	52 (53.1%)	1 (12.5%)	57 (58.2%)		
Present						
Flu	18 (64.3%)	36 (46.2%)	18 (64.3%)	30 (38.5%)	0.100	0.019
Absent	10 (35.7%)	42 (53.8%)	10 (35.7%)	48 (61.5%)		
Present						
Sore Throat	22 (68.8%)	32 (43.2%)	21 (65.6%)	27 (36.5%)	0.016	0.006
Absent	10 (31.3%)	42 (56.8%)	11 (34.4%)	47 (63.5%)		
Present						
Myalgia	32 (69.6%)	22 (36.7%)	30 (65.2%)	18 (30.0%)	0.001	0.001
Absent	14 (30.4%)	38 (63.3%)	16 (34.8%)	42 (70.0%)		

DISCUSSION

With the advancements in diagnostics and progress in the development of POCT, newly emerging pathogens are being detected with great accuracy. Furthermore, these innovations have led to establishing a timely provisional diagnosis, which helps in taking timely decisions about the isolation of patients and different treatment options.

The COVID-19 pandemic has led to an unprecedented public health crisis which has affected every country across the globe. Prompt detection of SARS-CoV-2 is pivotal in limiting the spread of the disease. Currently, two diagnostic tests are available to detect acute SARS-CoV-2 infection. One is the molecular test, which detects the genetic material of the virus, and the second is an antigen-based test which detects different proteins of the virus. Molecular tests are highly sensitive and specific for nucleic acid detection of virus and are considered the gold standard for COVID-19 diagnosis with a turnaround time of 4-5 hours.[11] It is a fact that Antigen tests are less sensitive and specific than molecular tests. WHO recommends the sensitivity of ≥ 80% and specificity of ≥ 97 for RDT test kits for diagnosis of acute COVID-19 infection compared to a Nucleic Acid Amplification Test (NAAT) as a reference assay. COVID-19 Ag Rapid test device offers accurate and quick results within 20 minutes; however, there is limited data available on the accuracy of this rapid test. RDT works on the principle of lateral flow immunoassay device and claims a sensitivity of 91.4% (94.1% for samples with Ct values ≤33)/specificity of 99.8%.[12]

A study was conducted in Madrid, Spain, on 255 patients suspected of acute COVID-19 infection to check the reliability of RDT in diagnosing acute SARS-CoV-2 infection. Two nasopharyngeal swabs were obtained from each patient, one was applied for RT-PCR testing, and the second was used to perform RDT. Out of 255 samples tested, 60 (23.5%) were positive for RT-PCR. Among these, 44 (17.2%) positive samples were correctly detected by RDT with a sensitivity of 73.3%.[13]

A study was carried out at the University Medical Center Utrecht (UMCU) in the Netherlands, and the Horacio Oduber Hospital in Aruba for real-life validation of RAD in community-dwelling subjects with symptoms of potential SARS-CoV-2 infection observed sensitivity of 72.6% and 81% respectively.[14] Another study was conducted in Hong Kong on 105 respiratory samples for COVID-19 from Public Health Laboratory Services (PHLSB) and new 13 non-SARS-CoV-2

respiratory samples. These samples were tested to evaluate the accuracy and cross-reactivity of the RDT kit. In this study, results of RDT were also compared with Standard Q COVID-19 Ag (SD Biosensor, Korea) in addition to RT-PCR. Both kits showed similar sensitivity of 68.6 % and 65.7–71.4 %, respectively.[15] A study on the diagnostic accuracy of RDT for SARS-CoV-2 infection was conducted in a private medical centre in Santiago, Chile. A total of 127 samples were tested for patients with respiratory symptoms related to COVID-19 or an epidemiological risk factor for COVID-19 infection (travel or contact with a case), with a reported sensitivity of 93.9%.[16]

This study was conducted in Pakistan to evaluate the Rapid antigen detection (RDT) test, and our study results observed sensitivity and specificity of RDT at 88.8% and 100%, respectively. The sensitivity observed in our study is relatively higher than that observed in the above three studies. However, these minor differences in sensitivity may be attributable to different sample collection and testing conditions. According to our results, the RDT correctly detected PCR-positive samples up to a Ct value of 29 (sensitivity of 91.0% for Ct value below 29.0). The high sensitivity of RDT in our study was also associated with high viral load in patients and those who were symptomatic and in the early phase of infection for SARS-CoV-2. However, false negative results were also observed with low viral load related to early infection (incubation period), before the virus established its replication peak and late infection due to decline of viral replication. However, a false negative result may lead to transmission of SARS-CoV-2 infection if missed with RDT and may greatly impact the spread of infection in the community. Therefore, false negative results must be confirmed by the RT-PCR test. The patients who are symptomatic of COVID-19 and require rapid screening tests with high sensitivity for arriving at clinical diagnosis for self-isolation and treatment, in this context, RDT can be used with caution due to potential false negative results.[17,18]

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CONCLUSION

COVID-19 Ag Rapid test device is less sensitive and specific than the PCR test and, therefore, should be used with caution in diagnosing acute SARS-CoV-2 infection in the clinical setting. However, it can be helpful in the early isolation of cases in an outbreak and a closed community and for case management in peripheral setups, where PCR facilities are unavailable. However, further large-scale studies are required to evaluate the analytical sensitivity and specificity of RAD test kits before their recommendations can be given as a screening tool in tertiary care setups.

Conflict of Interest: None.

Author's Contribution

EG: Conception of idea, Design of work and final approval, NS: Data collection & drafting, SKN: Correction of manuscript, analysis and critical revision, MN: Data collection, analysis & drafting, FA:, HH: Data collection and analysis

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